UNIVERSITÉ DU QUÉBEC À MONTRÉAL & UNIVERSITÉ DE MONTPELLIER

THE ROLE OF PHENOTYPIC VARIATION IN URBAN EVOLUTION

THESIS

PRESENTED

AS PARTIAL REQUIREMENT

OF THE DOCTORATE IN BIOLOGY (COTUTELLE)

 $\mathbf{B}\mathbf{Y}$

MEGAN JOY THOMPSON

AUGUST 2024

UNIVERSITÉ DU QUÉBEC À MONTRÉAL & UNIVERSITÉ DE MONTPELLIER

LE RÔLE DE LA VARIATION PHÉNOTYPIQUE DANS L'ÉVOLUTION URBAINE

THÈSE

PRÉSENTÉ(E)

COMME EXIGENCE PARTIELLE

DU DOCTORAT EN BIOLOGIE (COTUTELLE)

PAR

MEGAN JOY THOMPSON

AOÛT 2024

UNIVERSITÉ DU QUÉBEC À MONTRÉAL Service des bibliothèques

Avertissement

La diffusion de cette thèse se fait dans le respect des droits de son auteur, qui a signé le formulaire *Autorisation de reproduire et de diffuser un travail de recherche de cycles supérieurs* (SDU-522 – Rév.12-2023). Cette autorisation stipule que «conformément à l'article 11 du Règlement no 8 des études de cycles supérieurs, [l'auteur] concède à l'Université du Québec à Montréal une licence non exclusive d'utilisation et de publication de la totalité ou d'une partie importante de [son] travail de recherche pour des fins pédagogiques et non commerciales. Plus précisément, [l'auteur] autorise l'Université du Québec à Montréal à reproduire, diffuser, prêter, distribuer ou vendre des copies de [son] travail de recherche à des fins non commerciales sur quelque support que ce soit, y compris l'Internet. Cette licence et cette autorisation n'entraînent pas une renonciation de [la] part [de l'auteur] à [ses] droits moraux ni à [ses] droits de propriété intellectuelle. Sauf entente contraire, [l'auteur] conserve la liberté de diffuser et de commercialiser ou non ce travail dont [il] possède un exemplaire.»

ACKNOWLEDGEMENTS / REMERCIEMENTS

The biggest thank you goes to Anne and Denis who have provided me unwavering guidance, invaluable insight, and support both before and during my PhD; I lucked out with the most "super duper supervisory duo"! Denis' easy-going manner and excitment about science was infectious, and he contributed to my thesis journey feeling low stress and fun. Specifically, I would like to thank Denis for his big ideas and improving my *understanding* of science. Anne went above and beyond as a supervisor; helping us secure an apartment in France as a guarantor, inviting us along to family outings, and fighting admin battles on my behalf among many other things. Anne was vital in keeping momentum during my PhD as she made it a main priority to be available for meetings or reading drafts and, since it is not unusual for me to suffer decision paralysis, she played a main role in boosting my confidence and talking through some of the big (and not so big) research decisions during my thesis. Anne and Denis are such exemplary leaders and I admire them both a lot. I have tried to learn everything I can from them as mentors during my PhD, and I look forward to continuing to do so into the future!

I would also like to thank my CEFE and UQAM colleagues, including members of the E3CO team, CEFE tit team, and GRECA. Specifically, Barbara Class, Laura Gervais, and Tracy Burkhard were close colleagues and friends that provided a lot of professional and personal "galpal" support along the way. Amélie Fargevieille deserves a specific mention since she always made me feel welcome and included at the CEFE; she was a top officemate and founder of des belles gosses. Other important people include Erwan Harscouet, Nicolas Silva, Sonia Mai, Drew Sauve, Dhanya Bharath, Mathieu Videlier, Francesca Santostefano, Tom Ratz, Hélene Dion-Phénix, Mathias Gagnon-Barbin, Hassen Allegue, Mathilde Tissier, Catherine Capkun-Huot, and Elene Haave-Audet who were a pleasure to interact with inside and outside of work during my PhD. Thank you to Samuel Caro, Céline Teplitsky, Ségolene Delaitre, and Paul Cuchot, who were in different teams at the CEFE, but always made an effort to attend my talks and discuss my results. I would also like to thank everyone in the CEFE tit team who made fieldwork easy. Special thanks to Sam Perret for being my main field buddy and showing me the ropes (...and tolerating my manual driving, eek!). Christophe de Franceschi helped me pass my specialist mist netting license while in France and I am so appreciative for his one-on-one training for this. Many thanks to my committee members Pierre-Olivier Montiglio, Céline Teplitsky, and Ophélie Ronce for useful discussions and advice.

Outside of my two universities, I would like to thank those in the European urban tit network. I feel so lucky that I have been able to interact with so many talented and successful academics during my PhD; having access to diverse and expert perspectives has facilitated my growth as a researcher (and significantly

improved my organization skills!). Davide Dominoni and Pablo Capilla-Lasheras, in particular, were important collaborators from the beginning. Julien Martin very kindly agreed to guide me with some very complicated stats and I feel like I learned a lot from him in a short time, including how to scribble equations on zoom chats. The Montpellier Zoo staff, and specifically Marc Romans and Baptsise Chenet, were essential contributors to the organization and running of fieldwork and the common garden, and I am so appreciative to them for making our academic-industry collaboration so easy. A very pragmatic thanks to NSERC, FRQNT, and CeMEB who provided me with funding throughout my PhD.

I want to give a mandatory shout out to my family, especially my parents. With three children doing PhDs at the same time, it's obvious how instrumental their priority on learning was when we were growing up, and I am so fortunate for this. Further, I found myself with no where to go while on the way to my first field season in Spring 2020 when the pandemic hit, and they happily housed me while I started a PhD during a very uncertain time. So thank you to my family who I know support me no matter when or what!

Last, but certainly not least, is Matthew, who is proofreading for me as I write this. I mainly just want to thank him for listening. He had to endure daily "how was your day" chats that included all my thesis highs and lows, and I can't begin to express how important his steady encouragement was for me along the way.

TABLE DES MATIÈRES

ACK	NOWLEDGEMENTS / REMERCIEMENTS	iii
LIST	f of FIGURES / LISTE DES FIGURES	. viii
LIST	GOF TABLES / LISTE DES TABLEAUX	xi
LIST DES	T OF ABBREVIATIONS AND ACRONYMS / LISTE DES ABRÉVIATIONS, DES SIGLES ET ACRONYMES	. xiv
LIST	T OF SYMBOLS AND UNITS / LISTE DES SYMBOLES ET DES UNITÉS	xv
ABS	TRACT	. xvi
RÉS	UMÉ	xvii
CHA	APITRE 1 General Introduction	1
1.1	The nature and causes of variability	1
1.2	Urban Evolutionary Ecology	3
1.3	Study systems	4
1.1 1.1 1.1	 3.1 Great and blue tits	4 5 6
1.4	Main objective and research questions	6
1.4 1.4	4.1 How does urbanization affect phenotypic variation?4.2 How do genetic and plastic changes shape urban phenotypes?	7 8
CHA	APITRE 2 Phentoypic variation in urban environments: mechanisms and implications	10
2.1	Urban phenotypic variation	11
2.2 2.2 2.2 2.2 2.2 2.2	 Mechanisms shaping phenotypic variation in urban environments	13 14 14 15 16
2.3	Implications of urban phenotypic variation	19
2.4	Implications for species interactions, communities, and ecosystem processes	20
2.5	Implications for fitness, selection, and population dynamics	21
2.6	Implications for urban conservation management	23
2.7	Concluding remarks	24
2.8	Acknowledgements	25
CHA	APITRE 3 Continental patterns of phenotypic variation along urban gradients: a mega-analysis	26

3.1	Intro	oduction	27
3.2	Met	hods	31
3.	.2.1	Datasets and trait measures	31
3.	.2.2	Environmental variables	33
3.	.2.3	Statistical approach	34
3.	.2.4	Statistical approach : adult tarsus	35
3.	.2.5	Statistical approach : nestling tarsus	36
3.	.2.6	Statistical approach : lay date	37
3.3	Resi	ılts	37
3.	.3.1	Adult tarsus	37
3.	.3.2	Nestling tarsus	39
3.	.3.3	Lay date	41
3.4	Disc	sussion	42
3	4 1	Tarsus variation	43
3	42	I av date variation	
3	43	Average effect of urbanization on variation and possible consequences	45
3.	.4.4	Limitations and moving forward	47
35	Con	clusions	47
3.6	Ack	nowledgements	
5.0	ACK	nowledgements	40
CHA effe 4.1	APITI cts dr Intro	RE 4 The city and forest bird flock together in a common garden: genetic and environment ive phenotypic divergence in urban populations	tal 49 50
42	Met	hods	59
ч.2 Л	2 1	Study system and quantifying urbanization	
4. 4	2.1	Common garden manipulation : egg transfer to wild foster parents	
	2.2	Common garden manipulation : captive rearing	
4	2.4	Common garden manipulation : blood sampling and genotyping	67
4	2.5	Phentovnic measurements	62 62
4	.2.6	Statistical analysis	64
13	Dog		68
4.5	Rest		08
4.4	Disc	2USS10n	
4.	.4.1	Support for genetic change	72
4.	.4.2	Support for plastic change	74
4.	.4.3	Environmental and genetic contributions towards individual phenotypic variation	75
4.	.4.4	Caveats	
4.	.4.5	Conclusion	76
4.5	Ack	nowledgements	77
СН		PE 5 City tit with cognitive variation in wild and common garden contexts	78
		AL 5 City of wit. cognitive variation in who and common garden contexts	
5.1	Intro	oduction	79
5.2	Met	hods	81
5.	.2.1	Study system	81
5.	.2.2	Common garden	82

		~~		
5.2.	3 Genotyping	83		
5.2.	4 Cognitive assay	84		
5.2.	5 Statistical approach	86		
5.2.	6 Statistical approach : errors	86		
5.2.	7 Statistical approach : latency to escape	. 87		
5.3 R	Results	88		
5.3.	1 Number of errors	88		
5.3.	2 Latency to excape	. 91		
5.4 D	Discussion	92		
5.5 A	Acknowledgements	95		
СНАР	PITPE 6 General Discussion	06		
CIIAI		. 90		
6.1 S	ummary of results	96		
6.2 H	Iypothesized drivers of increased urban phenotypic variation	. 97		
6.3 F	Future directions	. 98		
6.4 T	The significance of urban research	101		
6.5 C	General conclusion	102		
ANNE	EXE A Supplementary materials for Chapter 2	103		
ANNEXE B Supplementary materials for Chapter 3				
ANNEXE C Supplementary materials for Chapter 4 123				
ANNEXE D Supplementary materials for Chapter 5 129				
APPENDICE E Capilla-Lasheras et al. 2022 Ecology Letters				
APPENDICE F Gervais et al. 2024 Under revision				
APPENDICE G Charmantier et al. 2024 Under revision				
BIBLIOGRAPHY / BIBLIOGRAPHIE				

LIST OF FIGURES / LISTE DES FIGURES

- Figure 4.3: ISA (impervious surface area) effect and 95% confidence intervals (CI) on phenotypic traits of common garden (blue) and wild (brown) birds across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. ISA effects varied clearly by sex only in one case (A: aggression in wild birds) and these sex differences are shown (wild males: orange, wild females: red); aggression over the ISA gradient in the common garden did not differ clearly by sex and so the common garden estimate has not been split by sex in A. Phenotypes were measured in the wild annually during the breeding season between 2011–2022, whereas we measured phenotypes in the common garden between 06 June 2022–31 January 2023.
- **Figure 5.1:** Cognitive assay used to measure inhibitory control in wild and common garden contexts. A) Side view of the cognitive assay cage in the field, B) diagram showing assay components from above including a possible route to the exit in red, and C) a video clip showing how the cognitive task was analyzed (note bird detouring barrier on right).
- **Figure 5.3:** The number of errors (A) and latency to escape (B) the detour task over trials (individuals assayed annually in the wild and every three months in common garden). A) the number of errors over trials separately for habitat type (urban vs forest) and context (common garden trials: purple to pink; wild trials: red to orange). B) the probability of escaping over trials (purple to pink) in the common garden experiment. Estimates from the cox proportional hazards models (Table 5.1.2B) were subtracted from 1 so that plots show the probability of escaping. Figures show model estimates and associated 95% confidence intervals when continuous predictors held at their means and categorical predictor held at the level included in the intercept.
- Figure S2.1 : Related to Figure 2.2. Means \pm standard deviations of morphological traits (wing length, tarsus length, and body mass) from each comparison in order of decreasing latitude for urban (blue) and nonurban (green) great tits separated by sex (male = dark shade, female = light shade)......108
- Figure S2.2 : Related to Figure 2.2. Means \pm standard deviations of morphological traits (wing length, tarsus length, and body mass) from each comparison in order of decreasing latitude for urban (blue) and nonurban (green) blue tits separated by sex (male = dark shade, female = light shade)......109

LIST OF TABLES / LISTE DES TABLEAUX

- **Table 4.1:** Synthesis of studies (N = 77; non-exhaustive) that have used a common garden approach to compare the phenotypes of urban and nonurban populations (e.g., rural, forest, mountain, agricultural) across a variety of different groups (amphibians, birds, invertebrates, fish, plants, reptiles) and traits (physiology, behaviour, morphology, life history, phenology). Information concerning the traits measured, approach taken, results, conclusions, and other notes are shown for each study. Studies were collated from Lambert et al. 2021 (Table 1) and from a literature search for articles since 2020 that included both "common garden" and "urban" (conducted March 22, 2024 using Google Scholar)...53
- **Table 4.2:** Summary of 1) wild and 2) common garden model structures that account for different fixed and random effects. Ranges for continuous fixed effects and number (N) of random effect levels for each trait are shown.

 66

- **Table S2.4** : related to Figure 2.2. Mean estimates and 95% confidence intervals of urban *versus* nonurban differences in mean (lnRR) and variance (lnCVR) effect sizes for models evaluating a) the overall effect, b) species, c) sex, and d) morphological traits. We show the relative heterogeneity (I^2) explained by each random effect in the overall model (a). As well, we show the estimated amount of variance explained by the fixed effects (marginal R^2) in models b-d. Model intercepts have been stripped from models b-d.
- **Table S3.1** : Summary of data sets used to examine patterns of phenotypic variation along urban gradients comprising urban (U) and forest (F) habitats across Europe in two species (GT = great tits, BT = blue tits) and across three traits (adult tarsus length = tars, nestling tarsus length = ntars, and lay date). 111

- Table S3.4 : Model estimates when examining the effect of continuous urbanization (i.e., impervious surface area; ISA) at 100 meters instead of the categorical effect of habitat (i.e., urban vs. forest). Effects highlighted in bold have changed conclusions (i.e., credible interval overlaps or not zero) for A) adult tarsus length and B) nestling tarsus length, and C) female lay dates from Table 3.1 in main text, but most conclusions remain the same.
- **Table S3.5** : Model estimates when not including the city of Munich location. Effects highlighted in boldhave changed conclusions (i.e., credible interval now overlaps or not zero) for A) adult tarsus lengthand B) nestling tarsus length from Table 3.1 in main text, but most conclusions remain the same. 115

- **Table S4.2**: Overview of phenotyping in the common garden experiment including number of observations, individuals, and mean and range of number of repeated measures per individual. The age when individuals were measured is shown as well as repeatability across wild and common garden contexts. Note that genotypic data was only available for 72 individuals and so animal models will exclude the one individual that wasn't sequenced.

 126
- **Table S4.3** : Common garden animal model comparison to Table 4.3 when including the genetic relatedness matrix (GRM) used to additionally estimate individual genetic variance (V_A). Fixed and random model estimates and 95% credible intervals (CI) across phenotypic traits (A: Aggression in hand, B: Breath rate index, C: Exploration, D: Tarsus length, and E: body mass). Exploration estimates are from a Poisson generalized mixed-effect model, while all other traits were fit with Gaussian mixed-effect models. Interactions between sex and habitat were not significant across traits and dropped from the model. The number of observations (obs) and individuals (ind) for each trait and context are shown in the top panel. We were missing genetic data on one individual and so our sample sizes and observations differ slightly from those reported in Table 4.3. We also report estimated heritability across all traits and Q_{ST} values for traits where we see a habitat difference (breath rate index and body mass)....... 127
- **Table S4.4** : Model comparisons to Table 4.3 when replacing the habitat effect with the proportion ISA
(impervious surface area at 100m; continuous urbanization). Fixed and random model estimates and
95% credible intervals (CI) for 1) wild and 2) common garden contexts across phenotypic traits (A:
Aggression in hand, B: Breath rate index, C: Exploration, D: Tarsus length, and E: Body mass).
Exploration estimates are from Poisson generalized mixed-effects model, while all other traits were fit
with Gaussian mixed-effects models. The number of observations (obs) and individuals (ind) for each
trait and context are shown in the top panel.
- **Table S5.1**: Model comparison to Table 5.1 in main text when examining the site-level proportion ISA effect (impervious surface area; 100m) instead of the habitat type (forest vs. urban) effect. There was one case where the interaction between ISA and trial was significant (wild errors model) where the number of errors decreased with increasing ISA in trial 1 and 3, but not trial 2. Otherwise, results are qualitatively similar.

 129
- Table S5.2: Model comparison to Table 5.1 in main text when including data from only the first wild trial.

 Model results showing habitat type and site-level proportion ISA (impervious surface area) effect are shown. Results are qualitatively similar with this reduced data set.

 130

LIST OF ABBREVIATIONS AND ACRONYMS / LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

approx.	approximately
CG	common garden
CI	credible or confidence interval
DHGLM	double hierarchical linear mixed-effect models
GRM	genetic relatedness matrix
LKJ	Lewandowski-Kurowicka-Joe
Ν	number
ind	individuals
ISA	impervious surface area
obs	observations
Х	times

LIST OF SYMBOLS AND UNITS / LISTE DES SYMBOLES ET DES UNITÉS

%	percent
°C	degrees celsius
β	model effect size
β_{m}	effect size from mean part of model
$\beta_{\rm v}$	effect size from dispersion part of model
h^2	narrow sense heritability
F_{ST}	amount of genetic variance among populations relative to total genetic variance
g	grams
G & E	genetic differentiation and plastic response to environmental variation
GxE	genetic differentiation of plastic response to environmental variation
km	kilometers
Qst	amount of genetic variance among populations relative to the total genetic variance in a trait
lnCVR	variance effect size : natural logarithm of the ratio between the coefficients of variation
lnRR	mean effect size : natural logarithm of the ratio between the means
m	meters
mm	milimeters
Р	P-value
R	repeatability
sec	seconds
r	correlation between random effects
r^2	Pearson correlation
rho	Spearman correlation
$V_{\rm A}$	additive genetic variance
V_{F}	fixed-effect variance
VI	among-individual variance
V_{P}	total phenotypic variance
V _R	residual variance

ABSTRACT

Evaluating how eco-evolutionary processes drive individual diversity in natural systems has been a central focus in Evolutionary Ecology and numerous studies now examine individual phenotypic differences along environmental gradients. Genetic differences between individuals and phenotypic plasticity can both shape the total phenotypic variation observed within wild populations, and distinguishing the relative contributions of genetic and plastic effects is crucial for establishing the eco-evolutionary processes affect variation. Urban systems, specifically, are useful contexts to test how eco-evolutionary processes affect variation as phenotypic differentation is occuring on contemporary time scales across replicated urban contexts. Although it has been widely recognized that urbanization can affect the *mean* phenotypes of populations, whether genetic or plastic changes drive these urban phenotypic shifts and how urbanization affects overall phenotypic variation in wild populations and to evaluate how genetic and environmental effects may contribute to differences in phenotypic variation along urban gradients.

My thesis uses both meta- and mega-analyses with long-term datasets on replicated wild populations and experimental approaches to address my objectives. I first used meta- and mega-analyses with data on great and blue tits (*Parus major, Cyanistes caeruleus*) along 14 urban gradients across the European continent (Chapters 2 and 3) to evaluate how urbanization impacts morphological and life history variation. I establish that urbanization increases phenotypic variation (Chapters 2 and 3). More specifically, I find that urbanization increases individual differences at fine local scales within subpopulations and can also drive higher differentiation among urban subpopulations at larger spatial scales (Chapter 3).

To examine how genetic and plastic change contribute to phenotypic divergences in urban populations, I used a common garden experiment with great tits along an urban gradient in Montpellier, France. I find that both genetic and plastic effects can contribute to wild urban phenotypes, and that their contributions are trait-specific. I show that genetic change likely contributes to smaller urban body size and faster breath rates, whereas plasticity to urban conditions most likely drives higher aggression and exploration behaviours in urban birds (Chapter 4). I also examine cognitive variation in wild great tits along the Montpellier urban gradient for the first time, and evaluate the genetic basis of this cognitive variation in the common garden experiment. I demonstrate that wild tits in more urbanized habitats have higher performance related to inhibitory control in a motor detour task compared to forest tits. However, these increased inhibitory control abilities are not maintained in the common garden context suggesting that the cognitive variation observed in the wild is likely driven by plasticity or experince in urban environments (Chapter 5).

Overall, my thesis establishes that urbanization is associated with increases in phenotypic variation in wild populations at multiple spatial scales, and that both genetic and plastic changes contribute to urban phentoypic divergences. Thus, my results suggest that phentoypic variation can play an important role in urban evolution. My thesis illustrates that both multi and single population research studies can make useful contributions to Urban Evolutionary Ecology moving forward, and I discuss that integrating community ecology and climate change themes in urban research could be especially effective research avenues to promote further advances in urban research.

Keywords : city, intraspecific variation, genetic variation, phenotypic plasticity

RÉSUMÉ

L'évaluation de la contribution des processus écoévolutifs à la diversité des organismes dans les systèmes naturels est un thème central de l'écologie évolutive et de nombreuses études examinent désormais les différences phénotypiques individuelles à travers les gradients environnementaux. Les différences génétiques et la plasticité phénotypique peuvent toutes deux façonner la variation phénotypique totale observée au sein des populations sauvages. Distinguer les contributions relatives des différences génétiques et plastiques est cruciale pour établir les conséquences écoévolutives de la variation dans les systèmes naturels. Les systèmes urbains, en particulier, fournissent un contexte intéressant pour examiner comment les processus écoévolutifs affectent la variation, car la différenciation phénotypique se produit sur des échelles de temps contemporaines dans les contextes urbains. Si l'on sait que l'urbanisation peut influencer les moyennes phénotypiques des populations, l'influence de l'urbanisation sur la variation phénotypique et les origines génétique ou plastique de ces changements phénotypiques urbains restent largement inexplorées. L'objectif principal de ma thèse était de déterminer comment l'urbanisation affecte la variation phénotypique et environnementaux peuvent déterminer la variation phénotypique le long des gradients urbains.

Ma thèse utilise des méta- et méga-analyses avec des données à long terme sur des populations sauvages répliquées ainsi que des approches expérimentales pour répondre à mes objectifs et à mes questions de recherche. En premier lieu, j'ai utilisé des méta- et méga-analyses avec des données sur les mésanges charbonnières et bleues (Parus major, Cyanistes caeruleus) sur 14 gradients urbains à travers le continent européen (chapitres 2 et 3) afin d'évaluer l'impact de l'urbanisation sur la variation morphologique et du cycle de vie. J'ai établi que l'urbanisation augmente la variation phénotypique (chapitres 2 et 3). Plus précisément, j'ai trouvé que l'urbanisation augmente les différences individuelles à des échelles locales fines au sein des sous-populations et peut également conduire à une plus grande différenciation entre les sous-populations urbaines à des échelles spatiales plus larges (chapitre 3).

Par la suite, pour examiner comment les changements génétiques et plastiques contribuent aux divergences phénotypiques dans les populations urbaines, j'ai utilisé une expérience de jardin commun avec des mésanges charbonnières le long d'un gradient urbain à Montpellier, en France. J'ai découvert que les effets génétiques et plastiques peuvent contribuer aux phénotypes urbains sauvages, et que leurs contributions sont spécifiques aux traits. J'ai montré que les changements génétiques contribuent probablement à la réduction en ville de la taille du corps et à une respiration plus rapide, tandis que la plasticité aux conditions urbaines est très probablement à l'origine de comportements d'agression et d'exploration plus élevés chez les oiseaux urbains (chapitre 4). J'ai également examiné pour la première fois la variation cognitive chez les mésanges sauvages le long du gradient urbain, et j'ai évalué la base génétique de cette variation cognitive dans l'expérience du jardin commun. J'ai trouvé que, comparé aux mésanges des forêts, les mésanges sauvages des habitats plus urbanisés ont des performances plus élevées en ce qui concerne le contrôle inhibiteur dans une tâche de détournement moteur. Cependant, ces capacités supérieures de contrôle inhibiteur n'ont pas été maintenues dans le contexte du jardin commun, ce qui suggère que la variation cognitive observée dans la nature est probablement le résultat de la plasticité ou de l'expérience dans les environnements urbains (chapitre 5).

Ma thèse dans son ensemble établit que l'urbanisation est associée chez la mésange, à des augmentations de la variation phénotypique dans les populations sauvages à plusieurs échelles spatiales, et que les changements génétiques et plastiques contribuent aux divergences phénotypiques urbaines. Mes résultats suggèrent donc que la variation phénotypique peut jouer un rôle important dans l'évolution urbaine. Ma thèse montre que les études sur des populations multiples et uniques apporteront des contributions utiles à

l'évolution de l'écologie évolutive urbaine, et je discute que l'intégration des thèmes de l'écologie communautaire et du changement climatique dans la recherche urbaine pourrait être une voie de recherche particulièrement efficace pour stimuler de nouvelles avancées dans la recherche en écologie évolutive urbaine.

Mots clés : ville, variation intraspécifique, variation génétique, plasticité phénotypique

CHAPITRE 1 General Introduction

1.1 The nature and causes of variability

Why do wild populations and the individuals that comprise them differ? This was a question that fascinated Charles Darwin who wrote: "At the present time, there is hardly any question in biology of more importance than this of the nature and causes of variability" (quoted in Weismann, 1882). Even today this is a central question in Evolutionary Ecology that has inspired numerous conceptual and fundamental advances over the last century (e.g., Falconer & Mackay, 1983; Fisher, 1919; Gause, 1947; Lande & Arnold, 1983; Lush, 1937; Lynch & Walsh, 1998; Via & Lande, 1985; Wright, 1921). Variation can be observed in most natural systems and can be studied across biological levels including between and within populations of the same species (i.e., intraspecific variation). The study of phenotypic variation that exists between individuals in wild populations has been a primary focus for evaluating how ecological contexts can lead to evolutionary change on ecological communities (Hendry, 2017). Phenotypic variation is the target of selection and directly affects how individuals interact with their environments (Lynch & Walsh, 1998). Thus, evaluating how eco-evolutionary dynamics shape variation in natural systems generally requires insights into how phenotypic variation arises.

Phenotypic differences between individuals are observed across diverse environmental gradients and establishing whether these differences have a genetic basis can inform on the evolutionary potential of wild populations (Mitchell-Olds et al., 2007). When certain phenotypes provide fitness benefits and are selected for, populations can adapt to local environmental conditions (i.e., local adaptation) if phenotypic change represents underlying genetic change that is passed on to the next generation (given gene flow is restricted, Bürger & Lynch, 1995; Hansen & Houle, 2008; Kawecki & Ebert, 2004; Lynch & Walsh, 1998). Organisms can also adjust to local environmental conditions through phenotypic plasticity or the ability of a given genotype to express different phenotypes in response to environmental variation (Pfennig, 2021). Plastic responses can both impede or facilitate genetic evolutionary change (Price et al., 2003) and plasticity may even evolve (Ghalambor et al., 2007; Via & Lande, 1985). Determining the "nature and causes of variability" by quantifying the contributions of genetic and environmental sources of variation towards total phenotypic variation is crucial since local adaptation and plasticity can have different demographic and evolutionary consequences (Ghalambor et al., 2007; Kawecki & Ebert, 2004; Nicolaus & Edelaar, 2018; Snell-Rood, 2013).

Statistical or experimental approaches are used to evaluate how genetic variation in wild populations contributes to phenotypic variation. These approaches can also determine the relative contributions of other sources of variation on phenotypes including plasticity resulting from early or permanent environmental variation, maternal variation (genetic or environmentally-induced), or genotype-environment interactions (G x E; Kruuk et al., 2008; Leroi et al., 1994). Examining the sources of phenotypic variation in wild populations requires immense effort (i.e., resource-intensive experiments or long-term datasets with pedigrees) and is not feasible when initiating most studies. A useful first step towards establishing the possible causes and consequences of variation would be to intially examine how local ecological conditions can impact phenotypic variation in wild populations.

Phenotypic variation in the wild is commonly evaluated by comparing differences in phenotypic means between populations along environmental gradients but, over the last few decades, several researchers have highlighted the importance of also considering how phenotypic variance can change with environmental variation (Bolnick et al., 2011; Des Roches et al., 2018; Sánchez-Tójar et al., 2020; Violle et al., 2012; Wolf & Weissing, 2012). Quantifying the phenotypic variation of wild populations, in addition to the phenotypic mean, would allow more comprehensive evaluations of the consequences of variation, which can have important impacts for populations, communities, and ecosystems. For example, individual differences in foraging strategies, social behaviour, or immunity can affect intra-specific interactions and demographic processes (Bolnick et al., 2011; Pelletier et al., 2007). Individual diversity can also buffer populations from rapid environmental changes and increase the probability of population persistance in novel environments (Gibert, 2016). At the community and ecosystem levels, individual differences in foraging niche or space use impact inter-specific interactions that can alter community species compositions, with cascading effects for the functioning of ecosystems (Gibert & Brassil, 2014; Violle et al., 2012; Wright et al., 2016). Despite abundant theory on the consequences of individual variation, the quantification of phenotypic variation and its biological significance are often neglected when studying wild populations (Capilla-Lasheras et al., 2022; Sánchez-Tójar et al., 2020).

Studies examining how ecological conditions drive individual diversity will provide a starting point to initate efforts that can determine the causes and consequences of variation, and so will be essential for establishing management approaches that conserve natural variation (Forsman, 2014). Management approaches that preserve phenotypic (and genetic) variation may be most pressing for populations exposed to novel environmental change, like urbanization (Carvalho et al., 2021; Mimura et al., 2017; Moran et al., 2016; Paquette et al., 2021). Preserving phenotypic variation will be important for conserving ecosystem

functioning and nature's contributions to people in variable and highly modified environments like cities (Des Roches et al., 2021); environments that are expanding globally.

1.2 Urban Evolutionary Ecology

Cities are expected to rapidly expand in the coming decades (United Nations, 2019). Urbanization is a process of rapid environmental change that highly modifies habitats by for example: i) replacing vegetated areas with sealed non-natural surfaces like roads or buildings (i.e., impervious surface area or ISA), ii) introducing ornamental and non-native vegetation, iii) introducing novel food resources via trash bins or recreational feeding, iv) increasing chemical, light, or sound pollution levels, and v) increasing environmental heterogeneity at landscape and local scales (Szulkin et al., 2020). Urban populations are exposed to novel and interactive selective pressures which presents an oppurtinity to study how these rapidly expanding environments alter fundamental eco-evolutionary processes (Alberti et al., 2020). Indeed, urban systems have been described as natural laboratories to study how multiple abiotic and biotic axes impact the evolutionary ecology of wild populations across replicated environmental gradients (Szulkin et al., 2020). There has been mounting concern about how cities impact wild organisms and, as the amount of urbanization around the world has continued to grow over time, so too has the field of Urban Evolutionary Ecology.

There is now an impressive abundance of research that has examined ecological and evolutionary questions across taxa that occupy urban environments (reviewed in Alberti, 2008; Diamond & Martin, 2021; Donihue & Lambert, 2015; Forman, 2014; Johnson & Munshi-South, 2017; Niemelä et al., 2011; Rivkin et al., 2019; Szulkin et al., 2020). This literature shows that the traits of organisms are changing rapidly in response to urbanization, with this phenotypic change being accelerated in urban compared to nonurban environments (Alberti et al., 2017). Indeed, urban animals have been shown to differ from their nonurban conspecifics in diverse ecological traits, including their life history, morphology, physiology, behaviour, and cognition (Alberti et al., 2017; Bonier, 2012; Griffin et al., 2017; Hahs et al., 2023; Lambert et al., 2021; McDonnell & Hahs, 2015; Miranda et al., 2013; Sol et al., 2013). This body of work has enabled broader generalizations about how urbanization impacts the mean traits of populations across species and studies. For instance, urban organisms tend to have earlier phenology (Capilla-Lasheras et al., 2022; Jochner & Menzel, 2015), be smaller (Hahs et al., 2023; Merckx et al., 2018), and may also have higher cognitive abilities than nonurban organisms (Griffin et al., 2017; Lee & Thornton, 2021; Sol et al., 2020). However, few studies have examined how urbanization affects variation of these phenotypes, which has limited the long-term applications of urban research.

A major and unanswered question in urban studies is whether observed phenotypic shifts in urban populations are shaped by plasticity or selection on traits with underlying genetic variation (Szulkin et al., 2020). Urban populations are commonly assumed to be adapting to novel conditions in urban environments, but only few examples have demonstrated adaptive urban evolution (Lambert et al., 2021). Instead, many urban phenotypic changes may result from phenotypic plasticity to novel environmental conditions (Hendry et al., 2008). Phenotypic plasticity can influence future evolution by increasing survival in new urban environments and influencing which individuals reproduce, but adaptive plasticity could also slow the rate of evolutionary change if maladaptive genotypes are shielded from selection (Pfennig et al., 2010). The eco-evolutionary mechanisms acting within urban environments are likely complex (Alberti et al., 2020), but decomposing phenotypic variation into its genetic and environmental sources is one useful approach to examine how these complex mechanisms shape phenotypic variation (Conover et al., 2009). Quantitative genetic and experimental approaches are useful tools to this end, but these are still not readily applied in urban research as time-intensive experiments and large datasets are required (Lambert et al., 2021).

Beyond this, it remains relatively unexplored whether urbanization impacts individual diversity more generally in wild populations. Previous meta-analyses have demonstrated that environmental stressors that often characterize urban habitats (i.e., heat, food, or pollution stressors) can increase individual differences across several types of traits (O'Dea et al., 2019; Sánchez-Tójar et al., 2020), but there have been few examinations into how urbanization affects phenotypic variation. Phenotypic differentiation is occurring in real-time along urban gradients (Alberti et al., 2017) and this offers an ideal system to better understand how different sources of variation (e.g., genetic or environmental) contribute to processes that shape diversity in replicated natural populations at multiple levels (e.g., interindividual or population levels). My thesis aims to fill this gap by combining quantitative and experimental approaches to address how urbanization affects variation in wild populations of European tits.

1.3 Study systems

1.3.1 Great and blue tits

In my thesis I examine how urbanization affects variation in great and blue tits (*Parus major, Cyanistes caeruleus*), passerine bird species at the center of urban research. Tits are songbirds in the Paridae family and these species are widespread and common throughout most of the European continent (Shirihai & Svensson, 2018). Great and blue tits occupy coniferous, deciduous, and mixed woodland habitats, but can also be found across a variety of additional habitat types including suburban and urban areas (del Hoyo et

al., 2007). These tit species are common visitors to backyard birdfeeders and are thus commonly recognized wildlife species by the wider public (Perrins, 1979). As cavity nesters, they use pre-existing holes in trees to breed each spring and will readily breed in nest boxes. The species' abundance and ease of monitoring in nest boxes has established great and blue tits as two of the most studied bird species of long-term monitoring projects in Evolutionary Ecology (Bailey et al., 2022; Clutton-Brock & Sheldon, 2010; Culina et al., 2020; Kluijver, 1951; Lack, 1955).

Females can produce up to two broods each breeding season (most nests have 5-11 eggs per brood in great tits; 8-12 eggs per brood in blue tits) with both females and males contributing to feeding and raising the chicks (del Hoyo et al., 2007; Perrins, 1979). Chicks are fed mainly arthropods (mainly caterpillars), and adults have a more varied diet that changes seasonally and includes arthropods, berries, and seed resources (del Hoyo et al., 2007). However, the diet of tits can be considerably different in urban areas by containing fewer arthropods and more anthropogenic food sources (Pollock et al., 2017). These species are mainly sedentary, but ringing data suggests that indviduals from more Northern populations can disperse great distances (Perrins, 1979; Shirihai & Svensson, 2018). Tits are territorial around their nest during breeding and become non-territorial during winter when they move around larger areas in search of food.

1.3.2 European datasets on great and blue tits

I use long-term datasets on great and blue tits to examine how urbanization affects phenotypic variation in populations along 14 replicated urbanization gradients in nine European countries. These datasets come from 11 different European research groups that monitor the occupation and reproduction of tits in nest boxes along these gradients during the breeding season each Spring. Although the number of study sites and years of data collection varies across the gradients (monitoring initiated between 1990s-2010s), the populations are monitored using standardized approaches (see Culina et al., 2020). During Spring, nest boxes at established study sites are visited at least weekly to monitor reproduction and, specifically, to record the date when the first egg is layed (i.e., lay date). Breeding tits are captured at nestboxes after their nestlings hatch and are individually-marked with metal rings. The morphological traits of individuals are measured upon capture including tarsus length, wing length, and body mass. At approximately 15 days old, nestlings are also ringed and measured before they fledge the nest.

Most of these datasets have been integrated into the SPI-Birds network, an established organization that promotes and standardizes data across individually-marked breeding bird species globally (Culina et al., 2020). There are numerous published studies ranging in topic on the ecology (and fewer on the evolution)

of these monitored tit populations but, more recently, collaborative efforts have been initated in an attempt to draw more general conclusions across populations (Caizergues et al., 2022; Salmón et al., 2021; Vaugoyeau et al., 2016). For instance, European tits in urban habitats tend to be smaller and have earlier lay dates compared to tits in forested areas (Thompson et al., 2022; Vaugoyeau et al., 2016), which aligns with findings from other urban bird species globally (Capilla-Lasheras et al., 2022; Hahs et al., 2023).

1.3.3 Montpellier great tits

I also use long-term data on and an experimental approach with great tits along the Montpellier urban gradient; one of the 14 European gradients mentioned above. Monitoring of great tits along this urban gradient in Montpellier, France has been ongoing since 2011 (Charmantier et al., 2017). Urban great tits are monitored across eight study sites in the city of Montpellier, while forest great tits are measured in a natural woodland site 20km northwest of the city. As outlined above, the occupation and reproduction of great tits are monitored at these urban and forest study sites using standardized approaches during the breeding season. Several morphological, life-history, physiological, and behavioural traits have been measured in breeding tits along this gradient since the onset of the study (Caizergues et al., 2018; Caizergues et al., 2022), and phenotypic divergences between urban and forest tits in these traits have been documented. Specifically, urban great tits are smaller, lay earlier and smaller clutches, are more aggressive and exploratory, and have faster respiratory rates under stress (physiological response to handling) than forest tits (Caizergues et al., 2018; Caizergues et al., 2022; Charmantier et al., 2017). However, estimated selection did not support the hypothesis that these phenotypic divergences between urban and forest great tits were adaptive and, in some cases, even suggested that urban phenotypes were maladaptive (Caizergues et al., 2018; Caizergues et al., 2022). Despite evident gene flow occuring between study sites, previous genomic work suggests that these phenotypic divergences could have a genetic basis as urbanization explained a small but significant proportion of genetic variation in this system (Perrier et al., 2018). During my PhD, I contributed significantly to data collection for the long-term monitoring of the Montpellier urban great tit system; I managed and led fieldwork in spring 2022 and conducted fieldwork in spring 2023.

1.4 Main objective and research questions

My thesis first aims to establish how urbanization affects phenotypic variation in replicated populations of great and blue tits at a continental scale. I then aim to evaluate whether phenotypic divergence between urban and forest great tits in Montpellier, France are driven by genetic change or plastic responses to urban

conditions. These objectives relate to two main research questions and four related research chapters outlined here (summary shown in Figure 1.1).

1.4.1 How does urbanization affect phenotypic variation?

In my second chapter, I conduct a review that synthesizes how urbanization may drive increased phenotypic variation through several processes, and I discuss how modified variation in urban environments can have important consequences for urban populations, communities, and ecosystems. Using an illustrative metaanalysis with effect sizes from the European great and blue tit populations, I demonstrate that urbanization increases morphological variation where urban birds are smaller and more variable among each other. This chapter is published in Trends in Ecology and Evolution and was completed in collaboration with Dr. Davide Dominoni and Dr. Pablo Capilla-Lasheras at the University of Glasgow.

In my third chapter, I build on findings from my first chapter by conducting a mega-analysis that combines long-term raw datasets from European great and blue tit populations. Taking this approach allows me to determine at which spatial scales urbanization increases morphological and phenological variation across these replicated urban gradients. I show that urbanization increased tarsus length and lay date variation within subpopulations at local spatial scales implying that fine-scale environmental heterogeneity in cities drives higher urban individual diversity. I also find evidence that urbanization can increase phenotypic variation at both local and larger spatial scales simultaneously (i.e., between and within subpopulations) depending on the trait. Collectively, these results provide insights into which scales the processes reviewed in chapter 2 may be driving phenotypic variation in cities. This chapter was conducted in collaboration with 21 co-authors from 11 different research groups studying European urban tits and Dr. Julien Martin from the University of Ottawa who advised on statistical analyses.

I am also a co-author on two research papers related to my first question. The first of these papers, published in Ecology Letters (appendix E), was led by Dr. Pablo Capilla-Lasheras at the University of Glasgow. Using a meta-analysis we show that urbanization increases variation in reproductive phenology in 35 bird species worldwide. The second paper, currently under review, was led by Dr. Laura Gervais at the CEFE, France (appendix F). Using the Montpellier urban great tit system, we show that urbanization can both increase and decrease individual differences in behavioural traits.

1.4.2 How do genetic and plastic changes shape urban phenotypes?

In my fourth chapter, I conduct a common garden experiment using great tits from the Montpellier system to evaluate whether established phenotypic differences between wild urban and forest tits are maintained when tits were reared under common environmental conditions. I find clear evidence that birds from urban origins were smaller and had faster respiratory rates in response to handling compared to birds from forest origins, indicating that these trait divergences are driven by genetic differences between wild populations. I do not find clear differences in aggression and exploration behaviours between urban and forest common garden birds suggesting that plasticity may shape these phenotypes in the wild. These results show that both genetic and environmental sources of variation contribute to phenotypic varation in urban systems, and that their relative contributions are trait-specific. This chapter was conducted in collaboration with a team of researchers at the CEFE, and animal care technicians and veterinarians at the Montpellier zoo who assisted in rearing the common garden birds.

In my fifth chapter, I evaluate how cognition varied among individual great tits along the Montpellier urban gradient, and examine the genetic basis of cognitive variation in common garden birds. I find that tits from more urbanized habitats have higher performance related to inhibitory control (i.e., ability to inhibit prepotent responses) than tits in less urbanized habitats, but urban and forest common garden birds did not differ in this cognitive trait. Our results suggest that there is limited potential for the observed cognitive difference to evolve in the wild as inhibitory control appears to be mainly environmentally determined. This chapter is planned to be part of a larger manuscript for a special issue in Animal Cognition that will include results on the genomics of this cognitive trait prepared by Dr. Laura Gervais at CEFE, France.

I am also a co-author on a review paper (appendix G) related to my second question that synthesizes evidence on the strength of natural selection in urban environments. Although selection pressures are assumed to be stronger in urban than nonurban habitats, we do not find clear evidence of stronger urban selection in the literature and conclude that more studies quantifying urban selection will be required before further generalizations can be made. This review is currently being revised for Functional Ecology and was led by Dr. Anne Charmantier and our lab group at CEFE, France in collaboration with Dr. Albrecht Schulte-Hostedde at Laurentian University.



Figure 1.1: Summary of research questions and thesis chapters.

CHAPITRE 2

Phentoypic variation in urban environments: mechanisms and implications

Authors: Megan J. Thompson, Pablo Capilla-Lasheras, Davide M. Dominoni, Denis Réale, and Anne Charmantier

Published in: Trends in Ecology and Evolution (https://doi.org/10.1016/j.tree.2021.09.009)

In the past decade, numerous studies have explored how urbanization affects the mean phenotypes of populations, but it remains unknown how urbanization impacts phenotypic variation, a key target of selection that shapes, and is shaped by, eco-evolutionary processes. Our review suggests that urbanization may often increase intraspecific phenotypic variation through several processes, a conclusion aligned with results from our illustrative analysis on tit morphology across 13 European city/forest population pairs. Urban-driven changes in phenotypic variation will have immense implications for urban populations and communities, particularly through urbanization's effects on individual fitness, species interactions, and conservation. We call here for studies that incorporate phenotypic variation in urban eco-evo research alongside advances in theory.

Keywords: intraspecific variation, trait variation, diversity, heterogeneity, city

2.1 Urban phenotypic variation

As urbanization (see Glossary) around the world has continued to grow over time, so too have the fields of Urban Ecology and Evolution. Numerous studies have now examined ecological and evolutionary questions across taxa that occupy urban habitats (Diamond & Martin, 2021; Johnson & Munshi-South, 2017; Szulkin, Munshi-South, et al., 2020). Urban organisms differ from their nonurban conspecifics in many characteristics, and diverse examples show how urbanization affects the mean phenotypes of populations (Lambert et al., 2021). However, we still know little about how urbanization shapes phenotypic variation, the target of selection that will determine the ecology and future evolution of urban populations. Few studies have compared phenotypic variation between urban and nonurban populations (Bókony et al., 2012; Eggenberger et al., 2019; Falvey et al., 2020; Gómez-Benitez et al., 2021; Gorton et al., 2018; Lazić et al., 2015; Littleford-Colquhoun et al., 2017; Multini et al., 2019; Rodewald & Arcese, 2017; Theodorou et al., 2021; Williams et al., 2019; Table S2.1) and, to our knowledge, no studies have directly examined the causal mechanisms and consequences of this variation.

Here we provide an overview of the mechanisms that shape phenotypic variation in urban systems and synthesize potential implications of this variation (Figure 2.1). Our review focuses on intraspecific phenotypic variation between urban and nonurban populations, while emphasizing the value in examining phenotypic variation among urban subpopulations within cities alongside environmental heterogeneity (Box 1). We discuss sampling considerations (Box 2) and show how urbanization increases phenotypic variation in an illustrative analysis (Box 3). We note throughout where knowledge is still lacking and recommend future research directions.

The eco-evolutionary processes that shape the expression of phenotypic variation, and thus diversity, in natural populations have been well studied in a variety of systems (Mitchell-Olds et al., 2007). We therefore only provide a brief overview on how different processes such as dispersal, selection, plasticity, and (epi)genetic mutations may shape phenotypic variation in an urban context (overview in Figure 2.1). We do not provide a general rule for how different processes affect phenotypic variation in urban environments as many factors likely contribute to variation in diverse and interactive ways. Overall, however, our synthesis suggests that urbanization has increased phenotypic variation in several urban systems (see also Box 3; Table S2.1).

Glossary

<u>Developmental canalization:</u> a developmental process that constrains phenotypic variation by buffering variation from genetic and/or environmental sources.

<u>Developmental plasticity:</u> the capacity of a genotype to alter its phenotype depending on environmental conditions during ontogeny.

Dispersal: the movement of individuals between geographical areas or habitats.

<u>Environmental heterogeneity</u>: diversity in the presence and arrangement of biotic and abiotic features over space and time.

<u>Epigenetics</u>: the study of any process that alters gene activity (e.g., gene expression) without changing the DNA sequence. These alterations can be heritable and reversible.

<u>Fitness</u>: the contribution of an individual to the gene pool of the next generation, relative to other individuals within a population. Fitness metrics are quantitative measures associated with survival or reproductive output.

<u>Fluctuating selection:</u> changes in the strength or shape of selection pressures through space or time.

<u>Functional traits</u>: Phenotypic traits of an individual that influence their fitness or performance and can affect ecological processes and functions.

<u>Founder effect:</u> a reduction in genetic variation because a population is established by only a few individuals from an ancestral population.

<u>Genetic drift</u>: changes in the frequency of gene variants in a population due to random sampling of individuals.

<u>Genetic mutation:</u> Permanent alteration of a DNA sequence that results in a genetic variant that may be passed to future offspring.

Habitat fragmentation: landscape-level process that leads to a habitat becoming discontinued.

Intraspecific phenotypic variation: the measurable or observable phenotypic variation within a species.

<u>Matching-habitat choice</u>: the tendency for individuals to settle in a habitat that improves their fitness according to their phenotype, thereby promoting local adaptation.

Phenotypic variation: the measurable or observable variation in a trait.

<u>Phenotypic plasticity</u>: the capacity of a genotype to express different phenotypes depending on environmental conditions.

Phenotypic differentiation: phenotypic differences between two or more (sub)populations.

<u>Relaxed selection:</u> reduction in the strength of the association between fitness and a given phenotype.

<u>Selection</u>: the relationship between fitness and a phenotypic trait. Directional or stabilizing selection can decrease phenotypic variation while divergent or disruptive selection can increase phenotypic variation within a population.

<u>Source-sink dynamics</u>: a model that links variation in habitat quality to population dynamics where population growth is expected in high quality or source habitats and population declines are expected in low quality or sink habitats.

<u>Species interactions</u>: Interactions between individuals of different species which broadly include interspecific competition, predation, herbivory, parasitism, mutualism, and commensalism.

Urbanization: a process of environmental change resulting from dense human presence and occupancy.



Figure 2.1: Hypothesized mechanisms that affect intraspecific phenotypic variation within an urban population, and examples of ecological, evolutionary, and conservation management implications of this variation.

2.2 Mechanisms shaping phenotypic variation in urban environments

The eco-evolutionary processes that shape the expression of phenotypic variation, and thus diversity, in natural populations have been well studied in a variety of systems (Mitchell-Olds et al., 2007). We therefore only provide a brief overview on how different processes such as dispersal, selection, plasticity, and

(epi)genetic mutations may shape phenotypic variation in an urban context (overview in Figure 2.1). We do not provide a general rule for how different processes affect phenotypic variation in urban environments as many factors likely contribute to variation in diverse and interactive ways. Overall, however, our synthesis suggests that urbanization has increased phenotypic variation in several urban systems (see also Box 3; Table S2.1).

2.2.1 Dispersal

Whether urbanization increases or decreases phenotypic variation will partially depend both on a species' dispersal abilities and on the direction of dispersal (e.g., source-sink dynamics; Lepczyk et al., 2017). Dispersal has the potential to promote phenotypic variation within populations through the immigration of new individuals, phenotypes, and genotypes (Lenormand, 2002). Nonurban populations might act as source populations (Johnson & Munshi-South, 2017) whereby constant movements of individuals into urban areas from more natural habitats results in higher phenotypic variation in urban populations. For less dispersive species, however, movements can be restricted by habitat fragmentation in urban habitats, which could contribute to declines in variation (via reductions in urban populations sizes or increased genetic drift; Figure 2.1; Johnson & Munshi-South, 2017), especially if new urban subpopulations are formed by founder effects (Littleford-Colquhoun et al., 2017; Mueller et al., 2018). As dispersal in urban environments is still not well studied, further work in this area will be conducive.

2.2.2 Selection

Phenotypic variation could be higher in many urban systems if both environmental heterogeneity (Box 1) and relaxed selection allow more diverse phenotypes to persist (Figure 2.1). Alternatively, by favouring adaptive phenotypes that provide a fitness advantage, both directional and stabilizing selection can deplete phenotypic variation in a population over time, via a reduction of the underlying genetic variance across generations or the selective disappearance of certain individuals within each generation. However, relaxed selection may be more pervasive in cities than previously thought (e.g., Rodewald & Arcese, 2017), including in humans (You & Henneberg, 2018). A recent meta-analysis found that anthropogenic disturbances in nonurban habitats reduce the strength of selection (Fugère & Hendry, 2018). On closer examination, the authors found that absolute fitness has increased and variation in fitness decreased because of human disturbance, thus weakening the opportunity for selection. Relaxed selection in urban environments may result from reduced predation pressures, access to supplementary food (Lahti et al., 2009), or a loss of fitness variation (Fugère & Hendry, 2018; Rodewald & Arcese, 2017). Novel and strong

selection pressures might affect some urban populations (Johnson & Munshi-South, 2017; Rivkin et al., 2019), but phenotypic variation could still increase in these populations if selection pressures vary with heterogeneity in cities (see Box 1). A future focus on phenotypic variation and selection (direction and strength) in and outside cities should provide new biological insights into the processes that affect urban phenotypes and adaptation. These efforts will be especially meaningful as selection is still rarely estimated in urbanized species (Johnson & Munshi-South, 2017).

2.2.3 Plasticity

Phenotypic plasticity (including developmental plasticity) promotes variation and diversification within and between populations (Gilbert et al., 2015; Pfennig et al., 2010), and may be one of the most common mechanisms allowing individuals to colonize and persist in urban environments (Hendry et al., 2008). Plasticity could reduce intraspecific phenotypic variation in an urban population if most individuals are capable of plastic shifts resulting in similar phenotypic expression (Figure 2.1). A well-studied urban trait that demonstrates this trend is flight initiation distance (FID); the distance an individual allows before retreating when approached by a risky stimulus. Most urban animals similarly reduce their phenotypic mean, and thus variation, in FIDs if they can adjust their behaviours by habituating to non-threatening stimuli like humans (via repeated exposures; Blumstein, 2016), while nonurban individuals display more variable responses (shown in blue-tailed skinks, *Emoia impar*; Williams et al., 2019). Similarly, a recent metaanalysis shows declines in variation of antipredator behaviours following contact with humans in domesticated, captive, and urban animals (Geffroy et al., 2020).

Conversely, phenotypic variation in urban populations could be higher than nonurban populations because of impaired development and developmental plasticity (Figure 2.1; Box 3). Developmental processes act to constrain phenotypic variation among and within individuals (e.g., developmental canalization; Willmore et al., 2007), and can be disrupted if the level of an environmental stressor passes a threshold (Hoffman & Hercus, 2000). A recent meta-analysis found that developmental stress decreases the mean, but increases the variation, in diverse phenotypic traits across several taxa (Sánchez-Tójar et al., 2020). Urban environments may increase variation in populations if the many environmental stressors in these habitats (e.g., noise, light pollution, chemicals, or increased temperature) disrupt developmental processes (Figure 2.1). For example, impaired head shape development and higher phenotypic variation across head shape indices was found in urban Common wall lizards (*Podarcis muralis*; Lazić et al., 2015). Early life experiences or environments (e.g., competition, diet, predation) contribute to phenotypic differences within

populations (e.g., Dirienzo et al., 2019; Nicolaus et al., 2016), but this is not well examined in an urban context.

2.2.4 Mutation and epigenetics

Pollution and environmental stress can increase rates of genetic mutation, hypermethylation, or other epigenetic marks (Eeva et al., 2006; Somers et al., 2004; Yauk et al., 2008; Yauk et al., 2000) suggesting that phenotypic variation could increase in urban populations where these processes are occurring at higher rates (Figure 2.1; Box 3; McNew et al., 2017; Riyahi et al., 2015; Watson et al., 2020). Epigenetics may be an important mechanism of adaptation for urban populations as it can move phenotypes closer to the fitness optimum and increase mutation rates (Perrier et al., 2020), but the role of (epi)genetic mutations in shaping urban phenotypic variation remains unexplored.

Box 1: Environmental heterogeneity and within-city comparisons

Urbanization could affect intraspecific phenotypic variation differently across subpopulations within a city, and these differences might be in part explained by variable contributions from environmental heterogeneity. Environmental heterogeneity likely plays a large role in urban evolution where interactions between natural and anthropogenic features affect processes such as selection and dispersal (Rivkin et al., 2019). For example, higher environmental heterogeneity within cities compared to natural habitats expose organisms to diverse local conditions or selective pressures that vary in space and time and can contribute to higher phenotypic variation at the city level if trait data are pooled across field sites within the city or across years (Multini et al., 2019). Fluctuating selection or differential plasticity in response to heterogeneity may also explain why urban subpopulations of common ragweed (*Ambroisa artemisiifolia;* Gorton et al., 2018) and Eastern water dragons (*Intellagama lesueurii;* Littleford-Colquhoun et al., 2017) have higher phenotypic differentiation than nonurban subpopulations; a pattern shown in a variety of systems at the genetic level (Miles et al., 2019).

Dispersal could also influence how phenotypic variation is quantified over space if individuals choose environments that best match their phenotype (i.e., matching-habitat choice; Edelaar & Bolnick, 2012). Although this is still a fairly unexplored idea in the urban context, there is evidence for habitat matching in urban swans (van Dongen et al., 2015) and grasshoppers (Camacho et al., 2020). Non-random dispersal could reduce phenotypic variation within urban subpopulations if like-individuals settle in similar urban habitat types, but increase city-level phenotypic variation and differentiation among urban subpopulations (Figure 2.1). Within-city comparisons are needed alongside urban *versus* nonurban comparisons to disentangle the complex interactions that exist between urban phenotypic variation and heterogeneity at different scales (Merckx et al., 2018; Oliveira Hagen et al., 2017).

There remains no consensus on whether urban habitats are more environmentally heterogenous, and this is likely because scale is an important, but overlooked factor (Alberti et al., 2020; Uchida et al., 2021). Urban habitats are known as more spatially heterogenous (Pickett et al., 2017), but less temporally variable (Shochat et al., 2006). In Table S2.2, we provide examples from the literature that illustrate how urban environmental features may increase or decrease environmental heterogeneity depending on the spatial or temporal scale in consideration. Due to these discrepancies, it will be important for urban studies to report local scale environmental data alongside phenotypic data, so that future work can begin to account for the role of urban heterogeneity at multiple scales.

Box 2: Sampling considerations

As phenotypic variation is a population attribute, it will be important for authors to define what they mean by "population" when comparing phenotypic variation. A population is often defined in relation to gene flow and drift, but directly calculating population size in many wild species is not feasible (Marko & Hart, 2011). We use the term population loosely in this review to refer to groups of urban and nonurban samples that are spatially close to one another (e.g., individuals in a city vs. surrounding area). The type of measurement, size of geographic range, and conspecific density are important sampling factors that could directly affect the amount of phenotypic variation estimated in a population. Therefore, the scale considered (see also Box 1) and the sampling design used might affect the amount of phenotypic variation measured, particularly in cross-sectional studies. Comparing variation between two samples requires standardized measures of variation (e.g., coefficient of variation) that consider the scale of the trait measured and the mean-standard deviation relationship (further discussion in Pélabon et al., 2020).

Many studies, including meta-analyses (Fugère & Hendry, 2018), focus on comparing two contrasting populations, one urban and one nonurban. This may create biases in comparative analyses or review syntheses if the definition of urban and rural sites differs between studies. For instance, sites that are defined as urban can differ in size or location within a city. Small green areas in city centres, and large parks or cemeteries, can equally be considered as urban, but they are likely to be different ecologically. Sometimes, the urban environment is sampled in a more heterogenous way than in more classic urban studies, which includes randomly selecting sampling locations (Sprau et al., 2017) or using hierarchical designs (Merckx et al., 2018). It is worth noting that such alternative designs could lead to larger
phenotypic variation in the urban population, because they are likely to sample a larger array of microhabitats.

We wish to both point out these sampling considerations and acknowledge that dealing with these issues uniformly across studies in free-ranging populations can be challenging. We recommend researchers control for unbalanced sampling and report relevant information about their study populations when comparing phenotypic variation, for instance by explicitly quantifying the level of urbanization at study sites. Urbanization or urban environmental features are still not well quantified in many studies that examine phenotypic shifts. In particular, environmental measures are often anthropomorphically biased and may not represent the environmental scales that urban organisms occupy (Szulkin et al., 2020).

Box 3: Morphological variation in urban versus forest tits

We conducted an illustrative analysis to examine how urbanization may affect phenotypic variation of morphological traits using data on great and blue tits (*Parus major, Cyanistes caeruleus*) from a collaborative network of researchers across 13 different European forest and city pairs (see Figure 2.2; Table S2.3; Figure S2.1 and S2.2). First, we expected urbanization to decrease the mean of morphological traits based on previous findings (Caizergues et al., 2021; Senar & Björklund, 2021). Second, we hypothesized an increase in phenotypic variation for morphology in urban tits because a) environmental stress can increase (epi)genetic mutations or disrupt developmental mechanisms, b) fluctuating selection pressures via environmental heterogeneity might increase morphological variation within urban tit populations, and c) European tits are good dispersers (Salmón et al., 2021), which should reduce the effects of fragmentation that act to decrease phenotypic variation in urban populations for other lessdispersive taxa. We used lnRR (ln $\frac{Mean_{urban}}{Mean_{nonurban}}$) and lnCVR ($\approx \ln \frac{CV_{urban}}{CV_{nonurban}}$) to compare morphological mean and variance, respectively, between urban and nonurban tits from multiple systems (see supplementary for details; Nakagawa et al., 2015).

Urbanization tended to decrease the mean (lnRR), but increase the variation (lnCVR), in morphology as predicted. Urban birds tended to be smaller, with this effect being stronger in mass and tarsus length than wing length (Figure 2.2; Table S2.4). Interestingly, tits tended to have more variable body sizes in cities, a trend driven mainly by tarsus length (Figure 2.2; Table S2.4). Estimates of mean and variance effect sizes were similar for the two species, and slightly stronger in females than males (see supplementary, Figure S2.3; Table S2.4). Multiple of the above hypotheses may explain this increased morphological variation in urban tits. For example, the morphological traits we examine have different developmental trajectories where the tarsus develops early in life and remains fixed, wing metrics can vary annually with moults, and body mass can fluctuate continuously. Disruptions in development could then only have

observable effects on variation for early developing and constant traits, like tarsus length. Fluctuating selection and high heterogeneity among urban habitats could also increase morphological variation in tits at the city level (Box 1). This analysis reveals that urbanization increases phenotypic variation in tit morphology. Further work is needed to determine the mechanisms that interact to affect shifts in phenotypic variation in urban environments, as well as the consequences of higher phenotypic variation in cities.



Figure 2.2: Urbanization increases the variance (lnCVR; right), but decreases the mean (lnRR; left), in European tit morphology. Models evaluating the overall effect of urbanization (top) and contributions from morphological traits (bottom) are shown. Individual effect sizes (n = 114) are shown and scaled by their sample size. See also supplemental information in Annex A, Table S2.3, Table S2.4, and Figure S2.1-S2.3 for detailed information and results.

2.3 Implications of urban phenotypic variation

As shown above, urban conditions can significantly impact phenotypic variation. These changes in phenotypic variation can have immense implications for populations, communities, and ecosystems, as well as for conservation programs (Figure 2.1).

2.4 Implications for species interactions, communities, and ecosystem processes

Species abundances and compositions are strongly modified during urbanization (Diamond & Martin, 2021; Faeth et al., 2005; Marques et al., 2019). Although largely ignored, intraspecific phenotypic variation and its effects on competition (Bolnick et al., 2011; Moran et al., 2016) may have important implications for urban community compositions (Alberti et al., 2020; Des Roches et al., 2018). High trait variation associated with niche expansion can reduce interspecific competition (Bolnick et al., 2011; Violle et al., 2012), potentially to a greater extent in urban environments where individuals adopt novel resources and widen their niches (De León et al., 2019; Falvey et al., 2020; Pagani-Núñez et al., 2019). Theory also predicts that higher trait variation associated with competitive ability or niche differentiation can lead to the exclusion of competitively inferior species (Hart et al., 2016). If increases in intraspecific phenotypic variation in urbanexploiters or urban-adapters contributes to competitive exclusions and declines in species richness (Barabás & D'Andrea, 2016; De León et al., 2019), we could see a parallel loss in unique functional traits in urban communities that contribute to ecosystem services and functioning (Paquette et al., 2021; Sol et al., 2020). For these reasons, invasive species research would benefit from approaches that consider phenotypic variation, particularly in an urban context where native species are confronted with a high frequency of invasions (Forsman, 2014; Gaertner et al., 2017). One exemplar study quantified trait variation associated with locomotor performance and temperature tolerance in invasive cane toads (Rhinella marina) under lab conditions, and then modeled this variation alongside fine-scale climate and landscape data to predict the cane toads fundamental niche and potential for expansion across Australia (Kolbe et al., 2010). Efforts that explore how intraspecific phenotypic variation shapes urban invasions and species compositions would be useful additions to the urban literature, particularly in cases where competitive exclusions disrupt urban ecosystem services (Des Roches et al., 2021; Sol et al., 2020).

Intraspecific phenotypic variation influences trophic interactions that promote ecological processes and services like pollination or seed dispersal (Carvalho et al., 2021; Snell et al., 2019; Sol et al., 2020; Zwolak, 2018). Wild urban bees, for example, have higher intraspecific variation in functional foraging traits than nonurban bees, which may be driven by introductions of non-native and diverse floral resources in urban gardens and parks (Eggenberger et al., 2019). Such higher phenotypic variation within species of urban bees might widen their foraging niche and reduce the amount of interactions they have with native flower species (i.e., decreased interaction strength, Bolnick et al., 2011; Gibert & Brassil, 2014). This would have cascading impacts on pollination and, thus, urban plant community composition. Multi-species approaches are needed to explore eco-evolutionary and community dynamics (Bolnick et al., 2011; De Meester et al., 2019), but these approaches are still rare in urban research. We recommend future studies quantify and associate

phenotypic variation in multiple species to address the consequences of intraspecific phenotypic variation on urban species interactions that are modified along urban gradients.

Urban species interactions can be modified or disrupted if the timing of key life cycle events (i.e., phenology) change, yet considering the role of phenotypic variation in these phenological mismatches has been overlooked so far. Urbanization has been shown to affect both the peak and duration (i.e., variation) of many phenological events (Jochner & Menzel, 2015) and, sometimes, urbanization can cause asynchronous phenological shifts in interacting species. For example, urban plant species tend to flower earlier, but urban pollinators may not show a similar advance in diapause emergence and, thus, their foraging activities can overlap less with key flowering resources (Fisogni et al., 2020). As well, caterpillar emergence in urban environments is more variable and has several small peaks instead of a single peak typical in natural forest habitats (Pollock et al., 2017). This could have consequences for urban insectivorous birds such as great tits (*Parus major*) who rely on caterpillar prey during nestling provisioning. Urban-modified phenological variation could cause mismatches between interacting species on multiple trophic levels, which would have run-off implications for selection and population dynamics (Visser & Gienapp, 2019).

2.5 Implications for fitness, selection, and population dynamics

Intraspecific phenotypic variation can also influence population dynamics (Gibert, 2016; Pelletier et al., 2007; Wright et al., 2016). For example, higher intraspecific trait variation can promote diverse individual responses to environmental fluctuations that buffer and stabilize population dynamics (i.e., portfolio effects, Bolnick et al., 2011); a process that is especially applicable to urban populations undergoing rapid environmental change (Alberti et al., 2020; Mimura et al., 2017). Phenotypic variation can also drive population dynamics through selection (Farine et al., 2015; Pelletier et al., 2007), particularly during colonization events (Duckworth & Aguillon, 2015). Therefore, we anticipate that *a priori* knowledge on how phenotypic variation influences fitness or performance metrics will be useful when exploring urban selection and population implications.

Variation among individuals in their sexual signals can alter reproductive behaviours and selection in urban populations. Higher variation in mate qualities can increase the benefits of choosiness, mate searching behaviours, and intrasexual competition. These dynamics may be especially relevant in urban environments where pollution (e.g., chemical, nutrient, noise, light) can disrupt the communication and mating behaviours of animals (Candolin & Wong, 2019; Snell-Rood et al., 2015). For example, several species experience increased access to key nutrients in urban environments which can reduce individual variation in the honesty

of sexual signals that indicate an individual's ability to acquire resources (see review, Snell-Rood et al., 2015). Mate choice can also be affected by urban pollution or stressors which can reduce the perceived variation in sexual signals. For instance, the visual mating signals of three-spined sticklebacks (*Gasterosteus aculeatus*) have been disrupted by human-induced algal blooms. As a result, females are unable to perceive variation among males in their sexual signals and are, thus, more likely to choose lower quality mates that produce less viable offspring (Candolin et al., 2016). Maladaptive mating in urban habitats could impede adaptation, contribute to population declines, or divergences in mean phenotypes, potentially resulting in hybridization or speciation (Candolin & Wong, 2019; Thompson et al., 2018).

Modified natural selection in urban environments could also alter phenotypic variation, potentially resulting in life-history trade-offs or changes in subsequent selective processes. Urban Túngara frogs (Physalaemus *pustulosus*), for example, experience reduced predation and parasitism risk while singing (relaxed natural selection) and higher competition for mates (stronger sexual selection, Halfwerk et al., 2019). As a result, urban frogs call at higher rates, sing more complex and attractive songs, and plastically adjust their songs to sound and light levels in the environment. The authors suggest that a broader range of sexual signalling in urban frogs (i.e., higher phenotypic variation) afford them reproductive and survival advantages over the more natural forest phenotype (Halfwerk et al., 2019). Phenotypic changes via plasticity can increase fitness and promote adaptation in novel or stressful environments, but plasticity likely incurs significant costs, for example by reducing growth rates, generation times, or fecundity (Snell-Rood et al., 2018). Thus, populations that show adaptive plastic responses or higher phenotypic variation in response to novel environments could also shift toward slower life histories (Snell-Rood et al., 2018). As many urban phenotypic changes may involve plasticity (Hendry et al., 2008), including the example above, it would be interesting to explore the role of urban-modified phenotypic variation in life-history trade-offs and pace-oflife (Brans & De Meester, 2018; Sepp et al., 2017). The selective and demographic consequences of urbanmodified life history variation are unexplored in most urban systems, hence efforts tackling these ideas will provide timely insights into how sexual and natural selection shape urban populations.

Beyond quantifying phenotypic variation in urban populations, urban studies should also aim to determine to what extent a phenotypic trait is heritable (Hoffmann & Merilä, 1999). This is especially important if the adaptive or evolutionary implications of urban phenotypic variation are to be explored. While intraspecific phenotypic variation has a key role in influencing urban eco-evolutionary dynamics, it is highly informative to decipher between its genetic and environmental origins, and their interactions (Brans et al., 2017; De Meester et al., 2019). Exploring the origins of urban phenotypic variation calls on quantitative genetic approaches using long-term data or experimental approaches like common gardens (Lambert et al., 2021). Fear of humans, for example, is commonly thought to decrease in urban animals via habituation to humans, a form of phenotypic plasticity. However, variation in behavioural responses to humans is more heritable than expected in urban burrowing owls (*Athene cunicularia*) suggesting that a reduced fear of humans could also result from an evolutionary response (Carrete et al., 2016). Efforts making these distinctions are not commonly applied as large datasets or intensive experiments are required, but we emphasize here the value of these efforts in urban research.

2.6 Implications for urban conservation management

The advantages of incorporating intraspecific variation into urban conservation management has been highlighted recently (Lambert & Donihue, 2020; Mimura et al., 2017). Increasing phenotypic variation in populations of conservation concern has been suggested as an effective management approach in urban contexts. For example, phenotypic restoration initiatives can help establish lost phenotypic variation through reintroductions of missing phenotypes. Simulations have shown how reintroductions of larger seed types in human-impacted forests can help restore seed profiles back to natural levels and maintain seed dispersal (Carvalho et al., 2021). Efforts that increase phenological variation by extending the duration of key events in particular species, like flowering time, might help interacting species, like specialist pollinators, that experience asynchronous shifts due to environmental change (Olliff-Yang et al., 2020). Promoting trait diversity through management programs has also been recognized in urban arboriculture where urban forests tend to consist of similar species (Paquette et al., 2021) or clones (Vanden Broeck et al., 2018). Low phenotypic variation among planted urban trees increases vulnerability to drought or pests and can hamper the ecosystem services provided by trees in cities, hence increasing trait diversity in trees can offer an easy urban management approach to counter these challenges (Paquette et al., 2021). Trait distributions likely have very different implications for population growth and stability than trait means, and so including intraspecific phenotypic variation in population monitoring is warranted (Moran et al., 2016).

Phenotypic variation can also be used as a tool to regulate urban populations that cause problems for native species and humans. Selective management approaches, for instance, decrease phenotypic variation within a population by targeting "problem individuals" that possess certain phenotypes associated with human impacts and conflicts (Swan et al., 2017). For example, efforts evaluating personality variation in deer populations show that deer with bolder personalities may be more likely to cause human harm through vehicle collisions, crop damage, or disease transmission (Honda et al., 2018). Simulations suggest that selective harvesting of deer with bolder personality types could mitigate human-wildlife conflicts while sustaining population sizes (Honda et al., 2018). These management programs would be especially useful

in urban environments at the human-wildlife interface, but they require some knowledge of the phenotypic variation contained within target populations. We expect that studies exploring the implications of phenotypic variation in human-wildlife conflicts will help ensure that management interventions are successful and have longer lasting impacts (Swan et al., 2017).

Conservation programs could aim to increase intraspecific variation in populations they want to conserve and decrease variation in populations they want to mitigate (Des Roches et al., 2021; Lambert & Donihue, 2020). This is because higher intraspecific variation should have positive ecological effects on populations, in particular when the population mean traits are not well matched to the fitness optimum of the environment (i.e., phenotype-environment mismatch, Gibert, 2016), which might be more frequently observed in urban populations. However, increasing phenotypic variation will not always benefit populations if individuals are already well adapted to environmental conditions. A theoretical study demonstrates this and shows that high amounts of phenotypic variation will have increasingly negative ecological consequences on populations as they become better adapted to the local environmental optimum (Dibble & Rudolf, 2019). It will be imperative for urban monitoring programs to evaluate how variation and means associate with local fitness optima to better anticipate the implications of efforts that manage variation (Gibert, 2016; Lambert & Donihue, 2020). We suggest that these evaluations occur on fine scales as phenotype-environment mismatches may differ substantially among urban subpopulations due to within-city heterogeneity (Box 1). Considering phenotypic variation in urban conservation has great promise and city municipalities could benefit from more focused research in this area. We, however, recommend caution and prior investigations to comprehend the possible ecological and evolutionary implications of such interventions.

2.7 Concluding remarks

Our synthesis suggests that urban conditions impact phenotypic variation through various processes, and that urban effects on phenotypic variation have ecological, evolutionary, and management implications. We expect phenotypic variation to increase in urban systems through dispersal, relaxed or heterogenous selection, developmental plasticity, (epi)genetic mutations, or a combination of these (Figure 2.1; Box 1; Box 3; Table S2.1). The synergistic and counteractive effects of these mechanisms could shape variation in diverse and complex ways, and care will need to be taken to ensure appropriate sampling design in urban studies (Box 2). Urbanization has significant impacts on species interactions and individual fitness, which may exaggerate the effects of intraspecific phenotypic variation in urban systems. Examining these hypotheses across cities, taxa, and traits will be important for further generalizing how urbanization affects phenotypic variation, and in turn how variation affects evolutionary and environmental change.

Most Urban Ecology/Evolution projects already have data on the variance around phenotypes in their study populations and we, therefore, hope to encourage the comparison of variation, beside means, of ecologically relevant traits in future work (Sánchez-Tójar et al., 2020). To this end, we show in a preliminary analysis that urbanization increases the variation in morphological traits in tit species across Europe (Box 3). There is a need to examine phenotypic variation both between and within cities (Box 1), and to examine contributions of environmental features and heterogeneity to phenotypic variation at spatial and temporal scales relevant to a species' biology (Box 2).

2.8 Acknowledgements

We would like to thank all data contributors that shared summary statistics from their study systems with us for the analysis presented in Figure 2.2. We are very grateful for their long-term efforts and comradery. MJT is supported by a Canadian Graduate Scholarship from the Natural Sciences and Engineering Research Council (NSERC) of Canada and a PhD mobility grant from le Centre Méditerranéen de l'Environnement et de la Biodiversité (CeMEB). This project was funded by the Agence Nationale de la Recherche (URBANTIT grant ANR-19-CE34-0008-05 to AC) and a Fonds de Recherche du Quebec Nature et Technologie to DR. Artwork in figures were created by MJT aside from the city skyline which was obtained under a creative commons license.

CHAPITRE 3

Continental patterns of phenotypic variation along urban gradients: a mega-analysis

Authors: Megan J. Thompson, Julien Martin, Clotilde Biard, Josepha Bleu, Claire Branston, Pablo Capilla-Lasheras, Niels J. Dingemanse, Davide M. Dominoni, Marcel Eens, Tapio Eeva, Karl Evans, Caroline Isaksson, András Liker, Sylvie Massemin, Erik Matthysen, Alexia Mouchet, Sam Perret, Juan Carlos Senar, Gábor Seress, Marta Szulkin, Ernő Vincze, Hannah Watson, Denis Réale, and Anne Charmantier

Environmental variation drives differences between individuals in wild populations, and this phenotypic variation influences how individuals interact with their environment, leading to important eco-evolutionary consequences. Urban systems present a unique opportunity to evaluate how environmental variation shapes phenotypic variation in wild populations since phenotypic differentiation along urban gradients is widely documented and can be studied on contemporary timescales. We combine several long-term datasets in a mega-analysis approach to determine how urbanization impacts tarsus length and lay date variation at different scales (i.e., among and within subpopulations) in replicated great and blue tit (Parus major, Cyanistes caureleus) populations across the European continent. We find that urbanization increased phenotypic variation at local scales (i.e., within subpopulations) by on average 11%, and by as much as 25%, across the species and traits examined. We also find that subpopulations in cities differentiated to a greater extent than subpopulations outside cities for tarsus length but not lay date. Collectively, our results indicate that urbanization affects phenotypes at very fine spatial scales in cities and, depending on the trait, urbanization could impact patterns of phenotypic variation between and within subpopulations at both local and larger spatial scales. The genetic and environmental effects underlying these patterns of phenotypic variation require further exploration, but we discuss potential processes that could drive these patterns of phenotypic variation.

Keywords: city, urbanization, intraspecific variation, environmental heterogeneity, great tit, blue tit

3.1 Introduction

Individuals in wild populations differ across environmental contexts. Environmental variation can drive differences between individuals as they adjust or adapt to local conditions and, in turn, these differences may affect how individuals interact with their environment. Thus, phenotypic variation can have important ecological and evolutionary consequences by, for instance, encompassing individual differences in resource use, predator defence, or parasite resistance (Bolnick et al., 2011; Des Roches et al., 2018; Violle et al., 2012; Wolf & Weissing, 2012). Selection across environmental contexts acts directly on phenotypic variation and can contribute to different adaptive responses if this variation results from genetic variation; a process that is especially important for populations occupying or invading novel environments (Bürger & Lynch, 1995; Falconer, 1996; Hansen & Houle, 2008; Lynch & Walsh, 1998; Walsh & Blows, 2009). An organism's capacity to adjust the expression of traits in response to environmental variation (i.e., phenotypic plasticity) can also contribute to phenotypic variation across contexts, and plays a major role in evolutionary processes and adaptation to novel environments (Ancel, 2000; Chevin et al., 2010; Ghalambor et al., 2007). Evaluating genetic and plastic contributions to phenotypic variation, however, requires following many identified individuals of known relatedness over time, which isn't always feasible in wild populations, especially at broad spatial and temporal scales. Investigating how ecological conditions affect phenotypic variation is, thus, an important first step to link sources of phenotypic variation (i.e., genetic variation and plasticity) to evolutionary outputs (e.g., result of selection acting on phenotypic variation).

Environmental conditions are dramatically altered in anthropogenic environments (e.g., novel resources, modified interactions, heat island effects; Szulkin et al., 2020), providing a unique scenario in which to test how ecological conditions affect phenotypic variation across replicated gradients. In cities, environmental heterogeneity, novel stressors, and fragmentation can modify processes of selection, development, (epi)genetic mutation, or dispersal with cascading effects on the phenotypic variation contained within urban populations (reviewed in Chapter 2, Thompson et al., 2022). Although divergences in mean phenotypes between urban and nonurban populations have been documented across diverse taxa and traits (Diamond & Martin, 2021; Lambert et al., 2021; Szulkin et al., 2020), population differences in phenotypic variation are less commonly studied. In populations of shrews, urban individuals of *Crocidura russula* were found to be more diverse in their aggressive and boldness behaviours compared to rural individuals (von Merten et al., 2022). In birds, two recent meta-analyses found that urbanization increases phenotypic variation in urban populations for morphological (tit species across Europe, Thompson et al., 2022) and life-history traits (birds worldwide, Capilla-Lasheras et al., 2022). Although there is emerging evidence that urbanization increases phenotypic variation, meta-analyses are limited in comparing effect sizes across diverse studies

and more standardized approaches are required to establish how urbanization impacts phenotypic variation at different scales across urban replicate populations.

Ecological processes shape phenotypic variation at different scales in wild populations, for example, by modifying variation both among and within the subpopulations that comprise them (a subpopulation is defined here as a clustered group of individuals occupying the same local environment). For instance, cities are thought to be more environmentally heterogeneous at larger spatial scales than surrounding areas (Cadenasso et al., 2007; Niemelä et al., 2011; Pickett et al., 2017), which could imply that urban subpopulations occupy a wider range of habitats and are exposed to more diverse conditions than nonurban individuals (Alberti et al., 2020; Gorton et al., 2018; Rivkin et al., 2019). Further, some urban populations are subjected to higher genetic drift resulting from reduced dispersal in cities, which can also lead to differentiation between urban subpopulations (Miles et al., 2019). We could then expect higher urban phenotypic variation if there are phenotypic differences between city subpopulations where, for example, some urban subpopulations contain very small individuals and others contain large individuals, while nonurban subpopulations tend to all contain large individuals (Fig 3.1.1; we term this the "among subpopulation heterogeneity" hypothesis). Indeed, there is support in the literature that subpopulations in cities are more differentiated among each other than subpopulations outside cities, at both the phenotypic (Gorton et al., 2018; Littleford-Colquhoun et al., 2017) and genetic levels (Miles et al., 2019; Munshi-South et al., 2016; Schmidt et al., 2020).

Novel resources and stressors can vary at fine spatial and temporal scales even within an urban sampling location (Charmantier et al., 2017; Corsini et al., 2019; Jensen et al., 2023; Monniez et al., 2022; Stofberg et al., 2019), which could contribute to specializations or differences among individuals occupying the same local area (Ghalambor et al., 2007). In such situations, phenotypic differentiation between individuals within urban subpopulations (e.g., small and large individuals) could drive larger variation in urban populations if individuals within nonurban subpopulations do not differentiate to the same extent (e.g., only large individuals in nonurban subpopulations; Fig 3.1.2; we term this the "within subpopulation heterogeneity" hypothesis). Bees in urban gardens, for instance, display higher individual variation in foraging morphology than nonurban bees presumably as the introduction of non-native flowers increases fine-scale resource heterogeneity in these contexts (Eggenberger et al., 2019). Further, environmental heterogeneity could vary in space across the urban matrix and so we could expect differences between urban subpopulations in the heterogeneity they contain. For example, i) some urban subpopulations occupy highly heterogeneous locations and contain less diversity, iii) whereas nonurban subpopulations may be exposed

to similar amounts of environmental variation and contain similar individual diversity (Fig 3.1.3; we term this the "heterogeneity in heterogeneity" hypothesis). These three hypotheses are not mutually exclusive and could explain larger urban variation, but it is unknown at which of these population levels processes like selection, development, or dispersal modify variation in cities. Exploring whether urbanization affects phenotypic variation among or within subpopulations, or both, would be an important step towards generating testable hypotheses on how and at which scales various processes (e.g., patchy, fine-, or largescale selection) contribute to phenotypic shifts in urban populations.



Figure 3.1: Visual representation of three hypotheses concerning patterns of phenotypic variation among birds in five sampling locations (i.e., subpopulations) along a theoretical urban gradient where urbanization increases from right to left. Subpopulations contain groups of urban (blue) and nonurban (green) individuals that differ in body size where individuals are smaller on average in urban locations. Larger phenotypic variation in cities could be driven by 1) among subpopulation heterogeneity: higher differentiation among urban subpopulations where there is higher variation among groups of urban individuals in their mean traits than nonurban ones, 2) within subpopulation heterogeneity: higher individual differences within urban groups compared to nonurban ones, 3) heterogeneity in heterogeneity: differences in heterogeneity between urban subpopulations drives higher variation among groups of urban individuals in the trait variation drives higher variation among groups of urban individuals in the trait variation drives higher variation among groups of urban individuals in the trait variation drives higher variation among groups of urban individuals in the trait variation drives higher variation among groups of urban individuals in the trait variation drives higher variation among groups of urban individuals in the trait variation drives higher variation among groups of urban individuals in the trait variation they contain.

Here we examine how phenotypic variation is spatially distributed along urban gradients by partitioning phenotypic variation at different population levels in two European tit species (great tits, *Parus major* and blue tits, *Cyanistes caeruleus*). To build on findings from previous meta-analyses (Capilla-Lasheras et al., 2022; Thompson et al., 2022), we use a mega-analysis approach (Eisenhauer, 2021; Koile & Cristia, 2021; Sung et al., 2014). Meta-analyses compare effect sizes from different study systems, while mega-analyses

are an extension of this approach that combine raw long-term data from multiple replicate study populations. Although mega-analyses are ambitious approaches requiring coordination and collaboration across different research groups, this approach allowed us to examine finer-scale patterns of phenotypic variation across replicated urban great and blue tit populations. These species are commonly studied in both urban and forest habitats, and the availability of multiple long-term datasets on these species provides a unique opportunity to address the above hypotheses in populations across most of the species' distribution. Our study focuses on exploring patterns of variation in tarsus length and lay date since it has been previously shown that urban populations tend to contain more variation in these traits than nonurban populations (Capilla-Lasheras et al., 2022; Thompson et al., 2022). We expect to find support for our first two hypotheses and predict that higher urban variation will be driven by a combination of higher differentiation between urban subpopulations (hypothesis 1; Figure 3.1.1) and higher individual variation within urban subpopulations (hypothesis 2; Figure 3.1.2) for both tarsus length and lay date. Less is known about how environmental variation changes across the urban matrix and so we explore whether differences in heterogeneity between urban subpopulations also contributes to individual variation in these traits (hypothesis 3; Figure 3.1.3).

Tarsus length is fixed early in life (approx. 15 days for both species; Björklund, 1997; Gebhardt-Henrich & Van Noordwijk, 1994) and is an indicator of body size or competitive ability in tits (Kempenaers et al., 1992; Oddie, 2000). Since early developmental conditions around the nest box are known to impact tarsus growth in nestlings (Dhondt, 1982; Merilä & Fry, 1998; Seress et al., 2020; Talloen et al., 2010), we predict a strong effect of urbanization at the nestling stage since this trait is measured in their natal environment. We predict a weaker effect of urbanization in adults since they are mostly measured after dispersing and we lack direct information on their developmental environment. In contrast to tarsus length, a female's laying date is highly plastic to annual environmental conditions like spring temperature and habitat phenology (Bourgault et al., 2010; Visser et al., 2009), even at small spatial scales (Cole et al., 2013; Nager & van Noordwijk, 1995). As great and blue tits are closely related species with similar niches, we expect parallel responses to urbanization and make similar predictions across both species for tarsus length and lay date. Besides comparing trait variation between categorically classified urban and nonurban locations, we also quantify urbanization in a standardized way across all European sampling locations to consider how the degree of urbanization impacts trait means and variation at small and large spatial scales.

3.2 Methods

3.2.1 Datasets and trait measures

Populations of urban and forest great tits and blue tits have been monitored as a part of long-term research programs that involve following the occupation and reproduction of individuals in nest boxes. We combined datasets from 14 urban gradients (cities and surrounding area; we term this here "study system") across Europe (Figure 3.2). We obtained three full standardized datasets from the SPI-Birds network (Culina et al., 2020) and the remaining datasets were received directly from data owners. These datasets varied greatly in the number of years of data collection, the number of study locations and nest boxes along each gradient (Figure 3.2A; Table S3.1), and the data collected for each species and trait (Figure S3.1-S3.3). We used individual-level data from capture events during the breeding season from both adults and nestlings. Since both species reproduce in their first year, adults were defined as individuals that had hatched the previous year or older. Nestlings were measured on average 15 days (range: 12-17) after hatching before they fledged the nest. Individual tarsus length was measured using the Svensson's Alternative Method (Svensson, 1992) by measuring from the intertarsal notch to the end of the tarsus by bending the foot. In some studies (see Table S3.1), tarsus was measured using the Svensson's Standard or Oxford Maximum methods and so were converted to the Svensson's Alternative method using the established equations from SPI-Birds: Svensson's alternative = Svensson's standard * 0.777 + 6.158 or Svensson's alternative = Oxford maximum * 0.72005+ 3.64549 (Culina et al., 2020; Culina et al., 2020). Nest boxes were monitored regularly during the breeding season and the lay date of each female was determined as the date in which the first egg of a clutch was laid (range: March 16-June 11). We only considered nestling tarsus and lay dates from first clutches as not all datasets included monitoring of second broods, and the breeding season length, the frequency of double brooding, and nestling traits differ across Europe and between urban and nonurban habitats (Bukor et al., 2022; Sinkovics et al., 2023; Verboven et al., 2001; Visser et al., 2003). First clutches were defined as the first clutch laid by a female in a breeding season and were within 30 days of the first lay date of a focal species in a focal year within a focal sampling location (Culina et al., 2020; Van Balen, 1973).

Adults could be caught and measured multiple times if they bred in multiple breeding seasons, but are on average caught and measured 1.4 times in the combined dataset. We chose to evaluate individual phenotypic variation at population levels rather than the individual level since repeated individual measures were low and limited the ability to estimate within-individual variation. Therefore, individual phenotypic variation here encompasses both variation due to between-individual differences and within-individual variation. We hence included only one observation per individual to estimate individual differences among and within

subpopulations while avoiding pseudoreplication. For adult tarsus, we computed the mean tarsus length for each individual if they had more than one measure. Tarsus length is fixed early in life and should not change over subsequent measures, so taking the mean of this trait should reflect an individual's size over their life while reducing measurement error. The number of adult tarsus measurements per individual was similar across habitats for both species (urban great tit: mean = 1.4, range = 1–7, forest great tit: mean = 1.6, range = 1–9, urban blue tit: mean = 1.3, range = 1–7, forest blue tit: mean = 1.6, range = 1–9, urban blue tit: mean = 1.3, range = 1–7, forest blue tit: mean = 1.6, range = 1–7) and, we found that selecting instead a random observation per individual did not change our results. To ease comparability with results of adult tarsus length, we followed a similar approach for nestling tarsus by selecting one observation from each nest box within each year by randomly selecting one nestling observation per brood. We avoided taking the mean nestling tarsus measure per brood as we did not want to reduce the influence of extreme values (small or large nestlings) on the variation observed within each brood. Female lay dates were included only if an individual observations for lay date (great tit mean = 1.5, range = 1–6; blue tit mean = 1.4, range = 1–6) and so we selected only the first appearance of each known female in the dataset and their corresponding lay date. A summary of the combined datasets for the three traits is shown in Figure 3.2C.

Α	Study system	tudy system Country		Year total: range (urban / forest)				
	Harjavalta	Finland	28: 1991	-2018/28	8: 1991 - 2	2018 8/12		
	Göteborg	Sweden	4: 2017 - 2020 / 3: 2017 - 2019				2/2	
	Helsingborg	Sweden	4: 2018 - 2020 / 3: 2017 - 2020			20	2/4	
	Glasgow	Scotland	6: 2015 - 2020 / 5: 2016 - 2020			20	2/3	
	Malmö	Sweden	7: 2013 - 2020 / 7: 2013 - 2020				4/5	
	Warsaw	Poland	5: 2016 - 2020 / 5: 2016 - 2020				5/2	
	Antwerp	Belgium	26: 1997 - 2022 / 24: 1994 - 2018				1/8	
	Strasbourg	France	6: 2016 - 2022 / 6: 2016 - 2022				8/1	
	Paris	France	10: 2012 - 2021 / 12: 2010 - 2021				9/5	
	Munich	Germany	2: 2014 - 2015 / 10: 2010 - 2019				1/12	
	Budapest	Hungary	3: 2019 - 2021 / 2: 2020 - 2021				3/3	
	Veszprém	Hungary	8: 2013 - 2020 / 8: 2013 - 2020				3/2	
	Montpellier	France	6: 2013 - 2018 / 28: 1991 - 2018				8/1	
	Barcelona	Spain	27: 1992 - 2018 / 24: 1998 - 2021			021	3/1	
С	TRAITS: Adult t		arsus Nestling tarsus		Lay date			
	Number of:	Great tit	Blue tit	Great tit	Blue tit	Great tit	Blue tit	
	Study systems	13	8	12	8	13	9	
	Individuals	11928	7439	7505	2905	6378	4419	
	Urban clusters	50	30	48	28	58	37	
	Forest clusters	49	28	47	26	53	36	



Figure 3.2: Summary of European urban gradient datasets showing A) list of datasets (N = 14; referred to by study system in decreasing latitude, range = $61^{\circ}31$ ' to $41^{\circ}23$ ' N) and their year range and number of clusters (i.e., sampling locations defined by clustering algorithm) in urban and forest habitats, B) map of Europe showing the location of each study system in A and whether the dataset included great and blue tits (circles) or great tits only (squares), and C) the three traits examined and the number of study systems, individuals, and clusters (urban vs. forest) of the full combined dataset. See also Table S3.1and S3.2 for further information.

3.2.2 Environmental variables

As study locations defined by data owners differed in shape and size across study systems, we aimed to standardize the definition of a study location by defining clusters of nest boxes using a DBSCAN clustering algorithm in QGIS (v3.22.0, QGIS Development Team, 2023). We use the term "cluster" here to represent study locations or subpopulations along urban gradients, which were defined to contain at least 5 nest boxes within a 300-meter distance of each other. Often, this definition grouped together nest boxes in a similar habitat and led to the same study locations as those defined by data owners (119 locations; Table S3.1). Clusters of nest boxes gave similar results when clustering nest boxes within 200 and 400 meters (range = 136-106 locations). In the case of Munich, urban nest boxes did not cluster together because nest boxes as one cluster and compared results when including and excluding this cluster. As clusters differed in their size and we wanted to control for the possibility that a cluster's size could impact the variation they contained, we calculated cluster area (m²) as the total area covered by nest boxes using Minimum Bounding Convex Hulls in QGIS.

We quantified the degree of urbanization at each nest box (N = 7210) across all the gradients at two scales. The biologically relevant scale at which we should quantify urbanization is rarely obvious, yet it is a crucial methodological choice (Uchida et al., 2021). For example, small- and large-scale urbanization quantified around a group of individuals occupying a small urban green space could be considerably different and may have varied effects on phenotypes depending on the species or trait under study (e.g., Kaiser et al., 2016; Strubbe et al., 2020; Waterschoot et al., 2023). We therefore chose to examine the effect of urbanization at both a small and large scale (100 vs. 1000 meters) relative to the species' home range around the nest box (approx. 60-160 meters, Jarrett et al., 2020; van Overveld et al., 2011; Wilkin et al., 2006) and natal dispersal distance (up to 900 meters on average in females, Dingemanse et al., 2003; Garant et al., 2005; Szulkin & Sheldon, 2008). We calculated the proportion of impervious surface area (ISA), defined as sealed nonnatural surfaces (e.g., roads, railway tracks, buildings), as a metric for urbanization using the imperviousness density raster datasets from the Copernicus online database (resolution 10m, see European Environment Agency, 2020). Using 100- and 1000-meter circular radius buffers around each nest box, we calculated the proportion of ISA by counting the number of pixels associated with imperviousness and divided this by the number of pixels within each buffer (range: 0-1, where 1 = all ISA). The proportion of ISA was highly correlated with the proportion of impervious built-up area (European Environment Agency, 2020) across all nest boxes in our dataset (rho = 0.92 and 0.97 for 100- and 1000-m scales, respectively), and so we chose to only use ISA measures moving forward. When considering all nest boxes together, the quantified ISA at

100- and 1000-meter scales were correlated (rho = 0.75). To determine the degree of urbanization at a cluster, we averaged the proportion ISA across all nest boxes within that cluster. In addition to examining quantified urbanization at each cluster, we also examined urbanization as a categorical predictor (urban vs. forest), where the habitat category of each cluster was defined following the data owner's categorization of their study areas. Urban clusters were within or close to city centers and tended to have larger values of proportion ISA (cluster mean = 0.71 proportion ISA at 1000m, range = 0.14–0.98), whereas forest clusters were in forested areas outside cities and had lower proportion ISA (cluster mean = 0.07 proportion ISA at 1000m, range = 0.071 proportion ISA at 1000m, range = 0.054).

We also extracted large-scale land cover heterogeneity around each nest box using the CORINE Land Cover dataset that includes 44 different classes of land cover types (resolution 100m, European Environment Agency, 2021). We calculated the number of pixels associated with each land cover type within 100- and 1000-m buffers around each nest box. Using these values, we calculated land cover diversity using the Shannon Diversity Index that accounts for both richness and evenness of land cover types at each nest box. To generate land cover diversity at the cluster level, we again averaged diversity values across all the nest boxes within a given cluster. In our dataset, ISA had a negative quadratic relationship with land cover heterogeneity at both the nest box and cluster levels suggesting that moderately urbanized clusters (between 0.2–0.6 proportion ISA) tended to have higher landscape heterogeneity (Figure S3.4). All spatial datasets were projected in the Lambert azimuthal equal-area European projection (EPSG: 3035) because impervious surface area and land cover heterogeneity were estimated and compared across Europe. The associated raster tiles used for impervious density for each study system are listed in Table S3.1. An overview of urban and forest cluster summary characteristics across each study system is shown in Table S3.2.

3.2.3 Statistical approach

Double hierarchical generalized linear models, or DHGLMs, can be used to quantify residual variation within groups (Cleasby et al., 2015; O'Dea et al., 2022). In ecology and evolution, these models have been used to quantify intra-individual residual variation or "individual predictability" (i.e., how consistent an individual is in a trait, Hertel et al., 2021; Martin et al., 2017). These models require many repeated observations per unit of analysis to estimate whether units are predictable and show low variation around their average characteristic, or unpredictable and show high variation. For example, this approach can explain variation within groups (i.e., clusters in an urban or nonurban environment) that comprise observations of individuals. In this context, we could evaluate how "predictable" or variable groups of

individuals sampled in different clusters are and if this phenotypic variation could be explained by environmental variables like urbanization.

We use DHGLMs to estimate three key parameters to address our main hypotheses. First, to test the among subpopulation heterogeneity hypothesis (Figure 3.1.1), we examined whether urban clusters' means are more variable than forest ones (parameter 1: urban > forest among-group variance in means). Second, to test the within subpopulation heterogeneity hypothesis (Figure 3.1.2), we determined whether more urbanized clusters contain individuals with more phenotypic variation (parameter 2: positive effect of urbanization on a group's residual variation). Third, to test the heterogeneity in heterogeneity hypothesis (Figure 3.1.3), we explored whether the amount of phenotypic variation within clusters differed by habitat type (parameter 3: urban > forest among-group variance in residual variation).

We fit DHGLMs using tarsus length or laying date as our response variable in R (v.4.2.1) with the package brms (Bürkner, 2017) using the Stan software (Carpenter et al., 2017; Stan Development Team, 2023). We fit fixed and random effects in both the mean (explains mean effects and among-level variation in means) and dispersion (explains residual variation) parts of the model to control for known effects on trait means and estimate our three parameters of interest. We fit the same model structures (described below) for great and blue tits separately to avoid fitting species interactions across model effects and ease interpretation. Response variables were standardized (Z-transformed; mean = 0, SD = 1) and fitted using a gaussian distribution. We also standardized all continuous fixed effects to help with model fit and convergence. We used weakly informative normal priors (N(location mean = 0, scale = 1)) for fixed effects, half-normal priors for random effects (N(0,1)), and a Lewandowski-Kurowicka-Joe correlation prior (LKJ prior; df = 2) for correlation of random effects since our response variables were Z-transformed (Hertel et al., 2021; McElreath, 2020). We ran four chains for 10,000 iterations each using a warm-up of 6,000 iterations and a thinning interval of 4. Thus, model estimates and credible intervals (CIs) used posterior distributions consisting of 4000 samples. All models had appropriate convergence with Rhat = 1 and effective sample sizes > 400 (Vehtari et al., 2021), and inspection of model diagnostic plots (traces, residuals, posterior predictive checks) confirmed good model fit.

3.2.4 Statistical approach : adult tarsus

We removed N = 4 clear outliers for adult tarsus measures that were visually outside the range of the other measures (11–28 mm; for both species) before analysis. Based on previously documented effects on mean tarsus length published on these species (Biard et al., 2017; Caizergues et al., 2021; Corsini et al., 2021;

Saulnier et al., 2023; Seress et al., 2020; Thompson et al., 2022) we included the fixed effects of cluster habitat type (urban vs. forest), mean latitude of each cluster (range = $41^{\circ}23'-61^{\circ}31'N$), and sex (female or male) in the mean part of the model. We included random intercept effects for each study system (i.e., N = 13, excludes Harjavalta dataset) and for each sampling year (range = 1991-2022) to estimate among-system and among-year variation. To evaluate whether urban clusters differentiate to a greater extent than forest ones in their means (i.e., parameter 1: urban > forest among-cluster variance in means; Figure 3.1.1), we fit heterogeneous variance (i.e., random intercepts) separately for urban and forest clusters.

In the dispersion part of the model, we fit the fixed effect of habitat type (urban vs. forest) to explain tarsus variation within clusters and test whether urban clusters contain more diverse individuals than forest locations (i.e., parameter 2: habitat effect on residual variance within clusters; Figure 3.1.2). Males are often more variable in their body sizes than females, especially in mammals (Zajitschek et al., 2020), but this trend could be reversed in birds (Reinhold & Engqvist, 2013). As great and blue tits are dimorphic species, we also explored potential sex differences in tarsus variation by fitting sex as a fixed effect. We included the fixed effects of Shannon diversity index of land cover heterogeneity and mean latitude to determine their contributions on variation within clusters. To account for differences between clusters in their size and number of years of data collection on the variation they contain, we also controlled for cluster area (range = 6456-226604340 meters²) and number of years each cluster was studied (range = 1-28) as fixed effects. We fit random intercepts for study systems to estimate within-system variation. We then also fit heterogeneous variance for urban and forest clusters in the dispersion model to examine whether urban clusters differ more in their tarsus variation (i.e., parameter 3: urban > forest among-cluster variance in residual variance; Figure 3.1.3). Following the format presented in O'Dea et al. (2022), we present mathematical model equations of the mean part of the model, the dispersion part of the model, and their covariance for the analysis of adult tarsus in equations S3.1–6. As an additional step, we ran supplementary models by replacing the categorical habitat effect with impervious surface area (ISA) at 100- and 1000meter scales in the mean and dispersion parts of the model to evaluate how the mean and variation of tarsus were affected along the continuous urbanization gradient. We did not fit heterogeneous habitat variance for clusters in these supplementary models.

3.2.5 Statistical approach : nestling tarsus

We removed N = 9 outliers that were visually outside the range of measures of both species for nestling tarsus length (great tits: range = 10.2–26.2 mm, blue tits: range = 10–21.8 mm). The DHGLM model structure for nestling tarsus included the same fixed and random effects in the mean and dispersion parts of

the model as for adult tarsus but, as nestlings are unreliably sexed this early in life, we did not examine the effects of sex. Instead, we controlled for the effect of nestling age on the mean tarsus length (i.e., as a fixed effect in the mean part of the model, range = 12-17 days old).

3.2.6 Statistical approach : lay date

The DHGLM model structure for lay date contained the same fixed and random effects in the mean and dispersion parts of the model as for adult tarsus, with one exception. As lay date is a female trait, we did not include sex as a predictor in the model. We instead included age category (2+ years of age vs. 1 year old) as a fixed effect in the dispersion part of the model. As experience and learning could play a role in a female's perception of environmental cues and lay date decisions (Bonamour et al., 2020), we explored whether first-time or experienced layers differed in their lay date variation. We included data from N = 13 study systems (excluding Barcelona) and the number of lay date observations was greater for juveniles in both species (great tits: N = 4503 juveniles vs. 1875 adults, blue tits: 3337 juveniles vs. 1082 adults), with this ratio being higher in forest habitats (great tits: 1.8x more juveniles in urban vs. 2.7x in forest; blue tits: 2.1x more juveniles in urban vs. 3.5x in forest).

3.3 Results

3.3.1 Adult tarsus

In the mean part of the model, adult tits had shorter tarsus lengths if they were from urban habitat types (20.08 and 19.79 mm for forest and urban great tits, 17.15 and 16.99 mm for forest and urban blue tits; back-transformed estimates from Table 3.1A) or from habitats with higher ISA (Table S3.3–4; Figure S3.5). The variance among urban clusters in their mean tarsus length was four times higher than the variance among forest clusters in both species providing support for the among subpopulation heterogeneity hypothesis; urban groups differentiated among each other to a greater extent than forest groups in their means, especially in great tits (parameter 1: urban > forest among-cluster variance in Table 3.1A; Figure 3.3.1A). The variance among study systems in mean tarsus length was high for both species and these study system differences explained at least four times more variation than that among years (Table 3.1A; Table S3.5).

In the dispersion part of the model, urbanization increased the residual variation within clusters providing support for the within subpopulation heterogeneity hypothesis (parameter 2: positive habitat effect in Table 3.1A, but note CIs cross zero; similar results for ISA in Table S3.3–4). The mean residual standard deviation

Table 3.1: Fixed and random effect estimates specified in the mean and dispersion (i.e., explains residual variation) parts of a double hierarchical linear model (DHGLM) when examining the effect of urbanization (forest vs. urban) on the mean and residual variation of three traits: A) adult tarsus length, B) nestling tarsus length, and C) female lay dates. Great and blue tit data were run in separate models (N = number of individuals / observations shown for each), and response variables and continuous covariates were standardized (mean = 0, standard deviation = 1). Model estimates are shown with their 95% credible intervals and effects in bold have credible intervals that exclude zero. Figure 3.3 shows the forest vs. urban cluster variance from the mean and dispersion parts of model (parameter [1] in Figure 3.3A and parameter [3] in 3.3C, respectively) and the habitat effect from the dispersion part of the model (parameter [2] in Figure 3.3B). Response and continuous variables were standardized before fitting models (mean = 0, variance = 1) and effects in the dispersion part of the model are on the log scale.

	A) Adu	lt tarsus	B) Nestli	ng tarsus	C) Lay date		
Mean part	Great tit	Blue tit	Great tit	Blue tit	Great tit	Blue tit	
-	N = 11928	N = 7439	N = 7505	N = 2905	N = 6378	N = 4419	
Fixed effects							
Intercept (β_{m0})	0.159	0.162	-1.021	-0.885	0.256	0.293	
	[-0.299-0.638]	[-0.701-1.054]	[-1.5720.458]	[-1.881-0.146]	[-0.011-0.535]	[-0.24-0.857]	
Habitat (urban)	-0.289	-0.173	-0.409	-0.172	-0.256 [-0.113	
	[-0.3710.209]	[-0.2780.073]	[-0.5340.284]	[-0.38-0.03]	-0.3340.176]	[-0.257-0.029]	
Latitude	0.598	0.566	0.518	0.594	0.678	0.726	
	[0.274-0.914]	[-0.015-1.108]	[0.155-0.832]	[-0.17-1.32]	[0.488-0.851]	[0.346-1.054]	
Sex (male) Age	0.523	0.587	0.085	0.065			
NA D L ECC ([0.505-0.541]	[0.562-0.611]	[0.064-0.105]	[0.038-0.091]			
<u>Random Effects</u>	0.04	0.248	0.112	0 127	0.205	0 506	
rear	0.04	0.240 [0 191_0 324]	0.112	0.137 [0.095_0.193]	0.395	0.500 [0 39_0 663]	
System	0.872	1 173	0 755	1 043	0 387	0.608	
System	[0.58-1.363]	[0.697-1.946]	[0.474-1.207]	[0.611-1.774]	[0.247-0.623]	[0.337-1.089]	
Cluster: [1]							
Forest	0.052	0.041	0.133	0.327	0.162	0.327	
	[0.032-0.077]	[0.004-0.095]	[0.082-0.201]	[0.211-0.484]	[0.122-0.212]	[0.227-0.458]	
Urban	0.223	0.162	0.286	0.151	0.118	0.063	
	[0.158-0.302]	[0.06-0.287]	[0.2-0.393]	[0.027-0.298]	[0.07-0.175]	[0.003-0.173]	
Dispersion part							
Fixed effects							
Intercept $(\beta v_{0,exp})$	-0.6	-0.553	-0.434	-0.48	-0.734	-0.506	
1 (7 (,0,0,1))	[-0.750.449]	[-0.9030.159]	[-0.6240.24]	[-0.7060.245]	[-0.8450.608]	[-0.7150.299]	
Habitat (urban) [2]	0.036	0.088	0.109	0.214	0.086	0.093	
	[-0.025-0.097]	[-0.019-0.195]	[0.005-0.215]	[0.046-0.398]	[0-0.174]	[-0.03-0.213]	
Heterogeneity	-0.009	0.001	-0.001	-0.017	0.008	-0.008	
	[-0.036-0.017]	[-0.045-0.05]	[-0.04-0.038]	[-0.088-0.059]	[-0.034-0.049]	[-0.068-0.045]	
Latitude	0.044	0.272	0.098	0.116	-0.039	-0.036	
~	[-0.054-0.15]	[0.026-0.521]	[-0.029-0.238]	[-0.064-0.274]	[-0.115-0.038]	[-0.192-0.103]	
Cluster area	0.03	0.08	0.01	0.183	-0.035	-0.024	
~	[0.012-0.047]	[-0.081-0.243]	[-0.024-0.044]	[-0.062-0.415]	[-0.094-0.023]	[-0.17-0.124]	
Cluster years	-0.008	0.009	-0.003	-0.10/	0.031	0.089	
Cov (mole) NA	[-0.062-0.047]	[-0.102-0.12]	[-0.085-0.075]	[-0.305-0.084]	[-0.055-0.101]	[-0.01/-0.182]	
$\Delta ge(1)$	0.027	-0.005			-0.05	10.055	
Random effects	[0.001-0.055]	[-0.037-0.027]			[-0.072-0.011]	[-0.00)-0.01)]	
System (intercept)	0.239	0.378	0.27	0.096	0.134	0.203	
System (intercept)	[0.146-0.399]	[0.154-0.818]	[0.154-0.462]	[0.003-0.325]	[0.061-0.25]	[0.063-0.488]	
System (<i>r</i> mean dispersion)	-0.102	-0.099	-0.15	-0.041	0.376	0.301	
(mean, anspersion)	[-0.589-0.417]	[-0.675-0.539]	[-0.648-0.407]	[-0.771-0.742]	[-0.223-0.801]	[-0.417-0.846]	
Cluster: [3]				-			
Forest (intercept)	0.064	0.061	0.121	0.205	0.151	0.067	
	[0.033-0.102]	[0.01-0.126]	[0.077-0.179]	[0.083-0.35]	[0.104-0.211]	[0.003-0.177]	
Forest ($r_{\text{mean, dispersion}}$)	-0.493	-0.118	-0.491	-0.543	-0.144	0.214	
	[-0.871-0.069]	[-0.796-0.694]	[-0.8340.005]	[-0.915-0.064]	[-0.518-0.267]	[-0.625-0.804]	
Urban (intercept)	0.065	0.062	0.172	0.173	0.1	0.161	
	[0.007-0.132]	[0.002-0.184]	[0.112-0.25]	[0.025-0.332]	[0.013-0.185]	[0.048-0.306]	
Urban ($r_{\text{mean, dispersion}}$)	-0.292	-0.038	-0.767	-0.232	-0.027	0.068	
	[-0.858-0.446]	[-0.803-0.762]	[-0.970.375]	[-0.85-0.586]	[-0.661-0.609]	[-0.753-0.808]	

for forest and urban great tits was estimated as 0.57 and 0.59 mm, respectively (a 3.5% increase in urban habitats; back-transformed model intercept $\exp(\beta_{v0})$ and $\exp(\beta_{v0})$ + habitat (urban) in Table 3.1A; Figure 3.3.2A). The difference in variation between habitat types was higher in blue tits where the estimated mean residual standard deviation for forest and urban blue tits was estimated as 0.50 and 0.55 mm (a 10% increase in urban habitats; Figure 3.3.2A). In great tits, sex had a clear effect on the variation in tarsus length where males tended to have more variable tarsus lengths than females (while considering differences in mean size; Table 3.1A).

We did not find support for the heterogeneity in heterogeneity hypothesis as urban and forest clusters were similar in the residual variation they contained (parameter 3: similar estimates of urban and forest amongcluster variance in residual variance; Table 3.1A; Figure 3.3.3A). The mean residual standard deviation of adult tarsus length also varied across study systems (Table 3.1A; Table S3.5). We found negative correlations between the mean and standard deviations of tarsus length across study systems and clusters (i.e., negative estimates for $r_{mean, dispension}$ of random effects in dispersion model of Table 3.1A) suggesting that systems and clusters that tend to contain individuals with smaller tarsi (lower mean) also contain individuals with variable tarsus lengths (larger standard deviation). However, there was high uncertainty around these negative correlations (i.e., large CIs overlapping zero) suggesting the relationship between the mean and variation of tarsus length either i) differed considerably among systems and clusters in this dataset or ii) we lacked power to estimate these correlations across 99 clusters. Excluding the Munich location in a subsequent analysis did not change our main conclusions (Table S3.5A).

3.3.2 Nestling tarsus

In the mean part of the model, nestlings in urban habitat types had smaller tarsi, but this effect was less clear in blue tits (CI crossing zero; 18.23 and 17.6 mm in forest and urban great tits, 16.0 and 15.8 mm in forest and urban blue tits; back-transformed estimates from Table 3.1B). The effect of ISA on tarsus length, however, was clear in both species with nestlings in more urbanized habitats having smaller tarsi than those in less urbanized ones (Table S3.2-3; Figure S3.5). For great tits, the among-cluster variance was twice as high for urban compared to forest clusters providing support for the among subpopulation heterogeneity hypothesis (parameter 1: urban > forest among-cluster variance in Table 3.1B; Figure 3.3.1B). However, we did not find the same support in blue tit nestlings as the estimated variation among forest clusters was slightly higher and the CIs between habitat types overlapped (Table 3.1B; Figure 3.3.1B). Similarly to adult tarsus length, the estimated variance in mean nestling tarsus length among study systems was high for both

species and these between study system differences explained at least six times more variation than the variation among years (Table 3.1A; Table S3.5).



Figure 3.3: Results of each tested hypothesis (columns) for A) adult tarsus length, B) nestling tarsus length, and C) lay date traits (rows). 1) Among subpopulation heterogeneity hypothesis (parameter 1) shows the variance estimated among forest and urban clusters on the mean of the traits, 2) Within subpopulation heterogeneity hypothesis (parameter 2) shows the habitat effect size (forest versus urban) on the residual variation of the traits, and 3) Heterogeneity in heterogeneity hypothesis (parameter 3) shows the variance estimated among forest and urban clusters on the residual variation of the traits. Forest clusters are shown in green and urban clusters in blue. Estimates for B in grey show the habitat effect where positive values indicate more residual variation in urban habitats. Estimates for great tits are represented by squares and blue tits by triangles. Model estimates are from Table 3.1 and their 95% (thin line) and 50% (thicker line) credible intervals are shown.

In the dispersion part of the model, urbanization more strongly increased the variation of nestling tarsus within clusters (within subpopulation hypothesis and parameter 2: strong and clearer positive estimates of habitat effect in Table 3.1B; and ISA in Table S3.2–3). The mean residual standard deviation for forest and urban great tits was estimated as 0.87 and 0.97 mm (an 11% increase in urban habitats; Figure 3.3.2B). The difference in variation between habitat types was greater in blue tits where the estimated mean residual standard deviation for forest and urban blue tits was 0.69 and 0.86 mm (a 25% increase in urban habitats; Figure 3.3.2B).

We did not find support for the heterogeneity in heterogeneity hypothesis as both species showed similar among-cluster variation for urban and forest habitats in their residual variation (parameter 3: urban ~ forest among-cluster variance in residual variation; Table 3.1B; Figure 3.3.3B). As shown for adult tarsus above, there was high uncertainty around the estimated correlations between the means and residual standard deviations of nestling tarsus across study systems (i.e., large CIs overlapping zero for system *r*_{mean}, dispersion effect). In great tits, urban and forest clusters had clear negative correlations between the mean and residual standard deviations of tarsus size indicating that clusters that tended to contain smaller great tit nestlings also tended to contain more variable individuals (i.e., strong negative Cluster *r*_{mean}, dispersion estimates). These strong negative correlations in great tits were robust when running the model with a correlation prior with higher degrees of freedom (df = 5). These cluster-level negative correlations were not as clear in blue tits. Excluding the Munich location in a subsequent analysis did not change our conclusions, and the effect of urbanization had a stronger effect on both the mean and residual variation of nestling tarsus in these models (Table S3.5B).

3.3.3 Lay date

In the mean part of the model, the effect of urbanization (categorical and continuous) had a clear effect on mean lay date in great tits, but the effect in blue tits was weaker (CI of habitat effect just crossing zero for blue tits in Table 3.1C, see also ISA effects in Table S3.3–4). The average female lay date for forest and urban great tits was 109 and 106 Julian days (April 19 and 16), respectively, and forest and urban blue tit females had average lay dates at 105 and 103 Julian days (April 15 and 13; back-transformed estimates in Table 3.1C). We did not find support for the among subpopulation heterogeneity hypothesis in great tits as there was similar among-cluster variance in mean lay date and overlapping CIs between the habitat types (parameter 1 great tits: urban ~ forest among-cluster variance in means, Table 3.1C; Figure 3.3.1C). We found opposite support for this hypothesis in blue tits, where the variation among forest clusters was five times higher than the variance among urban clusters; forest subpopulations of individuals differentiated to a greater extent in their mean lay dates compared to urban subpopulations (parameter 1 blue tits: urban < forest among-cluster variance in means, Table 3.1C). The estimated variance among study systems in mean lay date was high but, unlike the results for tarsus, the estimated variance among years was also high in both species (similar estimates of among-system and among-year variance in Table 3.1C).

Urbanization increased lay date variation within clusters for both species providing further support for the within subpopulation heterogeneity hypothesis (parameter 2: positive urban habitat effect on residual variation, but note CIs cross zero in Table 3.1C; same for ISA but clearer effect in great tits in Table S3.3–

4; Figure 3.3.2C). The mean residual standard deviation of lay date for forest and urban great tits was estimated as 5.6 and 6.1 days (8.9% increase in urban habitats; back-transformed estimates from Table 1C), and 6.1 and 6.6 days for forest and urban blue tits (an 8.2% increase in urban habitats). Among-cluster variance in the residual variation of lay dates were similar between urban and forest habitat types, which does not support the heterogeneity in heterogeneity hypothesis (parameter 3: urban ~ forest among-cluster variance in residual variation; Table 3.1C; Figure 3.3.3C). Estimated correlations between the mean and standard deviations of lay date across study systems and clusters (i.e., estimates for $r_{mean, dispersion}$ in Table 3.1A) were weak and highly uncertain.

3.4 Discussion

Using a mega-analysis approach with long-term data from fourteen European pairs of urban and nonurban tit populations, we confirm strong phenotypic divergence in morphology and breeding phenology, and find evidence that urbanization increases phenotypic variation at multiple population levels. Urbanization is associated with increased differences in tarsus length between subpopulations (i.e., clusters; hypothesis 1, Figure 3.1.1, Figure 3.3.1A&B), especially in great tits, but this evidence was lacking or even opposite when considering how urbanization affected lay date differences between subpopulations (Figure 3.3.1C). We also found support that urbanization increases the phenotypic variation contained within subpopulations (hypothesis 2; Figure 3.1.2, Figure 3.3.2), a result that was consistent across all traits, providing more general support that individual phenotypic differences exist at fine spatial scales within cities. We did not find any clear support for differences in variation between urban subpopulations (hypothesis 3; Figure 3.1.3, Figure 3.3.3) suggesting that subpopulations in cities do not differ more in their among-individual variation than subpopulations outside cities. Collectively, our results show that i) urban phenotypic variation is affected at local scales within subpopulations and ii) urbanization might also increase differences between subpopulations at larger landscape scales depending on the trait. Despite numerous hypotheses on how ecological and evolutionary processes can affect phenotypic change in urban populations (Alberti et al., 2020; Diamond & Martin, 2021; Rivkin et al., 2019; Szulkin et al., 2020; Thompson et al., 2022), there are still limited empirical examples (Lambert et al., 2021). Although this work does not directly evaluate the ecological processes acting in cities, it is a first step using a novel approach to generate hypotheses that may explain spatial patterns of urban phenotypic variation (discussed below). These results highlight exciting avenues to determine whether greater individual heterogeneity in cities may be associated with higher plasticity and/or higher evolutionary potential in urban birds.

3.4.1 Tarsus variation

In support of the among subpopulation heterogeneity hypothesis (hypothesis 1), we found greater differences in mean adult tarsus length between urban subpopulations than forest ones. These higher urban subpopulation differences were found in both species, and these patterns also extended to great tit nestlings. Differences between urban habitats in nestling food resources (and nutrients like carotenoids, Biard et al., 2006; Isaksson, 2009) might be a strong driver of larger urban variation at this population level as it has been shown that urban great tit nestlings develop longer tarsus lengths than forest nestlings when they are experimentally supplemented food compared to controls (Seress et al., 2020). Differences in nestling tarsus arising between urban subpopulations could also reflect genetic differences since tarsus length is heritable (h² between 0.3 and 0.8 in great tits, Young & Postma, 2023); another likely possibility as we see parallel patterns in adults at this population level. Quantitative genetic approaches could be used to evaluate the relative contributions of early environmental or additive genetic effects on nestling tarsus length to determine which processes likely contribute to stronger nestling tarsus differences between urban subpopulations. As tarsus length is fixed early in life, dispersal behaviours likely contribute to the higher urban subpopulation differences in adult tarsus where, for example, i) urban birds have limited dispersal after fledging and so phenotypic (and potentially genetic) differences between subpopulations reflect those seen in nestlings or ii) variation in environmental quality drives non-random dispersal where larger birds competitively exclude smaller ones from greener urban habitats with more food resources (Edelaar & Bolnick, 2012). Dispersal dynamics in city birds are still poorly studied because of technical challenges (but see Hanmer et al., 2022; Senar & Björklund, 2021) and further advances here would be conducive to explain these patterns of tarsus variation in adults.

We also found support for the within subpopulation heterogeneity hypothesis (hypothesis 2) since urbanization predicted higher tarsus diversity within urban subpopulations across both species and life stages. This fine-scale tarsus diversity estimated within urban subpopulations could be driven by the processes discussed above acting at finer spatial scales. Variation in the quality of breeding territories within an urban area, for example, could contribute to non-random distributions of adults where individuals with larger tarsi occupy higher quality habitats and can feed their nestlings higher quality food resources. Variation in body mass, for instance, is spatially variable in a woodland population of great tits and this fine-scale phenotypic diversity was argued to be primarily driven and maintained by non-random dispersal related to habitat quality (Garant et al., 2005). Modeling fitness landscapes (e.g., Camacho & Hendry, 2020) across urban gradients would help establish how trait variation correlates with environmental variation at fine scales, and whether these phenotypic patterns have selective consequences.

The estimated effect of urbanization on tarsus variation within subpopulations was stronger and clearer in nestlings than adults. This result was expected as we have direct urbanization data related to the environment that nestlings developed in, whereas adults were measured in the environment they choose after dispersing, which would create noise around urbanization's effect in adults. However, smaller among-individual variation in adult tarsi could also reveal that selective processes cause non-random disappearance from the population between the nestling and adult stages. Larger body mass after hatching is known to predict survival to fledgling in urban great and blue tit nestlings (Corsini et al., 2021) and so this might imply that smaller individuals are less likely to fledge and recruit into urban populations, thereby reducing the tarsus variation observed in urban adults. We observed an increase in average tarsus length from nestling to adult stages supporting this prediction of selective survival. Individual-based demographic models would be useful to evaluate how certain phenotypes affect survival probabilities in urban and nonurban populations (DeAngelis & Grimm, 2014; Dunlop et al., 2007).

3.4.2 Lay date variation

We found evidence that females lay earlier clutches in urban compared to forest environments, and that urban females have more diverse lay date strategies than forest females at local spatial scales (hypothesis 2). More diverse lay dates across breeding territories in an urban area could be driven by variation among urban females in lay date plasticity, resulting from varied responses to fine-scale heterogeneity in urban environmental conditions (e.g., temperature, artificial light, dominant tree species and their phenology; Bailey et al., 2022; Bonamour et al., 2019; Dominoni et al., 2020; Jensen et al., 2023; Matthysen et al., 2021; Shutt et al., 2019). This higher urban lay date variation could also reflect larger local genetic variation where urban subpopulations contain individuals with diverse genotypes that translate into more diverse egglaying phenotypes. Relaxed urban selection, for example, could drive the observed increases in lay date strategies (and associated genetic variation) within cities as there is evidence that selection on lay date is weaker in urban compared to forest populations of great and blue tits (Branston et al., 2021; Caizergues et al., 2018). Genetic differences between individuals at local scales could also be explained by larger urban effective population sizes or source-sink dynamics where constant dispersal of individuals from nonurban to urban areas increases phenotypic diversity (Johnson & Munshi-South, 2017). Unlike the patterns observed for tarsus variation, we did not find support that urbanization is associated with increased lay date differences between subpopulations (hypothesis 1). This implies that environmental conditions or underlying genetic variation related to lay date vary only at local scales within urban subpopulations, rather than also varying between subpopulations at a landscape scale. More years of data collection across these urban tit populations would allow a more ambitious approach that incorporates pedigrees in our models

allowing an evaluation of whether genetic variation follows a similar pattern as lay date (or tarsus) variation at these population levels.

In blue tits, we found opposite support for the among subpopulation heterogeneity hypothesis (hypothesis 1) where blue tit subpopulations differ more in their breeding phenology in forest areas than areas located in cities. This was a surprising result that has not been, to our knowledge, previously reported, and so we are unable to offer a solid explanation for this pattern. It is possible that forests across Europe may have more heterogeneity in the environmental conditions that drive differences in lay date timing (e.g., temperature, tree composition, Bailey et al., 2022). However, this would not fully explain why this result was unique to blue tits. Previous studies have reported habitat-dependent differences in lay date responses between these species and it is hypothesized that these results are driven by species differences in their foraging ecology, competitive abilities, territory quality, or environmental cue perception (Branston et al., 2021; Matthysen et al., 2021; Vaugoyeau et al., 2016). Differences between the species in the perception of environmental cues like spring temperatures, specifically, could explain our results if processes like the urban heat island effect homogenize these cues at landscape scales in cities. While these explanations are speculative, urbanization clearly affects the phenological distribution of urban tit populations, and it will be important to explore these patterns further to establish how urban and forest tit species are differentially responding to advances in spring phenology under a warming climate.

3.4.3 Average effect of urbanization on variation and possible consequences

For the six species-trait combinations, we found that urbanization increased phenotypic variation within subpopulations by 11% on average (range: 3.5–25%). More specifically, urbanization was associated with an 8% increase in lay date variation on average, which is a weaker effect than the average effect of urbanization on lay date variation reported across 34 bird species globally (19.2% increase, Capilla-Lasheras et al., 2022). Alternatively, the increase in variation that we report here is stronger than reported in a recent meta-analysis showing that human disturbances outside urban contexts (e.g., climate change, pollution, harvesting) have negligible effects on phenotypic variation with most wild populations showing changes of only ~1% in variation on average, and only a few cases where larger changes in variation were observed (Sanderson et al., 2023). It is unclear whether our urban tit populations represent an outlier case of how human disturbance impacts variation, like those few cases reported in Sanderson et al. (2023), or whether disturbance in urban environments like reported by Capilla-Lasheras et al.(2022) and here, specifically, can have similar impacts on phenotypic variation to manipulated stressors in experiments (O'Dea et al., 2019; Sánchez-Tójar et al., 2020). We show that differences between study systems explained four times more

variation in tarsus length than differences between years suggesting that tit morphology varies considerably in space across the continent. Differences between study systems and years explained a similar amount of lay date variation highlighting that the phenology of European tits varies across both space and time. Further mega- or meta-analyses that include studies from multiple taxa (especially outside aves) and traits in urban environments will be needed to generalize how ecological conditions related to urbanization (in space and time) impact phenotypic variation in the wild.

Beyond identifying plausible processes that shape variation in urban populations, we also discuss here the possible consequences of increased variation at different population levels (Bolnick et al., 2011; Mimura et al., 2017; Violle et al., 2012). At the within-subpopulation level, we show that urbanization increases lay date and tarsus variation. This fine-scale phenotypic variation could give urban subpopulations the ability to respond to new or fluctuating selection pressures and buffer environmental variation and, if this phenotypic variation is underlined by similar patterns in genetic variation, the ability to adapt to further environmental change (Mimura et al., 2017; Moran et al., 2016). For example, the cues predicting optimal lay date timing could be less clear in urban environments (Schlaepfer et al., 2002) and so fine-scale variation in lay date strategies could buffer unpredictable annual variation in how lay date timing relates to reproductive fitness in urban environments. More diverse urban lay date strategies could also impact urban food webs by, for example, having top-down selective consequences for the phenology of urban insect communities, which in turn could impact leafing phenology in urban tree communities (Jensen et al., 2022). At the among-subpopulation level, urbanization was associated with increased differences in tarsus, which could contribute to higher diversity at the urban population or species levels. In mammals, lower interpopulation variation in body size was correlated with a species' Red List status and vulnerability to extinction (González-Suárez & Revilla, 2013), which could suggest that larger tarsus differences observed here between urban subpopulations may also buffer overall population persistence in the face of local extinctions. Higher differences between urban subpopulations in the individual variation they contain compared to nonurban subpopulations could indicate that subpopulations in cities may vary more in their ability to plastically adjust or adapt to further environmental change, but we did not find clear evidence for this scenario (hypothesis 3). Evaluating dispersal between urban subpopulations will be particularly important for identifying further consequences at this population level. For example, if dispersal between subpopulations is limited within the urban matrix, this could lead to further phenotypic (or genetic) differentiation in cities, with possible implications for adaptive radiations or speciation (Littleford-Colquhoun et al., 2017; Thompson et al., 2018).

3.4.4 Limitations and moving forward

This study takes advantage of a large collaborative effort that is pushing to standardize research approaches and combine long-term data (Culina et al., 2020) and, although these large collaborative efforts will be key for establishing broad conclusions across replicate populations or cities, there are relevant limitations here that warrant discussion. First, our data comprises long-term studies on great and blue tits that are historically studied in forest habitats. Therefore, it is unclear whether differences in phenotypic variation reported here between urban and forest populations also extend to how variation differs between urban and nonurban (e.g., grassland, marsh, etc.) populations more generally. Second, we have more replicate populations that exist across a larger geographical range for great tits than blue tits, and so population-level patterns that differ between the species here should be interpreted with this in mind. Third, we found no clear associations between land cover heterogeneity and phenotypic variation across the traits and species examined. This negative result may be due to the cruder resolution of the Europe-wide land cover data we used (1x1 km classification), rather than a true absence of an effect. Environmental heterogeneity can occur at fine spatial scales (within meters) and could be more likely to drive the fine-scale trait variation we observed here. Studies measuring fine-scale environmental data will be needed to further explore how environmental variation translates into phenotypic variation. Establishing this link in urban environments will be especially meaningful as urban habitats are assumed to be more environmentally heterogenous, but this assumption may depend on the environmental axis and scale considered (Thompson et al., 2022). Accumulating data on different axes of environmental variation, such as temperature, vegetation cover or artificial light at night, and examining how these factors differentially impact traits would allow a more comprehensive examination of how urbanization impacts variation at local scales.

3.5 Conclusions

In conclusion, we find that urbanization is associated with increases in variation within subpopulations across both traits and species examined. This suggests that processes like selection, dispersal, or plasticity affect phenotypes at local scales in cities, and that modified variation will likely have consequences within subpopulations. We also find evidence that urbanization can impact variation at multiple population levels simultaneously, but this depended on the trait. It remains to be determined whether adaptive or neutral evolutionary processes, like responses to selection or drift respectively, could drive these patterns of phenotypic variation, and so identifying the genetic basis of this variation will be conducive to this end. Large collaborative efforts will be powerful approaches for identifying more generally how urbanization is impacting wildlife as these large datasets open new research possibilities and the ability to draw broad

insights across replicate cities. Single system research using experiments or finer scale data will be necessary complements to these mega-analyses, and together single and multi-population approaches can make timely fundamental and applied contributions in urban ecology and evolution. Determining how ecological conditions like urbanization affect phenotypic variation is an especially significant research avenue to establish the programs needed to conserve wild populations and ecological communities in cities (Mimura et al., 2017), so that nature's contributions to society (Des Roches et al., 2021) are maintained in the face of the Anthropocene.

3.6 Acknowledgements

This project would not have been possible without the coutnless students and researchers that have contributed to the ongoing long-term monitoring of the study populations included here. We would like to thank Stefan Vriend and Antica Culina at SPI-Birds for back and forth exchanges about datasets and completing data requests, and also Frank Adriaensen for preparing data from Antwerp. Thank you to Ned Dochtermann and Raphaël Royouté for an interesting initial discussion about the project and sharing resources on DHGLMs. MJT was supported by a Canadian Graduate Scholarship from the Natural Sciences and Engineering Research Council (NSERC) of Canada, a Fonds de recherche du Québec Nature et technologies (FRQNT) PhD scholarship, and a PhD mobility grant from le Centre Méditerranéen de l'Environnement et de la Biodiversité (CeMEB). This project was funded by the Agence Nationale de la Recherche (URBANTIT grant ANR-19-CE34-0008-05 to AC), the OSU-OREME, and a Fonds de Recherche du Quebec Nature et Technologie to DR.

CHAPITRE 4

The city and forest bird flock together in a common garden: genetic and environmental effects drive phenotypic divergence in urban populations

Authors: Megan J. Thompson, Denis Réale, Baptiste Chenet, Ségolene Delaitre, Amélie Fargevieille, Marc Romans, Samuel P. Caro, and Anne Charmantier

Phenotypic divergences between populations are common in nature, and especially frequent along urbanization gradients. Urban phenotypic shifts are documented across diverse taxa, but the underlying genetic and environmental drivers behind these phenotypic changes are unknown in most urban systems. We synthesize urban common garden studies in the literature (N = 77) across a range of taxa and, collectively, these studies show that both genetic and plastic responses can contribute to phenotypic divergence in urban populations. We conduct our own common garden experiment using genotyped great tits (Parus major; N = 73) along an urbanization gradient in Montpellier, France to: 1) determine whether documented morphological, physiological, and behavioural shifts in wild great tits populations are maintained in birds reared in a common garden from urban and forest origins and 2) evaluate how different sources of genetic, early maternal investment, and environmental variation contributed to individual variation in our experiment. In line with the phenotypic divergence in the wild, birds from urban origins have faster breath rates and are smaller than birds from forest origins suggesting genetic differentiation has driven these trait differences. Alternatively, wild differences in aggression and exploration are not maintained in the common garden indicating that plasticity to urban environmental conditions likely drive shifts in these traits. Individual-specific variation tended to explain the most trait variation in the experiment, especially for traits that had less evidence for genetic population differences, while brood-specific and social environmental variation had minimal contributions. Our results show that both genetic and plastic effects can drive urban phenotypic shifts in the wild and, thus, provide complementary support to previous urban common garden studies in a model species.

Keywords: urbanization, urban gradient, common garden experiment, plasticity, genetic differentiation, great tits

4.1 Introduction

Phenotypic differences between individuals of the same species are observed in wild populations that occur across diverse environmental gradients. Various evolutionary and ecological processes shape the phenotypic diversity we observe in nature and lead to phenotypic divergences between populations (Hendry, 2017; Mitchell-Olds et al., 2007). For instance, local selection pressures on heritable traits can lead to divergent adaptation to local environmental conditions (Kawecki & Ebert, 2004). Developmental or reversible phenotypically plastic responses to local environments can also drive phenotypic adjustments (Ghalambor et al., 2007). Determining how local adaptation and plasticity interact to shape phenotypes is crucial as these processes can have different impacts on demographic and evolutionary trajectories of wild populations (Ghalambor et al., 2007; Nicolaus & Edelaar, 2018; Snell-Rood, 2013).

Some of the most striking examples of environmental variation occur along urbanization gradients, such as urban shifts in multiple taxa towards smaller body size because of the heat island effect (Merckx et al. 2018). Such phenotypic shifts in urban populations are frequently documented across diverse taxa and traits (Diamond & Martin, 2021; Szulkin et al., 2020), through changes in both phenotypic means (Lambert et al., 2021; Miranda et al., 2013) and variation (Capilla-Lasheras et al., 2022; Thompson et al., 2022). Urban phenotypic shifts are commonly assumed to be driven by genetic change via selection and subsequent evolution, but there is still a lack of evidence that urban organisms are adapting to these novel urban conditions (Lambert et al., 2021) and plasticity could play a major role in urban phenotypic change (Hendry et al., 2008; Yeh & Price, 2004). Determining the mechanisms behind phenotypic changes in urban organisms could importantly inform on whether urban populations will continue to adjust in pace with further environmental change. For these reasons, there have been several calls for research that disentangle the genetic and plastic contributions on urban phenotypes and, more specifically, calls for urban common garden experiments (Lambert et al., 2021; Rivkin et al., 2019; Sanderson et al., 2023).

Common garden experiments are a useful approach for exploring the genetic basis of phenotypic differences between populations. These manipulations rear individuals from different populations under the same environmental conditions from very early life stages, and ideally across several generations. As individuals develop and mature under common conditions, any phenotypic differences that persist in this context are likely a result of underlying genetic differentiation rather than plastic responses to environmental conditions (Lambert et al., 2021). Thus, common gardens can be used by researchers to determine whether documented phenotypic divergences in wild populations are driven by genetic change and possible evolution, but can also be used to explore possible interactions between genetic and plastic changes acting in these systems

(Conover et al., 2009). For these reasons, common garden approaches are needed in urban evolution research to explore the potential processes acting in these contexts (Alberti et al., 2017; Sanderson et al., 2023).

We censused 77 common garden studies with urban populations in the literature; an impressive number despite the effort and resources these experiments require (see synthesis in Table 4.1). Most of these studies have been published within the last 10 years (83%, N = 64) and many of these studies support genetic divergence underlying shifts in urban phenotypes (86%, N = 66), which could indicate local adaptation to urban conditions via evolution. Fewer studies document plasticity to environmental conditions as a driver of phenotypic change (58%, N = 45), but this conclusion is especially common in multi-treatment common gardens. Experiments so far tend to use invertebrate or plant models, likely as these organisms are more easily reared, reproduced, and manipulated in captive environments. Many studies have focused on physiological phenotypes associated with tolerance to temperature as the urban heat island effect is known to increase temperature and heat stress in urban environments (Mohajerani et al., 2017). For example, urban damselflies (*Coenagrion puella*), water fleas (*Daphnia magna*), wood louse (*Oniscus asellus*), and acorn ants (*Temnothorax curvispinosus*) have higher heat tolerance compared to nonurban conspecifics when reared under common conditions providing evidence that urban invertebrates have adapted to these urban heat island effects (Brans et al., 2017; Diamond et al., 2018; Tüzün & Stoks, 2021; Yilmaz et al., 2021).

While common garden studies provide support in favour of urban evolution in some well-studied taxa, whether genetic change drives phenotypic shifts in other urban taxa and traits is not as well known. For instance, birds are one of the most studied taxa in urban environments and there are growing generalizations on how urbanization impacts the traits of birds globally (e.g., earlier lay dates, Capilla-Lasheras et al., 2022). Great tits (*Parus major*), specifically, have become a model species for studying urban evolution across Europe and Asia and, thus, research on this species is now contributing to large collaborative research efforts that evaluate trends across replicated urban gradients in continent-wide analyses (Salmón et al., 2021; Thompson et al., 2022; Vaugoyeau et al., 2016). Despite these exciting efforts, there exists a fundamental knowledge gap about whether urban phenotypic shifts in this model species are driven by genetic change between populations (and possibly evolution) or by plastic responses to urban conditions. Common garden experiments in urban dark-eyed juncos (*Junco hymemalis*) and European blackbirds (*Turdus merula*) suggest that genetic change could at least partially play a role in phenotypic differences across morphological, physiological, and behavioural traits (Table 4.1; Atwell et al., 2012; Miranda et al., 2013; Reichard et al., 2020), but it remains to be seen whether this holds for other urban bird species and, specifically, the great tit where urban phenotypic shifts have been well documented.

This study uses a common garden experiment to disentangle the mechanisms that shape urban phenotypes in populations of great tits in and around Montpellier, France. In this system, we have documented several phenotypic differences between urban and forest populations in life history, morphology, physiology, and behaviour (Caizergues et al., 2018; Caizergues et al., 2022; Charmantier et al., 2017); trends that tend to be consistent across other European populations (Biard et al., 2017; Corsini et al., 2021; Thompson et al., 2022; Vaugoyeau et al., 2016). In the wild, urban great tits are smaller, have faster breath rates under constraint, show higher aggressiveness when handled, and are faster explorers than their forest counterparts, although estimates of selection gradients suggest that these urban phenotypic shifts are not favoured by natural selection (Caizergues et al., 2018; Caizergues et al., 2022). Genomic studies have revealed that, despite evident gene flow in this system, a small but significant proportion of genetic variation is explained by urbanization. This result suggests some genetic divergence between the urban and forest populations (i.e., significant F_{ST} = 0.006–0.009 between urban and forest comparisons; Caizergues et al., 2022; Perrier et al., 2018). A common garden approach is the next logical step to decipher the genetic and plastic influences on the phenotypic divergences documented in this urban system, but also in others across the species distribution.

In this study we reared individuals from urban and forest sites around the Montpellier area in Southern France under common conditions to evaluate whether documented morphological, physiological, and behavioural differences persist under the same environmental conditions. We had two major aims. The first was to compare the phenotypes of birds from urban and forest origins reared in a common garden. We hypothesized stronger genetic change for lowly labile traits (e.g., morphology, physiology) than for labile ones (e.g., behaviour; Dingemanse & Wolf, 2013; Stirling et al., 2002) and, therefore, that these morphological and physiological differences would be more likely to persist under common conditions. More specifically, we predicted that birds from urban origins would be smaller and more stressed (phenotypic difference persists), but not more aggressive or exploratory (phenotypic difference does not persist), than birds from forest origins. Our second aim was to evaluate how different sources of variation (i.e., genetic and environmental variation) shaped phenotypes in the experimental context. We present phenotypic estimates from wild populations alongside those from the common garden for comparison.

Table 4.1: Synthesis of studies (N = 77; non-exhaustive) that have used a common garden approach to compare the phenotypes of urban and nonurban populations (e.g., rural, forest, mountain, agricultural) across a variety of different groups (amphibians, birds, invertebrates, fish, plants, reptiles) and traits (physiology, behaviour, morphology, life history, phenology). Information concerning the traits measured, approach taken, results, conclusions, and other notes are shown for each study. Studies were collated from Lambert et al. 2021 (Table 1) and from a literature search for articles since 2020 that included both "common garden" and "urban" (conducted March 22, 2024 using Google Scholar).

Group	Urban vs.	Traits	Approach ¹	Urban results ²	Conclusion ³	Notes	Ref
AMPHIBIANS							
Agile frog (Rana dalmatina)	Woodland	Physiology	Eggs	Lower heat tolerance	E (& G)	Difference in common garden opposite to wild divergence	(Bókony et al., 2024)
Common toads (<i>Bufo bufo</i>)	Agricultural & natural	Physiology	F1 generation	No differences in toxicity and potency (or in opposite directions)	E	Urban and agricultural higher toxicity and potency in wild	(Bókony et al., 2019)
		Physiology	F1 generation	No differences in stress	Е	Urban and agricultural higher stress response in wild	(Bókony et al., 2021)
Guttural Toad (Sclerophrys gutturalis)	Natural	Behaviour	F1 generation	No differences in boldness	Е	Higher urban boldness in wild	(Mühlenhaupt et al., 2022)
BIRDS	Mountain		Fledglings	Higher boldness	G	Same results in wild	(Atwell et al., 2012)
Dark-eyed juncos (Junco hymemalis)		Behaviour Physiology		and stress response			
		Morphology	Nestlings	Urban birds have shorter wing and tail lengths, and whiter tails	G	Same results in wild	(Rasner et al., 2004; Yeh, 2004)
		Physiology	Fledglings	No differences in testosterone	Е	Higher urban testosterone	(Atwell et al., 2014)
		Behaviour	Fledglings	Higher minimum frequency, no difference in peak or max frequency	G	Same results in wild	(Reichard et al., 2020)
		Physiology	Fledglings	Higher gonadal sensitivity	G	Advanced reproduction in wild	(Fudickar et al., 2017)
European blackbirds (Turdus merula)	Rural	Behaviour	Nestlings	More neophobic and seasonally less neophilic	G	Higher urban repeatability	(Miranda et al., 2013)
	Forest	Physiology	Nestlings	Attenuated stress responses	G	Trends varied seasonally	(Partecke et al., 2006)
		Behaviour Physiology	Nestlings	Lower migratory disposition and earlier gonadal development	G	Loss of migration in wild Observed in males only	(Partecke & Gwinner, 2007)
INVERTEBRATES			52	TT 1 4 4			
Water fleas (Daphnia magna)	Rural	Physiology	F2 generation 2 temperature treatments	Higher thermal tolerance, haemoglobin, and smaller body size	G & E	Higher thermal tolerance at warmer rearing temperature	(Brans et al., 2017)
		Morphology	F2 generation 2 temperature treatments	Smaller body size	G & E	Smaller body sizes at warmer rearing temperatures	(Brans et al., 2017)
		Physiology Life-history	F3 generation 2 temperature treatments	Higher energy reserves and lower enzyme activity	G & E	Rearing temperature affected some traits Pace of life syndrome in urban fleas	(Brans et al., 2018)
--	-----------------	----------------------------	--	--	-------	---	--
		Life-history Morphology	F2 generation 2 temperature treatments	Faster maturation, smaller, increased fecundity, early release of progeny	G & E	Trait changes in response to rearing temperature in same direction Faster pace of life in urban fleas Some evidence of G x E for fecundity	(Brans & De Meester, 2018)
		Physiology	F2 generation 2 temperature treatments Toxicity treatment	Higher survival	G	No differential impacts of temperature	(Brans et al., 2021)
Water fleas (<i>Ceriodaphnia</i> <i>cornuta</i>)	River / Lake	Life-history Morphology	F2 generation Food treatments	Reduced dietary tolerance to cyanobacteria	G x E	Genomic differentiation between populations	(Zhang et al., 2022)
Mosquito (Anopheles gambiae)	Rural	Physiology	Larvae	Agricultural sites higher tolerance to carbamates	G		(Antonio- Nkondjio et al., 2011)
		Physiology	Larvae	Higher tolerance to ammonia	G		(Tene Fossog et al., 2013)
		Physiology	Larvae	Agricultural sites higher tolerance to DDT	G		(Tene Fossog et al., 2013)
Asian tiger mosquito (Aedes albopictus)	Rural	Phenology	F1 generation Light and dark treatment	No difference in diapause incidence	Е	Diapause lower and more variable under light treatment	(Westby & Medley, 2020)
Blowfly (Calliphora vicina)	Rural	Morphology	F2 and F3 generation 4 temperature treatments	Smaller and faster growth rates at higher temperatures	G x E	Sex differences across temperatures	(Hwang & Turner, 2009)
Wormlion fly (Vermileonidae sp.)	Natural	Behaviour	Larvae	Higher foraging investment and more selective habitat choice	G		(Samocha & Scharf, 2020)
Damselflies (Coenagrion puella)	Rural	Life-history Physiology	Larvae with immune challenge Control and heat wave treatments	No differences in survival, growth rates, immune response, or bioenergetics. No reduction in energy budgets when exposed to a simulated heat wave.	G x E	Heat wave experiment significantly influenced all traits measured	(Tüzün & Stoks, 2021)
		Life-history Physiology	Larvae 5 temperature treatments	Lower growth rates and higher survival	G	No large differences across temperatures Some evidence for	(Tüzün et al., 2017)

						countergradient	
		Behaviour	Larvae Control and pesticide treatments	Less active, bolder, and have slower growth rates when exposed to pesticides	G x E	variation	(Tüzün et al., 2017)
(Ischnura elegans)		Behaviour	Larvae 2 temperature treatments Control and pesticide treatments	More active when exposed to pesticides, but no differences in exploration or food intake	G x E		(Tüzün et al., 2015)
		Life-history	F1 generation 3 temperature treatments 2 predator treatments	Lighter and slower growth rate	G x E	Central and high latitude origin populations Different responses across latitudes	(Palomar et al., 2023)
		Behaviour	F1 generation 2 temperature treatments	Higher encounter and predation rates, but only on prey from rural origins	G x E	Daphnia prey also reared in common garden	(Brans et al., 2022)
Spindle ermine moths (<i>Yponomeuta</i> <i>cagnagella</i>)	Pristine	Behaviour	Larvae	Urban moths less attracted to light	G	Males more likely to be attracted to light than females	(Altermatt & Ebert, 2016)
		Morphology	Larvae	Smaller wings and less attracted to light	G		(Van de Schoot et al., 2024)
Latticed heath moth (Chiasmia clathrate)	Rural	Physiology	F2 and F3 generation	Higher heat tolerance in adults	G	Parallel patterns across three countries Divergence not evidence in larval stage	(Merckx et al., 2024)
		Phenology	F3 generation Light treatments	Reduced diapause induction	G & E	Light increases direct development and reduces development time	(Merckx et al., 2023)
& green-veined white butterfly (<i>Pieris napi</i>)		Life-history	F1 generation Photoperiod treatments	Lower day length threshold for direct development and reduced diapause induction	G x E	2 replicate cities Longer and later urban flight period in the wild	(Merckx et al., 2021)
Speckled wood (Pararge aegeria)	Rural	Behaviour	Eggs	No differences in personality	Е	Urban males who were more active as larvae were more exploratory as adults	(Kaiser et al., 2018)
	Agricultural & woodland	Behaviour	F1 generation	No differences in boldness or activity	Е	Covariation between boldness and activity existed in urban butterflies	(Kaiser et al., 2020)
Acorn ants (Temnothorax curvispinosus)	Rural	Phenology	F1 generation 5 temperature treatments	Urban earlier reproduction	G & E	Earlier reproduction in colonies from lower latitudes and those reared under warmer temperatures	(Chick et al., 2019)

		Physiology	F1 generations- 3 cities 5 temperature treatments	Higher heat tolerance (2 out of 3 cities)	G & E	Higher fitness under treatment like source environment Higher heat tolerance under warmer rearing temperatures	(Diamond et al., 2018)
		Physiology	F1 generation 2 temperature treatments	Higher plasticity in heat tolerance when reared under warmer temperature	G x E	•	(Diamond et al., 2018)
		Physiology	F1 generation 2 temperature treatments	Higher heat tolerance and lower cold tolerance	G x E		(Diamond et al., 2017)
		Physiology	F1 and F2 generations	Higher heat tolerance and lower cold tolerance	G	Maintained over F1 and F2 so likely not transgenerational plasticity	(Martin et al., 2019)
		Physiology Behaviour	F1 generation	Higher metabolism, and faster running speed at higher temperatures	G x E		(Chick et al., 2021)
Ant (Temnothorax nylanderi)	Forest	Life-history Morphology	F1 generation Cadmium treatment	Higher cadmium resistance	G & E	Difference not observed in field	(Jacquier et al., 2021)
		Life-history Morphology	F1 generation Cadmium treatment	Higher emergence rate and larger worker size when exposed do cadmium	G & E	Cadmium negatively affected life- history traits	(Jacquier et al., 2021)
Common woodlouse (Oniscus asellus)	Rural	Physiology	F1 generation 2 temperature treatments	Higher heat tolerance, but no difference in cold or desiccation tolerance	G & E	Tolerance varies between temperature treatments	(Yilmaz et al., 2021)
		Behaviour	F1 generation 2 temperature treatments	Higher running speed	G x E	Reduced running speed when reared at higher temperatures	(Yilmaz et al., 2021)
Grasshopper (Chorthippus brunneus)	Rural	Morphology Life-history	F1 generation 2 temperature treatments	Longer femur and wing lengths Higher body mass and growth rate in females only	G & E	Rearing temperature affected measured traits, but generally sex dependent	(y Gomez & Van Dyck, 2012)
(Chorthippus biguttulus)	Non- roadside	Behaviour	Nymphs 2 noise treatments	Higher frequency vocal signals and increased syllable to pause ratio	G & E	Higher frequency signals in noisy treatment	(Lampe et al., 2014)
Bend-legged ground cricket (Dianemobius nigrofasciatus)	Rural	Life-history	F2 generation Light and noise treatments	Higher survival and smaller body size, but no other differences	G & E	Light and noise treatments affected growth, diapause, and number of progeny	(Ichikawa & Kuriwada, 2023)

		Behaviour	F1 generation	Higher frequency and shorter chirp vocal signal	G	Vocal differences not more preferred by females	(Kuriwada, 2023)
Synanthropic spider (Steatoda triangulosa)	Rural	Behaviour	Egg-sacs	Lower avoidance of light	G		(Czaczkes et al., 2018)
Fruit flies (Drosophilia tripunctata)	Rural	Physiology	F2+ generation	Higher heat tolerance and lower cold tolerance	G	Sex differences Compare mid and low urbanization	(Diamond et al., 2022)
(Drosophilia suzukii)	Rural	Physiology	F3 generation	Lower thermal limit tolerance, but no difference in heat tolerance	G	Difference not found in wild	(Sato & Takahashi, 2022)
FISH							
Atlantic killifish (Fundulus heteroclitus)	Clean	Physiology	F2 generation	Higher tolerance to pollutants	G	Transcriptional mechanism underlies divergence	(Whitehead et al., 2012)
Creek chub (Semotilus atromaculatus)	Rural	Behaviour	Fry 2 water velocity treatments	Higher swimming efficiency	G & E	Same results in wild Marginal support for G x E for one urban location	(Kern & Langerhans, 2019)
		Morphology	Juvenile 2 water velocity treatments	More streamlined body shape	G	Marginal support for G x E for one urban location	(Kern & Langerhans, 2018)
MAMMALS							
Egyptian fruitbat (<i>Rousettus</i> <i>aegyptiacus</i>)	Rural	Behaviour	Pups	Bolder, faster learners, and less exploratory	E	Cross-fostering revealed that maternal rather than genetic effects may drive differences	(Harten et al., 2021)
PLANTS							
Holy hawksbeard (Crepis sancta)	Rural	Life-history	Seeds	Higher number of non- dispersing seeds	G	Same results in wild	(Cheptou et al., 2008)
		Phenology Morphology Physiology	Seeds	Later phenology, larger, and higher water-use efficiency and photosynthesis	G	Most patterns same direction as selection in urban field site	(Lambrecht et al., 2016)
Common ragweed (Ambrosia artemisiifolia)	Rural	Phenology Life-history	Seeds Urban and rural treatments	Earlier phenology and lower fitness	G & E	Reciprocal transplant Trend for G x E for life-history traits, but not phenology	(Gorton et al. 2018)
		Phenology Morphology	Seeds Germination experiment	Higher percent germination, smaller height, and higher leaf desiccation index	G & E	Other traits, including flowering time, showed no differences	(Kostanecki et al., 2021)
Common milkweed (Asclepias syriaca)	Rural	Life-history Phenology	Seeds	Limited support for differences	Е	Heritable genetic variation and weak phenotypic divergences in wild	(Breitbart et al., 2023)
White clover (Trifolium repens)	Nonurban	Phenology Morphology Life-history	F1 generation	Later phenology, larger flowers and biomass, thinner stolons,	G	Some differences may be driven by pollinators along urban gradient	(Santangelo et al., 2020; see also Thompson et al., 2016)

				reduced cyanogenesis, and greater seed			
Virginia pepperweed (<i>Lepidium virginicum</i>)	Rural	Phenology Morphology Fecundity	Seeds 5 cities	Earlier phenology, taller, and higher fecundity	G	City populations more genetically related	(Yakub & Tiffin, 2017)
Chitinolytic fungi (Chrysosporium pannorum, Trichoderma koningii, Torulmocyes lagena, Penicillium bilaii)	Rural	Physiology	F1 generation 5 temperature treatments	Higher growth rate at higher temperatures	G x E	Evidence for cogredient or countergradient variation in growth rate	(McLean et al., 2005)
Southern crabgrass (digitaria ciliaris)	Farmland	Life-history	Seeds 2 competition treatments	More shoots, shorter, and higher growth rates in high competition treatment	G x E		(Fukano et al., 2020)
Yellow jessamine (Gelsemium sempervirens)	Wild	Morphology	F1 generation	Larger floral traits	G	Patterns in same and opposite direction to those observed in wild	(Irwin et al., 2014)
Asian dayflower (Commelina communis)	Rural	Morphology	Seedlings	Lower leaf number and specific leaf area, no differences in other traits	G & E	Different patterns observed in the wild	(Taichi & Ushimaru, 2024)
Pineappleweed (matricaria discoidea)	Rural	Life-history Morphology	Seeds Temperature treatments Soil treatments	Limited support for differences	Е	Reciprocal common garden as treatments mimicked urban conditions	(Géron et al., 2022)
Common dandelion (<i>taraxacum officinale</i>)	Suburban Rural	Phenology Morphology Physiology	Seeds Herbivore treatment	Reduced early seed production following herbivory	G x E	Increased herbivory resistance after exposure to herbivores	(Pisman et al., 2020)
Thale cress (Arabidopsis thaliana)		Morphology Physiology	Seeds & F1 generation Herbivory treatments	Larger and higher tolerance to caterpillars, but not aphids	G	No wild size difference and higher urban aphid herbivory	(Qu et al., 2022)
		Phenology Life-history	F1 generation Growth treatments Indoor & Outdoor	Several differences including earlier flowering time	G	Genomics identifies set of clonal lineages	(Schmitz et al., 2024)
Reed canary grass (Phalaris arundinacea)	Natural	Physiology Morphology	Seeds Stress treatments	Greater stress tolerance characteristics	G x E	Some traits adaptive in treatments	(Weston et al., 2021)
REPTILES							
Australian water dragons (Intellagama lesueurii)	Semi- natural & natural	Behaviour	F1 generation	Higher boldness, but no difference in exploration or neophilia	G	Boldness only repeatable trait	(Baxter- Gilbert et al., 2019)
Crested anole (Anolis cristatellus)	Forest	Physiology	F1 generation 3 temperature treatments	No difference in heat tolerance	E	Higher urban heat tolerance in wild Higher urban gene expression change after exposed to heat challenge	(Campbell- Staton et al., 2021)
	Natural	Morphology	F1 generation	Longer limbs and more lamellae	G	Same results in wild	(Winchell et al., 2016)

¹Generation measured in the common garden (F1–F3) and, in cases where the F0 generation was used, the life stage individuals were brought to common conditions is shown. In the case of multi-treatment common garden approaches, details on the rearing treatments are also shown. ²Comparitive results using the urban group as the reference category. In the study listed last, urban anoles were found to have longer limbs and more lamellae than natural anoles.

4.2 Methods

4.2.1 Study system and quantifying urbanization

Populations of urban and forest great tits have been monitored at nest boxes in and around the city of Montpellier, France as a part of a long-term study (Charmantier et al., 2017). The forest population has been monitored since 1991 in La Rouvière forest located 20 km northwest of Montpellier where the number of nest boxes (32 mm diameter entrance) ranged from 37–119 because of theft/replacement. The urban population has been monitored since 2011 throughout the city of Montpellier at study sites that differ in their degree of urbanization (163–208 urban nest boxes across 8 study areas). During each spring, nest boxes are visited once per week to follow the reproduction of breeding pairs. We catch adults at nest boxes when nestlings are around 12 days old, ring them with a unique metal band, age them based on plumage as either yearling (born previous year) or adult (born the year before last), take a blood sample, and measure several phenotypes (see "phenotyping" section, Caizergues et al., 2018; Caizergues et al., 2022).

We quantified urbanization at each nest box using impervious surface area (ISA; sealed non-natural surfaces) from the imperviousness density raster dataset from the Copernicus online database (resolution 10 m, tiles: E38N22/E38N23, projection: LAEA EPSG 3035; European Environment Agency, 2020). We calculated the proportion of ISA using 100-meter radius circular buffer around each nest box in QGIS (v3.22.0; QGIS Development Team, 2023) and was a ratio between the number of ISA pixels over the number of pixels contained in the buffer area (range = 0-1, where 1 is all ISA). We used the proportion of ISA at each nest box as a continuous urbanization metric to characterize the territory of breeding wild birds (captured at nest boxes) and the territory of origin for the birds born and raised during the common garden experiment.

4.2.2 Common garden manipulation : egg transfer to wild foster parents

Between April 5 -22, 2022, we collected eggs from urban (N = 50 eggs from 4 sites) and forest (N = 40 eggs from 1 site) populations (Figure 4.1A; Table S4.1; see map in Perrier et al., 2018). We collected three to four unincubated eggs (cold to the touch) from each origin nest box. We insured eggs were unincubated by

³Conclusion of underlying mechanisms responsible for phenotypic differences between populations recorded as either G = genetic differentiation between populations, E = plasticity to environment, G & E = both genetic differentiation and plasticity, and G x E = genetic differentiation in plastic responses to environment.

collecting eggs from nest boxes where we were confident that females had initiated laying within the three to four days before collection and the collected eggs were still covered. We replaced collected eggs with false eggs to encourage the origin female to continue its reproduction and we moved collected eggs into foster nest boxes at our Montpellier Zoo study site where wild females had just commenced incubation (Figure 4.1B). The Montpellier Zoo is considered an intermediate site along our urban gradient because it is natural in its vegetative characteristics, but is exposed to humans and related stimuli (Demeyrier et al., 2016). Often we transferred eggs from their origin to foster nests within 6 hours. In one case, we transferred eggs two days later and we kept these clutches in a dark room and rotated eggs every 12 hours until their transfer (foster ZOO46; Table S4.1). On average, the collected urban eggs weighed; forest: mean = 1.70 g, variance = 0.0075, N = 40 eggs weighed; Welch's t-test: -4.87, df: 52.88, *P* < 0.001; Table S4.1).



Figure 4.1: Procedure of common garden experiment including (A) egg collection, (B) transfer of eggs to wild foster parents, (C) transfer of nestlings to nursery for hand rearing under common conditions, and (D) phenotyping of common garden birds across i) handling aggression, ii) breath rate index, iii) exploration in a novel environment, iv) tarsus length, and v) body mass traits.

Foster nest boxes contained eggs from two origin broods (six to eight eggs total; Table S4.1) and, due to the advanced urban lay dates in our system (urban origin nests laid on average 7.5 days earlier; Table S4.1), we did not mix urban and forest eggs in the same foster broods. The percentage of unhatched eggs was similar across habitat of origin (18% urban and 20% forest). Unhatched forest eggs were all from one abandoned

foster nest (invaded by hornets), whereas unhatched urban eggs were distributed across successful foster clutches (Table S4.1). Of the 90 eggs transferred, we had N = 73 nestlings hatch (41 urban and 32 forest; Table S4.1). We did not have mortality events after hatching or during hand rearing (see next section), so these sample sizes are representative of the number of individuals phenotyped (Table S4.2).

4.2.3 Common garden manipulation : captive rearing

Once nestlings could thermoregulate on their own at 10 days of age (Mertens, 1977), we transferred nestlings to the Montpellier Zoo nursery between April 29–May 16, 2022 (Figure 4.1 C; Table S4.1). Due to advanced urban lay dates in our system, urban nestlings entered captivity on average six days before forest nestlings (urban mean = 126 Julian days, urban range = 119-135; forest mean = 132 Julian days, forest range = 128-136; Table S4.1). Upon arrival, we ringed and weighed nestlings before placing them into artificial nests with their foster broods. We kept them in incubators that mimicked a dim cavity and kept chicks in a quiet environment (one to three broods per incubator; Figure 4.1C.i). At this stage, we handraised nestlings by feeding individuals every 30 minutes between 7:00 and 21:00. Diet consisted of handrearing powder solution (Nutribird A21 and A19, Versele-Laga, Deinze, Belgium), alternating with dead wax moth larvae and live mealworms. Once chicks were 15 days old, the diet was enriched with a cake made of eggs, sunflower margarine, sugar, wheat and protein-rich pellet flours (Country's Best Show1-2 Crumble, Versele-Laga, Deinze, Belgium). Cake was supplemented with commercial powders containing mostly vitamins and minerals (Nutribird A21, Versele-Laga; and Nekton-S, Nekton GmbH, Pforzheim, Germany). Individuals began to "fledge" their brood nests at an age of 18 days. We transferred these individuals in groups of two to three birds into small wire cages in the order of when they fledged (irrespective of sex, foster brood, or habitat of origin), where we trained them to feed by themselves (Figure 4.1C.ii). At this stage, we still fed individuals every 30 mins. Once birds were approximately 23 days old, we transferred them to larger cages (0.8 x 0.35 x 0.4 m) that allowed more movement (hops and flights) in groups of two individuals. At this stage their feeding schedule became less frequent, and birds were considered independent at an age of approximately 35 days. Early June, we transferred individuals to large outdoor aviaries (N = 8; size = $2.2 \times 4.4-5.5$ meters; Figure 4.1C.iii) and individuals were randomly organized into groups blind of habitat of origin and sex (N = 6-10 individuals per group). At this stage birds were eating independently a diet made of cake (see above) and live mealworms. Food and water were provided ad libitum.

All individuals were hand-reared by the same caretakers during the experiment and therefore received the same rearing conditions. Caretakers and experimenters were blind to the origin of the birds and birds from both origins were mixed through all stages of the rearing protocol.

4.2.4 Common garden manipulation : blood sampling and genotyping

We took blood samples from individuals to determine sex and nest of origin to control for genetic relatedness and assign each bird with an ISA of origin. For each foster brood, nestlings had two possible nests of origin from which eggs were collected so parents of nests of origin were also blood sampled and genotyped to assign nest of origin for each common garden bird. We took blood samples the day before birds were transferred to outdoor aviaries and we extracted DNA from these samples using DNeasy blood and tissue kits. Extracted DNA was sent to the Montpellier GenomiX platform (MGX) for RAD sequencing following a similar protocol as described in (Caizergues et al., 2022). We had a total of N = 343 individual samples for sequencing (N = 270 wild and 72 common garden individuals), which generated 10.1 M reads and an average depth of 19.8x per individual using paired-end RAD sequencing (2*150pb). We obtained 185321 SNPs on autosomal chromosome after filtering and we randomly subsampled 600 independent SNPs with a minor Allele Frequency (MAF) of 0.4 to reconstruct the genetic relatedness between all birds (using R package 'Sequoia', Huisman, 2017).

In one foster brood, we could identify relatedness between individuals, but not their origin nest as we were missing information on parental identity (Table S4.1; foster nest ZOO46 which received nestlings from FAC2 and CEF7). As we could not identify the urbanization level of the origin habitat with certainty for these cases, we used the average proportion ISA between the possible origin nest boxes as their urbanization value. Genotyping revealed that the ZOO46 foster nest contained three pairs of siblings (i.e., 6 individuals with 3 unique mothers and fathers). The was presumably because the four eggs collected from CEF 7 origin nest contained two separate pairs of siblings (i.e., 2 individuals with same mother and father and 2 individuals with different mother and father). Therefore, we coded individuals from foster nest ZOO46 as being from three different origin nests to account for relatedness between individuals.

4.2.5 Phentoypic measurements

Phenotypic measurements of common garden birds (Figure 4.1D) followed the same protocols used to phenotype wild birds (Caizergues et al., 2018; Caizergues et al., 2022). Here we examine five phenotypic traits that range across behavioural, physiological, and morphological traits: handling aggression, breath

rate index, exploration in a novel environment, tarsus length, and body mass. We took all phenotypic measures of common garden birds indoors at the nursery, so all individuals were phenotyped under similar conditions (i.e., constant temperature, noise, and light levels). We took measures between 06 June 2022 and 31 January 2023 and all observers were blind to habitat of origin while phenotyping individuals during four separate phenotyping sessions. Following our phenotyping protocol in the wild where birds are mainly measured annually during the breeding season (02 April–16 July), we measured phenotypes at the same time in the following order:

Handling aggression: We measured handling aggression immediately following capture (from nest box in the wild, cage or aviary in common garden) by provoking the bird while holding it pointing away from our bodies (Figure 4.1D.i). We scored their aggressive response between 0 (no reaction) to 3 (tail and wings extended, pecking, and vocalization) on a scale that increased in increments of 0.5 (see Caizergues et al., 2022; Dubuc-Messier et al., (2018) for further details). On average, we measured individuals 1.69 times in the wild (range: 1–8) and 3.89 times in the common garden (range: 1–4; approx. 44 and 264 days old; Table S4.2).

Breath rate index: We placed the bird in a cloth bag and allowed a 5-minute standardized period of rest before measuring its stress response to handling. Once removing the bird from the bag and properly holding the bird (Figure 4.1D.ii), we recorded the time it took for a bird to take 30 breaths (i.e., movements of the breast). We took this measurement twice in immediate succession and took the average between these measures to represent an individual's breath rate index. On average, we measured individuals 1.32 times in the wild (range: 1–6) and 3.89 times in the common garden (range: 1–4; approx. 44 and 264 days old; Table S4.2).

Exploration: We then placed birds into a small compartment next to a novel environment arena where they had a standardized two-minute rest period. We then initiated the novel environment exploration trial by coaxing birds into the novel arena. We recorded their behaviours in this arena for 4 minutes on video then an observer later counted the number of hops and flights birds took while exploring this novel environment (Figure 4.1D.iii). Only one observer scored videos from the common garden (MJT) while multiple observers have scored videos from the wild populations (inter-observer reliability rho > 0.95, including MJT). On average, we measured individuals 1.23 times in the wild (range: 1-4) and 2.89 times in the common garden (range: 1-3; approx. 74 and 264 days old; Table S4.2).

Tarsus length and body mass: Finally, we measured tarsus length (millimetres) with pliers to determine the length between the intertarsal notch and the end of the bent foot (i.e., Svensson's alternative method; (Svensson, 1992); Figure 4.1D.iv) and body mass (grams) using an electronic scale (Figure 1D.v; Caizergues et al., 2021). On average, we measured individuals 1.7 times in the wild (range: 1–8) and 2.92 times in the common garden (range: 1–3; approx. 44-264 days old; Table S4.2).

4.2.6 Statistical analysis

We examined wild and common garden (CG) data using separate Bayesian mixed-effect models since model structures between contexts accounted for different fixed and random effects, while examining a similar main effect of interest (i.e., habitat type; aim 1). All models included habitat type (urban vs. forest) and sex (male and female), and their interaction, as fixed effects in the model to evaluate how phenotypic differences vary across habitats and sexes. If the interaction between habitat and sex was weak and largely overlapped zero, we dropped this effect and refitted the model to evaluate the phenotypic differences between habitats and sexes independently. In subsequent models, we replaced the habitat type effect with proportion ISA to further evaluate changes in the wild and CG phenotypes along a gradient of urbanization.

We have already published results on the phenotypic differences between wild urban and forest populations (Caizergues et al., 2018; Caizergues et al., 2022), but here we report estimates from wild populations that i) include more years of data (3 additional years, year range: 2011–2022) and ii) use data only from the study sites used in our common garden experiment (i.e., 1 forest and 4 urban study sites). All wild models included random effects that accounted for differences between individuals, study sites, and years, and we additionally accounted for differences between observers for handling aggression, breath rate index, and tarsus length (Table 4.2). In addition to examining habitat and sex differences in these models, we also accounted for fixed effects such as time of day, date of measurement, and protocol type following previously established model structures for these traits (outlined by trait below, Table 4.2; Caizergues et al., 2018; Caizergues et al., 2022).

Besides examining habitat of origin effects in common garden models (aim 1), we also explored how different components of genetic and environmental variation shaped traits in the common garden (aim 2). All common garden models included the same random effects (Table 4.2): Individual ID accounted for variance among individuals (V_{ID}), nest of origin ID accounted for variance among origin nests (V_{NO}), and foster nest ID accounted for variance among foster nests (V_{NF}). We also included aviary ID as a random effect that accounted for variation among social groups in the experiment (V_{AV}) for the behavioural and

physiological traits considered (i.e., aggression, breath rate). Since individuals in the common garden experiment were genotyped, we also analyzed common garden data using mixed-effect animal models (Charmantier et al., 2014) by fitting a genetic relatedness matrix (GRM) between individuals in our common garden context (de Villemereuil et al., 2018). Since we collected eggs for the experiment, the nest of origin random effect (V_{NO}) may capture genetic differences between individuals (i.e., whether they are siblings) or differences related to early maternal investment in the eggs. Therefore, the GRM approach allowed us to further evaluate how the variation of each trait was partitioned when accounting for individual genetic variation and nest of origin variation (V_A and V_{NO} , respectively) at the same time. Although there was uncertainty around these variance estimates (i.e., credible intervals close to zero), we present this approach in supplementary materials as it provides additional insights on the minimal contributions from early maternal effects in the experiment (i.e., low V_{NO} estimates, supplementary methods and Table S4.3).

We computed the repeatability (R) of each trait in the common garden experiment as:

$$R_{CG} = \frac{V_I}{V_P} \tag{1}$$

where
$$V_I = V_{ID} + V_{NO} + V_{NF} + V_{AV}$$
 (2)

where
$$V_P = V_I + V_R + V_F$$
 (3)

where V_I is the among-individual variance that comprises variance across individuals (V_{ID}), nests of origin (V_{NO}), foster nests (V_{NF}), and aviaries (V_{AV}). V_P is the total phenotypic variance and includes sources of among-individual variance (V_I), residual variance (V_R) and fixed effect variance (V_F). We included fixed effect variance generated by non-experimental effects in the model that included habitat and sex (de Villemereuil et al., 2018). For comparison, we computed *R* in wild models as:

$$R_{WILD} = \frac{V_{IND}}{V_P} \tag{4}$$

where
$$V_P = V_{IND} + V_{SITE} + V_{YEAR} + V_{OBS} + V_R + V_F$$
 (5)

where V_{IND} is the among-individual variance estimated by individual ID in the model. V_P is the total phenotypic variance and includes variance among individuals (V_{IND}), study sites (V_{SITE}), years (V_{YEAR}), observers (V_{OBS}), and residual variance. We also include variance from habitat, sex, and age fixed effects (V_F) in the total phenotypic variance. For the Poisson models (exploration), we used the QCglmm package (de Villemereuil et al., 2016) to convert the variance components and repeatability estimate from the latent scale to the data scale.

Table 4.2: Summary of 1) wild and 2) common garden model structures that account for different fixed and random
effects. Ranges for continuous fixed effects and number (N) of random effect levels for each trait are shown.

1) WILD MODELS					
	A) Aggression	B) Breath rate	C) Exploration	D) Tarsus length	E) Body mass
	0–3 score	7.7-21.7 sec	0-320 hops	17.32–21.70 mm	14–20.4 g
Fixed effects					
Habitat	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban
Sex	female vs. male	female vs. male	female vs. male	female vs. male	female vs. male
Age	adult vs. yearling	adult vs. yearling	adult vs. yearling	-	adult vs. yearling
Time of day	Range: 7.16-17.13	-	Range: 6.47-16.67	-	Range: 7.17-17.13
Date of measure	Range: 101-196	Range: 108-190	Range: 109-190	-	Range: 101-197
Protocol	-	new vs. old	new vs. old	-	-
Temperature	-	Range: 8-33.7	-	-	-
<u>Random effects</u>					
Individual ID (VIND)	N = 772	N = 531	N = 472	N = 837	N = 810
Site ID (V _{SITE})	N = 5	N = 5	N = 5	N = 5	N = 5
Year ID (V _{YEAR})	N = 12	N = 9	N = 9	N = 12	N = 12
Observer ID (VOBS)	N = 29	N = 12	-	N = 27	-
2) COMMON GARDEN	MODELS				
	A) Aggression	B) Breath rate	C) Exploration	D) Tarsus length	E) Body mass
	0-3 score	8.7-22.3 sec	0-253 hops	17.99-21 mm	12.9-19.3 g
<u>Fixed effects</u>					
Habitat	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban
Sex	female vs. male	female vs. male	female vs. male	female vs. male	female vs. male
Time of day	Range: 7.42-11.53	Range: 7.60-11.97	Range: 7.67-12.02	-	Range: 8.22-11.53
Assay number	1-4 (categorical)	1-4 (categorical)	1-3 (categorical)	-	1-3 (categorical)
Observer ID	1-2 (categorical)	-	-	1-2 (categorical)	-
Random effects					
Individual ID (VID)	N = 73	N = 73	N = 70	N = 72	N = 71
Origin nest ID (VON)	N = 23	N = 23	N = 23	N = 23	N = 23
Foster nest ID (V _{NF})	N = 11	N = 11	N = 11	N = 11	N = 11
Aviary ID (VAV)	N = 8	N = 8	N = 8	N = 8	N = 8

We conducted all analyses in R v4.3.3 (R Core Team, 2024) using Bayesian mixed-effect models in the MCMCglmm package (Hadfield, 2010). We used weakly informative inverse-Gamma priors (V = 1, nu = 0.002) for fixed and random effects. We ran all models for 1000000 iterations, with a thinning of 500 and a burn-in period of 10000, which achieved effective sample sizes > 1000 across all estimates. We verified model fit by visually inspecting histograms and QQPlots of model residuals, and the relationship between the residuals and fitted values. We confirmed convergence of models by visually inspecting trace plots, verifying low autocorrelation, and by using Heidelberg stationary tests (de Villemereuil, 2018).

Aggression in hand: We fit aggression in hand scores as the response variable using Gaussian mixed-effect models. For the wild model, we also controlled for age category (adult vs. yearling), the time of day in

continuous format (minutes divided by 60), and the Julian date of measure (days since Jan 1) as fixed effects. For the CG model, we fit the fixed effects of time of day in continuous format, observer ID, and the assay number. Assay number in the CG combines age effects, date or seasonal effects, and habituation effects simultaneously as these factors are correlated with repeated assays over time in our experiment.

Breath rate index: We fit breath rate index as the response variable in Gaussian mixed-effect models. For the wild model, we also fit age category, Julian date of measure, and protocol type (new vs. old) as fixed effects. Initially breath rate index was measured as the number of breaths for 30 seconds (between 2013-2016) and so we account for this difference in protocol since these initial measures have been converted to approximate the amount of time for 30 breaths (Caizergues et al., 2022). As time of day and temperature were correlated in this dataset ($r^2 = 0.48$, P < 0.001), we chose to only include temperature as an additional fixed effect since this variable explained more variation in this trait than time of day. For the CG model, we fit time of day (as temperature conditions were constant) and the assay number as fixed effects.

Exploration: The number of hops and flights were fit as the response variable in a Poisson generalized mixed-effect model. For the wild model, we fit age category, Julian date of measure, time of day, and protocol type (old vs. new) as fixed effects. For the CG model, we fit time of day and assay number as fixed effects. We excluded three individuals from this analysis; one individual was injured before the exploration assay (forest female) and two individuals did not regrow all their wing feathers after moulting which affected movement during the exploration assay (urban female and forest male).

Tarsus length: Tarsus length was fit as the response variable in Gaussian mixed-effect models. As tarsus length is fixed early in life and should not be affected over time, we controlled for fewer confounding effects for this trait. In the wild models, we did not add additional fixed effects. In the CG model, we included observer as a fixed effect, which accounted for both differences between observer measures and differences between life stages as the first observer measured individuals earlier in life and the other observer past 152 days old. We excluded one urban female from this analysis that was a clear outlier for this trait; this individual was very small and undeveloped at 10 days post hatching when entering captivity (e.g., weight = 4.6 g vs. average weight = 13.6 g).

Body mass: Body mass was fit as the response variable in Gaussian mixed-effect models. For the wild model, we also controlled for age category, date of measure, and time of day as fixed effects. For the CG model, we also included the time of day and assay number as fixed effects. Besides the individual outlier

that we excluded for tarsus length, we also excluded a forest female that was injured before body mass measurements.

4.3 Results

Aggression in hand: We found clear evidence that wild urban birds were more aggressive than wild forest birds with this habitat difference being driven by higher aggression in urban males (habitat * sex effect Table 4.3.1A; Figure 4.2A). Results in the wild were qualitatively similar when examining how phenotypes changed along the urban gradient; there was clear evidence that wild males increased their aggressive responses with increasing proportion ISA, while wild females did not show this same increase in aggression (Table S4.4; Figure 4.3A). There was no clear evidence that this phenotypic difference was maintained in the common garden (weak habitat * sex or habitat effect; Table 4.3.2A; Figure 4.2A). and no clear evidence that handling aggression increased with proportion ISA in the origin habitat (credible interval crosses zero; Table S4.4). Individual ID explained 26% of the variation in aggression in the common garden, nest of origin explained 2%, foster nest explained 2%, and housing aviary explained 1% of the variation (Table 4.3.2A).

Breath rate index: We found weak evidence that wild urban birds had faster breath rates than wild forest birds (6% posterior less than zero; Table 4.3.1B; Figure 4.2B), but clear evidence that breath rate decreased across the urbanization gradient in the wild populations (credible interval excluded zero; Table S4.4; Figure 4.3B). We found clear evidence that this phenotypic difference was maintained in the common garden birds where birds from urban origins had faster breath rate across the urbanization gradient in the common garden birds where birds from urban origins had faster breath rates than birds from forest origins (Table 4.3.2B; Figure 4.2B), but there was no clear change in breath rate across the urbanization gradient in the common garden (credible interval crosses zero; Table S4.4; Figure 4.3B). Individual ID explained 40% of the variation in breath rates in the common garden while origin nest (11%), foster nest (4%), and housing aviary (4%) explained less variation in this trait (Table 4.3.2B).

Exploration: We found clear evidence that exploration in the wild was higher in urban compared to forest birds (Table 4.3.1C; Figure 4.2C) and less clear evidence that exploration increased with increasing urbanization (5% posterior less than zero; Table S4.4; Figure S4.3C). There was no evidence that these differences were maintained in the common garden (credible crosses zero; Table 4.3.2C; Figure 4.2C), and no clear evidence that exploration of common garden birds increased with proportion ISA in the origin habitat (Table S4; Figure 4.3C). Individual ID explained 17% of the variation in common garden

exploration behaviours while origin nest (7%), foster nest (10%), and housing aviary (2%) explained less variation in this trait (Table 4.3.2C).



Figure 4.2: Habitat effect and 95% credible intervals (CI) on phenotypic traits of common garden (blue) and wild (brown) birds across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. Habitat differences varied clearly by sex only in one case (A: aggression in wild birds) and these sex differences are shown (wild males: squares, wild females: triangles); aggression did not differ clearly by sex in the common garden and so the common garden aggression estimate has not been split by sex in A. Phenotypes were measured in the wild annually during the breeding season between 2011- 2022, whereas we measured phenotypes in the common garden between 06 June 2022-31 January 2023.

Tarsus length: We found clear evidence that tarsus length was significantly shorter in wild urban birds than wild forest conspecifics (Table 4.3.1D; Figure 4.2D) and with increasing urbanization in the wild (Table S4.4; Figure 4.3D). In the common garden experiment, this phenotypic divergence was in the same direction as the wild where birds from urban origins tended to have shorter tarsi than birds from forest origins, but we find no clear evidence that tarsus length differed by habitat of origin (credible interval crosses zero). The habitat effect size was slightly weaker in the common garden than the wild (i.e., CG: $\beta_{habitat} = -0.22$, Table 4.3.2D vs. wild: $\beta_{habitat} = -0.29$, Table 4.3.1D), but was stronger when examining how tarsus length decreased over the urban gradient (i.e., CG: $\beta_{ISA} = -0.29$ vs. wild: $\beta_{ISA} = -0.20$, Table S4.4). Out of the random effects considered, individual ID explained 46% of the variation in tarsus in the common garden, followed by origin nest and foster nest which explained 25% and 7% of the variation (Table 4.3.2D).

Table 4.3: Fixed and random model estimates and credible intervals (CI) for 1) wild and 2) common garden contexts across phenotypic traits (A: handling aggression, B: breath rate index, C: exploration, D: tarsus length, and E: body mass). Exploration estimates are from a Poisson generalized mixed-effect model, while all other traits were fit with Gaussian mixed-effect models. Common garden models estimated Individual ID (V_{ID}), origin nest ID (V_{NO}), foster nest ID (V_{NF}), aviary ID (V_{AV}), and residual variance (V_R). The number of observations (obs) and individuals (ind) for each trait and context are shown. Shown in bold are fixed and random effects whose credible intervals exclude zero (or 0.01 for random effects; Bonnet et al., 2022). Computed among-individual variance (V_{IND} & V_I) and repeatability are shown for both contexts for comparison.

1) WILD										
	A) A	ggression	B) E	Breath rate	C)]	Exploration	D) 7	arsus length	E)	Body mass
	N = 130	8 obs, 772 ind	N = 702	2 obs, 531 ind	N = 58	81 obs, 472 ind	N = 14	37 obs, 837 ind	N = 13	75 obs, 810 ind
Fixed effects	Est	CI	Est	CI	Est	CI	Est	CI	Est	CI
Intercept	2.29	1.68-2.84	12.74	10.53-15.19	3.32	0.78-5.97	19.42	19.25-19.58	15.99	15.35-16.65
Habitat (urban)	0.03	-0.3-0.46	-0.70	-1.71-0.35	1.60	0.84-2.2	-0.29	-0.480.11	-0.41	-1.14-0.18
Sex (male)	0.13	-0.04-0.3	0.06	-0.3-0.4	-0.06	-0.47-0.32	0.54	0.48-0.62	0.60	0.5-0.7
Age (yearling)	-0.09	-0.2-0.02	-0.01	-0.32-0.29	0.13	-0.19-0.48			-0.31	-0.390.24
Time of day	-0.04	-0.060.01			-0.01	-0.1-0.07			0.04	0.02-0.06
Date of measure	0.00	0-0	0.00	-0.02-0.01	-0.01	-0.03-0.01			0.00	0-0
Protocol (old)			0.16	-0.39-0.71	0.16	-0.35-0.63				
Temperature			0.09	0.06-0.13						
Habitat * Sex	0.28	0.03-0.53								
Random effects										
Individual ID (V _{IND})	0.43	0.34-0.52	2.61	2.06-3.19	2.73	1.99-3.5	0.28	0.25-0.31	0.39	0.33-0.45
Site ID (V _{SITE})	0.02	0-0.08	0.25	0-0.74	0.08	0-0.3	0.01	0-0.02	0.09	0-0.3
Year ID (V _{YEAR})	0.03	0-0.07	0.05	0-0.19	0.06	0-0.22	0.00	0-0	0.06	0.01-0.13
Observer ID (V _{OBS})	0.04	0.01-0.08	0.52	0.1-1.26			0.00	0-0.01		
Residual variance	0.55	0.48-0.62	1.75	1.4-2.1	1.61	1.08-2.14	0.02	0.02-0.02	0.27	0.24-0.31
R _{WILD}	0.39	0.30-0.46	0.50	0.34-0.60	0.54	0.39-0.66	0.69	0.60-0.74	0.41	0.25-0.49
2) COMMON GARDE	2) COMMON GARDEN									
	A) A	ggression	B) E	B) Breath rate		Exploration	D) Tarsus length		E) Body mass	
	N = 283	5 obs, 73 ind	N = 28	3 obs, 73 ind	N = 2	03 obs, 70 ind	N = 2	11 obs, 72 ind	N = 2	10 obs, 71 ind
<u>Fixed effects</u>	Est	CI	Est	CI	Est	CI	Est	CI	Est	CI
Intercept	2.17	0.98-3.34	9.43	7.21-11.62	4.02	1.91-6.07	19.43	19.1-19.76	15.84	14.71-16.98
Habitat (urban)	0.10	-0.21-0.45	-1.16	-2.320.02	-0.05	-1.06-0.81	-0.22	-0.64-0.17	-0.50	-0.960.03
Sex (male)	-0.19	-0.48-0.07	0.81	0.03-1.6	-0.36	-0.84-0.14	0.46	0.24-0.67	1.03	0.75-1.33
Time of day	-0.03	-0.15-0.08	0.47	0.27-0.67	-0.05	-0.24-0.12			-0.10	-0.2-0.02
Measurement (2)	-0.10	-0.42-0.19	0.94	0.5-1.37	-0.18	-0.67-0.24			0.12	-0.04-0.28
Measurement (3)	0.36	-0.11-0.83	0.50	0.04-0.9	-0.13	-0.58-0.3			0.50	0.37-0.67
Measurement (4)	0.26	-0.21-0.75	-0.38	-0.8-0.02						
Observer (2)	-0.69	-1.120.25					0.07	0.04-0.11		
<u>Random effects</u>										
Individual ID (V_{ID})	0.19	0.08-0.32	2.15	1.1-3.18	0.47	0-0.98	0.18	0.11-0.27	0.26	0.13-0.41
Origin nest ID (V _{ON})	0.02	0-0.07	0.58	0-1.66	0.20	0-0.71	0.10	0-0.22	0.12	0-0.3
Foster nest ID (V _{NF})	0.02	0-0.08	0.15	0-0.68	0.28	0-1.1	0.03	0-0.1	0.04	0-0.13
Aviary ID (V _{AV})	0.02	0-0.07	0.14	0-0.62	0.05	0-0.21				
Residual variance	0.51	0.42-0.61	1.58	1.29-1.87	1.66	1.22-2.17	0.01	0.01-0.02	0.18	0.14-0.23
(V_R)										
$V_{I} (= V_{ID} + V_{NO} + V_{NF} + V_{AV} + V_{F})$	0.23	0.12-0.41	2.92	1.95-4.68	0.91	0.33-2.18	0.30	0.20-0.49	0.40	0.26-0.68
R_{CG}	0.30	0.17-0.45	0.55	0.42-0.69	0.35	0.14-0.55	0.78	0.62-0.91	0.43	0.32-0.60



Figure 4.3: ISA (impervious surface area) effect and 95% credible intervals (CI) on phenotypic traits of common garden (blue) and wild (brown) birds across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. ISA effects varied clearly by sex only in one case (A: aggression in wild birds) and these sex differences are shown (wild males: orange, wild females: red); aggression over the ISA gradient in the common garden did not differ clearly by sex and so the common garden estimate has not been split by sex in A. Phenotypes were measured in the wild annually during the breeding season between 2011–2022, whereas we measured phenotypes in the common garden between 06 June 2022–31 January 2023.

Body mass: We found weak evidence that wild urban birds were lighter than wild forest birds (7% posterior less than zero; Table 4.3.1E; Figure 4.2E), but clear evidence that body mass decreased with increasing urbanization (credible interval excluded zero, Table S4.4; Figure 4.3E). We found clear evidence that common garden birds from urban origins were lighter than birds from forest origins (Table 4.3.2E; Figure 4.2E) despite all the birds being fed *ad libitum*. Results in the common garden across the urban gradient were consistent with this conclusion where the weight of common garden birds decreased with increasing urbanization of the origin habitat (Table S4.4; Figure 4.3E). The variation explained by random effects showed a similar pattern to tarsus length, where individual ID explained 28% variation in common garden body mass, followed by origin nest which explained 12% variation, and foster nest effects which explained 4% (Table 4.3.2D). On average common garden birds were significantly lighter than wild birds, reflecting a captive effect on body mass (Welch's t-test: t = -22.30, df = 272, *P* < 0.001, Figure 4.2E; Figure 4.3E).

4.4 Discussion

Our first aim was to determine whether phenotypic divergences between wild urban and forest populations of great tits were maintained in a common garden experiment, thus providing evidence of genetic differences between these populations. We found evidence that both genetic and plastic changes have contributed to the documented phenotypic shifts in wild urban tits, but the relative contributions of these drivers are trait specific. Specifically, we found that genetic differences between populations likely contributes to breath rate and body mass divergences, and that plasticity to urban conditions contributes to divergences in aggression and exploration. Our second aim was to explore how different sources of genetic and environmental variation shape the phenotypes considered in our experimental context. We found that individual differences tended to explain the most trait variation in the experiment, whereas nest of origin and foster nest variation had minimal contributions.

4.4.1 Support for genetic change

We find clear statistical support that genetic change (or very early maternal investment) drives population divergence in breath rate index as phenotypic differences between birds from urban and forest origins were maintained in our experiment (but note not along the ISA gradient). In line with findings in the wild populations (Caizergues et al., 2022), birds from urban origins had faster breath rates than birds from forest origins. As breath rate index correlates with heart rate under constraint (Dubuc-Messier et al., 2016) and has previously been associated with physiological stress responses in this species (Carere & van Oers, 2004; Krams et al., 2014), our results could indicate that genetic change in urban populations has contributed to a more proactive coping style in urban environments (Koolhaas et al., 2010, 2011). Our results differ from those in urban European blackbirds (*Turdus merula*; Partecke et al., 2006) and juncos (*Junco hyemalis*; Atwell et al., 2012) where lower stress responses were maintained when individuals were reared under common conditions. Indeed, there is no general consensus on how urbanization impacts stress responses in birds (reviewed in Bonier, 2012) and so our results make a useful contribution towards understanding how endocrinology might impact adaptation to urban contexts.

We also find support that being smaller may have a genetic basis in cities. Specifically, birds from urban origins were lighter than birds from forest origins, despite being fed the same diet *ad libitum*. This habitat difference in body mass was statistically clear and higher in the common garden than the wild (i.e., 0.5 g difference in common garden vs 0.41 g difference in wild) supporting a genetic basis for shifts to smaller urban body mass, rather than plasticity to environmental conditions which could possibly reduce this

phenotypic difference in the wild. Lighter urban body mass in the experiment could also be explained by early maternal investment in the egg, especially since the urban eggs collected for the experiment were on average lighter than forest eggs. Since egg size is highly heritable (e.g., egg volume $h^2 = 0.6-0.8$, Hõrak et al., 1995; Van Noordwijk et al., 1981; see also Christians, 2002), and female body size can positively correlate with their egg size in this species (Hõrak et al., 1995) it is possible that genetic differences between females in maternal egg investment could also shape body mass in the wild.

Birds from urban origins also tended to have smaller tarsi than birds from forest origins in line with the phenotypic shift from the wild (Caizergues et al., 2018) but, the wild habitat difference is small and we lack statistical power to make firm conclusions on whether this difference in tarsus length was clearly maintained in the common garden. A post-hoc power analysis suggests we had 48% power to detect the tarsus difference in the common garden and that future common garden efforts would need at least 75 individuals per urban and forest habitat group (cohen's d = 0.46 with 80% power, using package "pwr"). The habitat difference for tarsus length was weaker in the common garden than the wild (i.e., difference of 0.22 mm in common garden vs 0.29 mm in wild) which may indicate that a combination of plastic and genetic effects explain the wild tarsus length difference. Tarsus length and body mass are heritable traits and tend to strongly correlate with each other in this species (Gebhardt-Henrich & Van Noordwijk, 1991; Gosler & Harper, 2000; Hõrak, 1994; Young & Postma, 2023), suggesting that parallel genetic change for tarsus alongside body mass is possible. However, tarsus development is strongly influenced by environmental conditions in early life (Dhondt, 1982; Merilä & Fry, 1998; Talloen et al., 2010) and, urban great tit nestlings have been shown to develop longer tarsi when they are experimentally supplemented with food during growth (Seress et al., 2020). Therefore, we hypothesize that genetic and plastic effects both contribute to smaller tarsus lengths in urban birds. Quantitative genetic approaches using long-term datasets on wild populations, and observed or genetically reconstructed pedigrees, will provide a useful complementary exploration on the underlying drivers behind shifts to smaller urban tarsus lengths. This remains a future research objective for the Montpellier great tit system as quantitative genetic studies of wild populations require large datasets that follow individuals of known relatedness over several generations.

Decreases in traits associated with body size are documented across diverse taxa in cities (Hahs et al., 2023; Merckx et al., 2018) and this phenotypic shift is hypothesized to facilitate heat dissipation and be an adaptive response to rising global temperatures that are pronounced in urban areas via the heat island effect (Sumasgutner et al., 2023; Youngflesh et al., 2022). For example, *Daphnia* from urban origins had smaller body sizes and higher heat tolerance in a common garden experiment than those from nonurban origins, and there was evidence that smaller urban body sizes could indirectly increase heat tolerance in this species

(Brans et al., 2017). Further, city great tits tend to be lighter than forest tits across Europe (Thompson et al., 2022) and, in Veszprem, it has been recently shown that city tits are less affected by extreme temperature than their forest counterparts who experience declines in reproductive success during warmer springs (Pipoly et al., 2022). These results suggest that city great tits could be better adapted to warming conditions and since we find support that smaller urban body mass is driven by genetic change, our results could imply that decreases in urban body size are an adaptive response to heat island effects in cities. Smaller body size does not appear to afford urban great tits in Montpellier reproductive benefits (Caizergues et al., 2018), hence further work will be needed to evaluate whether smaller body sizes, or other correlated traits, are associated with higher survival in urban habitats.

Genetic change between populations that contribute to phenotypic differences can also arise via neutral evolutionary processes like genetic drift or founder effects (Leinonen et al., 2013), and differentiating these processes from local adaptation is informative to evaluate whether populations are adapting in pace with environmental change (de Villemereuil et al., 2020). We found evidence that breath rate and body mass differences are likely driven by genetic change or very early differences in maternal investment, but we are unable to completely dismiss the role of neutral evolutionary processes towards genetic differences between populations. Using a complementary quantitative genetics approach, we estimated higher genetic differences underlying these traits (computed Q_{ST} values in appendix C: 0.11 and 0.12) than could be explained by neutral genetic variation between these urban and forest populations (F_{ST} values between 0.006–0.008; Perrier et al., 2018). However, the high uncertainty around these Q_{ST} estimates (credible intervals crossed 0.006) prevent us from completely excluding neutral evolutionary processes here. In future, rearing individuals across multiple city and forest comparisons in a common garden experiment would further strengthen our evidence against processes of neutral evolution for these traits and possibly demonstrate parallel evolutionary trajectories across multiple city populations.

4.4.2 Support for plastic change

We did not find evidence that genetic change has contributed to urban behavioural shifts as birds from urban and forest origins did not clearly differ in their aggressive or exploratory behaviours in the experiment. It is commonly assumed that urban populations are evolving and adapting to novel urban conditions (Lambert et al. 2021), and behavioural adaptations may be particularly important in this process (Miranda et al. 2013). Alternatively, it has also been argued that phenotypic adjustments through plasticity are probably more frequent (Hendry et al. 2008), especially for behavioural traits (Sol et al. 2013; Caspi et al. 2022). Our results provide support for the latter argument and contrast findings in urban blackbirds and juncos where behavioural differences were assumed to be a result of local adaptation (Atwell et al. 2012; Miranda et al. 2013). We conclude that more aggressive and exploratory behaviours of wild urban great tits are most strongly driven by plastic adjustments to life in cities. Habitat matching behaviours (Edelaar et al. 2008; Camacho et al. 2020) could also explain these urban behavioural differences in the wild. Dispersal dynamics and habitat matching in an urban context are still poorly understood but, as more aggressive and exploratory phenotypes in this urban population do not afford survival or reproductive benefits (Caizergues et al. 2022), habitat matching may be less likely to contribute to these urban phenotypic shifts than plasticity.

4.4.3 Environmental and genetic contributions towards individual phenotypic variation

Our second aim was to investigate how different sources of environmental and genetic variation contributed to repeatable individual differences across traits in our experimental context. The estimated amongindividual variance and repeatability of traits in the common garden were remarkably similar to those estimated in the wild (i.e., similar estimates and overlapping credible intervals). Although credible intervals were overlapping between contexts, we estimated lower among-individual variance and repeatability for aggression and exploration in the common garden compared to the wild. Wild aggression and exploration divergences were not maintained in the common garden, and so it is likely that reduced environmental variation in the experiment decreased individual differences in these traits. Besides underlying genetic differences between populations for breath rate and body mass, trait variation in the experiment tended to be shaped predominately by differences between individuals (i.e., Individual ID). Individual ID in the experiment could comprise both individual-specific genetic and environmentally induced individual differences, and our complementary quantitative genetic analysis suggested that individual genetic variation contributed to individual differences across most traits (i.e., estimates of VA in Table S4.3). Foster nest and aviary variation, which likely explain variation related to early foster brood and social environmental conditions respectively, had minimal impacts on individual differences in our experiment. Specifically, early environmental conditions can affect tarsus development and growth (e.g., Seress et al., 2020), but we found limited support that foster parents and nests contributed to individual differences in tarsus in our experiment. Overall, estimated individual differences were similar between common garden and wild contexts, especially in those traits where we find evidence for underlying genetic differences between populations.

4.4.4 Caveats

Finally, we want to highlight a few caveats that should be considered when interpreting our results. First,

we are unable to fully discount the contribution of very early maternal effects towards the maintained breath rate and body mass differences in the common garden. By collecting unincubated eggs we limited maternal contributions to egg investment, which could influence morphological traits like body mass (Hõrak et al., 1995). Although we found limited (but unclear) support for maternal effects in our experiment (i.e., negligible V_{NO} ; Table S3), our results should be interpreted with this in mind. Second, birds in our experiment were assayed at a relatively young age (between 38 and 261 days old) compared to when they are usually assayed in the wild (73% observations at one year old), which may affect how our common garden estimates compare to our wild populations. However, measuring phenotypic traits earlier in our experiment seemed to have limited impact on results as most common garden phenotypes were similar to wild phenotypes. Body mass in the common garden was the only trait that seemed to differ from the wild. Wild juvenile birds (one year of age) tend to be on average 0.3 g lighter than wild adults in this population, so this may indicate that age could at least partially contribute to the observed difference in mass between contexts. Third, we monitor forest great tits in one larger study site and, although this nonurban area contains different forest types, we lack replication to draw broad inferences on the phenotypes of forest great tits more generally. Finally, rearing individuals under the same restricted and benign conditions (e.g. ad libitum food supply) may have prevented us from detecting phenotypic differences if they are impacted by genetic and environmental interactions (G x E; Conover et al., 2009). For instance, bioenergetic differences between urban and nonurban damselfly (Coenagrion puella) were only revealed in a multi-treatment common garden experiment when individuals were exposed to a simulated heat wave treatment (Tüzün & Stoks, 2021). Although difficult to conduct, multi-treatment common garden experiments where food or temperature are manipulated could be especially valuable for teasing apart G, E, G & E, or G x E acting on phenotypic shifts in urban great tits (see examples in Table 4.1).

4.4.5 Conclusion

In conclusion, our survey of the literature for urban common garden experiments indicates that both plastic and genetic divergences between urban and nonurban animal and plant populations are common. In our study we find evidence that urban phenotypic divergences in stress physiology and morphology are a result of genetic change or very early maternal investment in eggs. Common gardens are not able to affirm local adaptation, unless realistic multi-treatment or reciprocal transplant approaches are used (e.g., Gorton et al., 2018; Tüzün & Stoks, 2021), and evaluating reproductive and survival benefits of our common garden birds in aviaries would not be appropriate. Thus, investigating whether these genetic differences between populations are adaptive remains an avenue for future research. We did not find that genetic change has driven urban behavioural shifts (i.e., more labile traits), which provides contrary evidence to urban common garden studies in other bird species (Atwell et al., 2012; Miranda et al., 2013). Further work will be needed to uncover whether plasticity predominantly drives other urban behaviours in great tits (e.g., neophilia or boldness) and determine the mechanisms underlying discrepancies with other studies. Our results highlight that phenotypic shifts in urban populations can be impacted by both genetic and plastic changes and makes a valuable contribution in filling a fundamental gap concerning the urban evolution of a model species. Examining how evolutionary processes in urban contexts impact phenotypic and genetic variation will have important applications for conserving urban wildlife populations and their ecological roles in communities (Des Roches et al., 2021; Lambert & Donihue, 2020), but will also improve our fundamental understanding of ecology and evolution in wild systems more broadly, especially in light of global environmental change.

4.5 Acknowledgements

We would like to thank everyone who participated in the common garden and is not an author: Laura Gervais, Barbara Class, Dhanya Bharath, Christophe de Franceschi, Sam Perret from the CEFE and Vivian Espinasse, Thibault Pujol, Flavien Daunis, Cathie Troussier, Jérôme Brière, Laetitia Boscardin, Sébastien Pouvreau, Lucas Boussioux, Charlotte Gay, Marion Darde, Alexandre Lhuillier from the Montpellier Zoo. Thanks to Pierre de Villemereuil for useful discussions, coding help, and encouraging us towards a quantitative genetic approach. MJT was supported by a Canadian Graduate Scholarship from the Natural Sciences and Engineering Research Council (NSERC) of Canada, a Fonds de recherche du Québec Nature et technologies PhD scholarship, and a PhD mobility grant from le Centre Méditerranéen de l'Environnement et de la Biodiversité (CeMEB). This project was funded by the Agence Nationale de la Recherche (URBANTIT grant ANR-19-CE34-0008-05 to AC), the OSU-OREME, and a Fonds de Recherche du Quebec Nature et Technologie to DR.

CHAPITRE 5

City tit wit: cognitive variation in wild and common garden contexts

Authors: Megan J. Thompson, Laura Gervais, Dhanya Bharath, Samuel P. Caro, Charles Perrier, Denis Réale, and Anne Charmantier

In response to accelerating urbanization worldwide, the phenotypes of urban wildlife populations are changing. Phenotypic shifts in cognition, specifically, could ease adjustments to life in cities and, if these cognitive shifts result from genetic change, cognitive abilities could facilitate adaptation and evolution in urban environments. The genetic basis of cognition, especially in wild contexts, is not well studied and so there is a major knowledge gap concerning the role of cognition in adaptation to novel contexts. We evaluate cognitive variation in inhibitory control (i.e., the ability to inhibit prepotent responses) in wild great tits (*Parus major*; N = 393) along an urban gradient, and determine the genetic and environmental drivers of cognitive variation among urban and forest tits (N = 73) raised in a common garden experiment. We examine cognitive performance related to inhibitory control using a modified motor detour task and find that wild tits in more urbanized habitats make significantly fewer errors during the task than tits from less urbanized habitats, but we do not find that urbanization significantly affects the latency to complete the task in the wild. When examining performance related to inhibitory control in the common garden experiment, we find that habitat of origin does not affect the number of errors or latency to complete the task. Our results suggest that wild urban great tits are better able to inhibit prepotent responses than forest tits but, rather than having a genetic basis, that these abilities are driven by plasticity or experience in urban habitats. Although higher inhibitory control could facilitate adjustments to urban environments, we show that the evolutionary potential of this cognitive ability in an urban population may be limited.

Keywords: urbanization, individual variation, phenotypic variation, inhibitory control, common garden experiment, great tit

5.1 Introduction

Cities are expected to expand significantly in the coming decades (United Nations, 2019). This rapid expansion of urbanization is a process of novel environmental change that is transforming the habitat of wild populations worldwide by, for instance, increasing impervious surfaces, environmental pollution (chemical, light, sound), and introducing novel species and resources (Szulkin et al., 2020). Wild populations occupying urban environments need to adjust or adapt to these novel urban conditions to persist in these areas, and indeed phenotypic changes in urban populations are common and taxonomically widespread (Diamond & Martin, 2021; Johnson & Munshi-South, 2017; Lambert et al., 2021). For example, in comparison to their nonurban counterparts, some urban species are smaller (Hahs et al., 2023), less fearful of humans and associated stimuli (Geffroy et al., 2020), and behaviourally more aggressive (Miranda et al., 2013). Shifts in cognitive traits associated with the collection, storage, and use of environmental information could be especially important for facilitating adjustments to novelty and adaptation to urban ecological contexts (Lee & Thornton, 2021; Sol et al., 2020).

Wildlife populations in cities are confronted with novel opportunities and challenges that are usually very different from those in other environments, and successful urban organisms tend to be those that learn to exploit new resources and avoid harmful threats (Sol et al., 2013). There are several historical and modern examples of organisms innovating and exploiting new urban resources, including UK great tits (Parus major) opening glass milk bottles (Fisher & Hinde, 1949) or Australian sulphur-crested cockatoos (Cacatua galerita) opening household waste bins (Klump et al., 2021). Some cognitive traits can reduce the risk of population extinction (Ducatez et al., 2020) and, specifically, learning, problem solving, and decisionmaking could be especially important for adjusting to novel conditions in cities (Griffin et al., 2017; Lee & Thornton, 2021; Sol et al., 2020). Previous studies show that urban species and populations tend to have higher cognitive performance, especially on innovation, problem solving, or foraging related tasks, but high heterogeneity in results among few studies prohibits broad generalizations on this conclusion (Sol et al., 2020; Vincze & Kovács, 2022). Shifts in cognitive abilities could theoretically facilitate or inhibit adaptive evolution in urban environments by, for example, exposing populations to novel selection pressures or weakening the strength of selection (reviewed in Sol et al., 2020). At present, however, a major fundamental knowledge gap exists on the genetic basis of cognition and the potential for cognitive traits to evolve in wild populations.

Evaluating the evolutionary potential of cognitive traits requires examinations of the fitness consequences and genetic basis of these traits (Morand-Ferron et al., 2016). In wild birds higher cognitive performance

on reversal learning, problem solving, and spatial memory tasks is associated with fitness benefits (Cauchard et al., 2012; Cole et al., 2012; Sonnenberg et al., 2019), but also fitness costs (i.e., reversal learning; Madden et al., 2018). Evolutionary change in response to natural selection requires phenotypic variation to comprise underlying genetic variation and, to date, most estimates of heritability in cognition come from human or captive populations (Croston et al., 2015). Few studies have examined the heritability of cognitive traits in wild populations and, so far, heritability estimates range from high (Branch et al., 2022), to moderate (Langley et al., 2020), to low (De Meester et al., 2022; McCallum & Shaw, 2023; Quinn et al., 2016; Van Den Heuvel et al., 2023). For example, wild toutouwai (North Island robin; *Petroica longipes*) in New Zealand showed repeatable individual differences in inhibitory control (i.e., the ability to inhibit predominant responses) over a one year period, but the estimated heritability of this cognitive trait was low and non-significant suggesting this trait is shaped strongly by environmental effects (McCallum & Shaw, 2023). Despite growing evidence that the cognitive traits of urban organisms differ from their nonurban conspecifics (Lee & Thornton, 2021; Sol et al., 2020), no studies have evaluated the evolutionary potential of cognition in urban populations. Recently, Sol et al. (2020) called for more studies that examine cognition under an urban evolution lens and, specifically, highlighted the usefulness of common garden experiment.

We first aimed to compare inhibitory control between wild urban and forest populations of great tits (Parus *major*) in and around the city of Montpellier, France using a modified motor detour task that is easily administered in the field. Inhibitory control, or the ability to inhibit prepotent responses (Kabadayi et al., 2018; MacLean et al., 2014), is a cognitive trait that has been suggested to affect several fitness-related behaviours including foraging flexibility (Coomes et al., 2021), dietary choices (van Horik et al., 2018), premature attacks on prey (Miller et al., 2019), or conspecific resource sharing (MacLean et al., 2014). Recent studies on the genetic basis and evolutionary potential of inhibitory control in wild or recently wilddescended populations have found low to moderate heritability estimates for this trait (e.g., Langley et al., 2020; McCallum & Shaw, 2023; Prentice et al., 2023). Environmental variation can also affect inhibitory control when, for example, unpredictable environmental conditions during development increase inhibitory control performance (van Horik et al., 2019) and heat stress and traffic noise decrease performance (Blackburn et al., 2022; Soravia et al., 2023; Templeton et al., 2023). The ability to inhibit predominant responses when confronted with novel resources or challenges could play an important role in how organisms adjust to urban environments (Lee & Thornton, 2021), but currently no studies have compared how these abilities differ between wild urban and nonurban populations. Since urban animals tend to have higher cognitive performance than their nonurban conspecifics (e.g., problem solving; Vincze & Kovács, 2022), we hypothesize that wild urban great tits will have higher inhibitory control than forest tits. More

specifically, we predict that urban great tits will make fewer errors and reach the goal more quickly in a motor detour task presented in the field.

Our second aim was to evaluate the genetic basis of inhibitory control, so we further compared this cognitive trait between urban and forest great tits reared in a common garden experiment. Common gardens rear individuals from different populations under the same environmental conditions from very early life stages and assume that phenotypic differences that persist in this context are driven by underlying genetic differentiation, rather than plasticity to environmental conditions (Lambert et al., 2021). Common gardens have revealed genetic differences between urban and nonurban populations for physiological, morphological, and behavioural traits (e.g., Brans et al., 2018; Diamond et al., 2018; Partecke et al., 2006; Winchell et al., 2016), but urban cognition is yet to be examined in common garden experiments. Since inhibitory control can be heritable (Langley et al., 2020; McCallum & Shaw, 2023; Prentice et al., 2023), we hypothesized that genetic change and possible evolution may drive higher inhibitory control in urban populations. Specifically, we predicted that tits from urban origins would have higher cognitive performance on a motor detour task (i.e., fewer errors and faster latency to goal) than tits from forest origins, in line with the phenotypic divergence observed in the wild.

5.2 Methods

5.2.1 Study system

We monitored the occupation and reproduction of great tits at nest boxes in and around the city of Montpellier as a part of a long-term study (Charmantier et al., 2017). The forest population is located 20km northwest of Montpellier in La Rouvière forest (monitoring initiated in 1991; between 37–119 nest boxes with 32 mm diameter entrance) and the urban population is monitored across eight study sites throughout the city of Montpellier that differ in their degree of urbanization (monitoring initiated in 2011; between 163–208 nest boxes). Fluctuations in nest box numbers between years were a result of either theft or altering the proportion of large and small diameter nest boxes (28 vs 32 mm) to accommodate changing research objectives; the latter being more common in La Rouvière forest where blue tits are continuously monitored. During the Spring breeding season, we visited each nest box weekly to follow the reproduction of tits. When nestlings were approximately 12 days old, we caught parents in the nest boxes to ring them with a unique metal band, age them based on plumage (adult: 2+ year old, juvenile: 1 year old), take a blood sample, and measure several morphological and behavioural phenotypes (for more information see Caizergues et al.,

2018; Caizergues et al., 2022). During the 2021–2023 breeding seasons, we also assayed individuals on a cognitive task in the field (see 'Cognitive assay' section below).

We quantified the level of urbanization at each study site using the proportion of impervious surface area (ISA; sealed non-natural surfaces including sidewalks, roads, and buildings). We used the imperviousness density dataset from the Copernicus online database (resolution 10m, tiles: E38N22/E38N23, projection: LAEA EPSG 3035; European Environment Agency, 2020) and quantified the number of ISA pixels within a 100-m-radius circular buffer around each nest box in QGIS (v 3.220; QGIS Development Team, 2023). We computed the proportion of ISA by dividing the number of ISA pixels in this buffer by the total number of pixels (range: 0-1, where 1 = all ISA). We averaged the proportion ISA across nest boxes for a given study site to obtain a continuous urbanization metric at the site level (urban mean: 0.50, urban range: 0.21-0.98, forest mean: 0, forest range: 0-0).

5.2.2 Common garden

During the spring of 2022, we conducted a common garden experiment by collecting eggs from the urban and forest great tit populations and rearing nestlings under common conditions. We collected unincubated eggs that were covered and cold to the touch from urban and forest study sites between April 5–22 (N = 50 urban eggs from 4 sites, N = 40 forest eggs from 1 site). We collected between three and four eggs from each origin nest box (N = 23 origin nests) and transferred these eggs to wild foster nests at the Montpellier Zoo where females had just initiated incubation (N = 11 foster nests). The Montpellier Zoo is an established urban site with low to moderate levels of urbanization (average proportion ISA at 100m: 0.21) and is exposed to humans and associated stimuli. Foster nests contained eggs from two nests of origin (between six-eight eggs total) and, since urban origin lay dates tended to be earlier than forest ones (average 7.5 days earlier), we did not mix urban and forest eggs in the same foster brood. Of the 90 eggs transferred to foster nests, we had 73 nestlings hatch (N = 41 urban and 32 forest) that remained in foster nests until 10 days of age when they could thermoregulate on their own. We transferred 10-day old nestlings to captivity at the Zoo Nursery between April 29 and May 16 where they were ringed with a unique metal band and reared under the same captive conditions. Further information on origin and foster nest procedures can be found in Chapter 4 methods and Table S4.1.

We initially reared nestlings in artificial nest boxes (i.e., open wooden boxes) that were kept in incubators to mimic a dark cavity (one to three broods per incubator). We fed nestlings every 30 minutes between 7:00 and 20:00 on a diet that consisted of hand-rearing powder solution (Nutribird A21 and A19, Versele-Laga,

Deinze, Belgium), dead wax moth larvae, and live mealworms. At 15 days old, nestling diets were enriched with a cake made of eggs, sunflower margarine, sugar, wheat and protein-rich pellet flours (Country's Best Show1-2 Crumble, Versele-Laga, Deinze, Belgium) that was supplemented with commercial powders containing mostly vitamins and minerals (Nutribird A21, Versele-Laga; and Nekton-S, Nekton GmbH, Pforzheim, Germany). At approximately 18 days old, individuals began to "fledge" their captive nests and started flying around the nursery. We transferred these fledglings in the order they fledged to wire cages (two to three individuals per cage irrespective of habitat origin, foster brood, sex) where we started to train individuals to feed by themselves. We initially fed individuals every 30 minutes, but at approximately 23 days old feeding became less frequent (every hour, then every three hours) and individuals had access to food *ad libitum* in their cages. We considered birds independent at approximately 35 days old and in early June we transferred all individuals to large outdoor aviaries in randomly assigned groups of six to eight birds blind to habitat of origin, foster brood, or sex. Birds were eating independently at this stage on a diet made of cake (see above) and live mealworms. Food and water were provided ad libitum. All common garden birds were reared by the same caretakers during the experiment that were blind to habitat of origin as birds from urban and forest habitats were mixed throughout captive rearing. Temperature (25°C) and humidity (60%) was kept constant through rearing. See Chapter 4 methods for more specific information on the rearing protocol.

5.2.3 Genotyping

We took blood samples from each individual so we could determine their relatedness and exact nest of origin. This was necessary as foster broods contained eggs from two origin nests from the same habitat type and, apart from knowing whether individuals came from urban or forest habitats, we did not know the exact origin nest or relatedness of individuals after they hatched. We took blood samples a day before individuals were transferred to outdoor aviaries and we extracted DNA from these samples using DNeasy blood and tissue kits. DNA extracts were sent to the Montpellier GenomiX platform (MGX) for RAD sequencing following a similar protocol as described in (Caizergues et al., 2022). We included N = 343 individual samples for sequencing, including 270 and 73 samples for wild and common garden individuals, respectively. Paired-end RAD-sequencing (2*150pb) generated 10.1 M reads and an average depth of 19.8x per individual before filtering. We obtained 185321 SNPs on autosomal chromosomes after filtering, and we randomly subsampled 600 independent SNPs with a minor Allele Frequency (MAF) of 0.4 to reconstruct the genetic relatedness between all birds. We could determine the nest of origin of individuals and the corresponding proportion ISA of their origin study sites (continuous urbanization metric, see 'Study system' section) using genetic relatedness between individuals. In one foster brood, we could determine relatedness

among individuals (i.e., whether they were siblings) but not their exact nest of origin because we were missing information on parental identity from origin nests. For these individuals where we lacked information, we averaged the proportion ISA between the two possible urban origin sites.

5.2.4 Cognitive assay

We used the same cognitive assay to measure and compare cognition in both wild and common garden contexts. We designed a field assay similar to a motor detour task to evaluate inhibitory control and we administered this cognitive assay just before releasing individuals. Motor detour tasks have previously been adapted and administered successfully in the field in great tits, and it was found that individual performance was repeatable across the modified field task and the classic "cylinder task" in captivity (Davidson et al., 2022). We used a different field task that required individuals to escape a cage (i.e., the goal; Figure 5.1) by inhibiting their predominant impulse to hit a transparent barrier to reach the exit in front of them and instead move laterally around the barrier to reach the exit. We modeled the modified detour task after classic tasks used to measure inhibitory control but, unlike previous approaches, we did not have a habituation or training period. Thus, our task measures the ability of individuals to escape a challenging situation and performance during the task likely reflects processes related to inhibitory control, exploration, and stress sensitivity.



Figure 5.1: Cognitive assay used to measure inhibitory control in wild and common garden contexts. A) Side view of the cognitive assay cage in the field, B) diagram showing assay components from above including a possible route to the exit in red, and C) a video clip showing how the cognitive task was analyzed (note bird detouring barrier on right).

We first placed individuals in an enclosed opaque plastic tube on the outside of the task to standardize their position before the assay and to give them one minute of standardized rest in the dark (Figure 5.1A). We then opened a sliding door entrance that allowed access between the tube and the cage. In most cases, the bird entered the cage immediately and, in rare events where the bird did not enter, the observer would gently tap the end of the tube to encourage the individual into the cage. Upon entering, individuals were presented with an opening in the cage directly in front of them, but their direct path to this exit or goal was blocked

by a transparent plexiglass barrier (Figure 5.1B). To escape the cage (goal of the task), individuals needed to inhibit their predominant impulse to hit into the clear barrier and instead make a lateral motor detour towards gaps located at the side of the barrier to reach the exit. We allowed individuals up to 180 seconds to escape the cage before ending the trial and coaxing the bird to the exit.

We video recorded cognitive trials from above the cage (Figure 5.1C) that we later scored to extract two variables: the number of errors and the latency to escape. We recorded the number of errors as the number of hits an individual made into the transparent barrier during the trial before escaping (wild mean: 9.6 hits, wild range: 0–130; common garden mean: 4.9 hits, common garden range: 0-35). The average number of errors on the task was significantly lower in the common garden than in the wild (Welch's t-test: t = 5.9, df = 553, P < 0.001). We also recorded the latency for individuals to escape the task as the amount of time in seconds from when >50% of the individual's body entered the cage to the time when >50% of the body moved past the clear transparent barrier (wild mean: 22.81 sec, wild range: 0–180; common garden mean: 62.55 sec, common garden range: 0-180). The average latency to escape the cage was significantly higher in the common garden than in the wild (Welch's t-test: t = -7.3, df = 255, P < 0.001). These latency values were highly correlated when instead considering the latency to exit the cage (wild rho = 0.99 and common garden rho = 0.91) but, as there were occasional instances where a bird would perch at the exit before leaving, we decided to use the time to the barrier to measure latency to escape. Videos were scored by two observers (DB: wild 2021; MJT: wild 2022-2023 and common garden) who had high inter-observer reliability (rho > 0.97) for both traits on a set of 10 practice videos before initiating video analysis. We compared individual performance between our field detour task and the classic cylinder task administered in captivity in a subset of individual great tits from another study population in Moulis, France (Crouchet, 2023). The latency to escape, but not the number of errors, was significantly correlated between our field task and the classic cylinder task suggesting that inhibitory control affected part of the response of the birds to our field assay (N = 15 individuals, latency: $r^2 = 0.52$, P = 0.048; errors $r^2 = -0.19$, P = 0.50).

In both wild and common garden contexts, we administered this assay after conducting a standardized phenotyping protocol where we measured other behavioural and morphological traits (see methods in Chapter 4; Caizergues et al., 2018; Caizergues et al., 2022). In the wild, we conducted the assay in a location close to the nest box where individuals were captured. We placed the cage in a location facing away from roads or sidewalks in the wild, and the cage was positioned away from the sun to avoid reflections on the plexiglass window that could affect the bird's response. In the common garden, we captured individuals for phenotyping using mist nests and we conducted the cognitive assay in the aviary where individuals were being housed. To separate the focal individual, visually and physically, from individuals that had already

been released in aviaries, we used a thin sheet above the assay in the aviary that allowed light to pass through (similar to tree cover) and shaded the cage from the sun. Of the total individuals assayed (N = 393 wild and 72 common garden), N = 14 wild and 26 common garden individuals (4 and 36%, respectively) did not complete the task in 180 seconds in their first trial. In the common garden, we assayed individuals repeatedly and this improved to N = 5 individuals (8%) by the third trial.

5.2.5 Statistical approach

We evaluated differences between urban and forest tits in their cognitive performance (number of errors and latency to escape) in both wild and common garden contexts using frequentist mixed-effect models in R v4.3.3 (R Core Team, 2024). We analyzed wild and common garden data in separate models as they required different model structures and so we present four main models where we examine the two traits of interest in both contexts.

5.2.6 Statistical approach : errors

We evaluated the number of errors for individuals that successfully escaped the task within the 3-minute period. The number of errors was right-skewed and overdispersed due to a few large error values in both contexts (Figure S5.1A&B). We therefore analysed the number of errors during the task using negative binomial generalized mixed-effect models with the package glmmTMB (Brooks et al., 2017). In the wild model, we tested whether there were differences between urban and forest birds in the number of errors they made by fitting habitat type (forest vs. urban) as a fixed effect. We also included trial (1–3, categorical), sex (female vs. male), and age (adult vs. juvenile), and their interactions with habitat type in the full model to determine whether there were different impacts across trials, sexes, or ages depending on habitat type. We also accounted for the date of testing (in Julian days since Jan 1), the time of day (continuous format: minutes divided by 60), year (2021–2023), and whether birds had a blood sample taken before testing (yes vs. no). We included individual ID and study site ID as random effects since some individuals had multiple trials and individuals were grouped within sites. Since we had few individual repeated measures in the wild, we evaluated the robustness of our results by using only the first trial of the test across individuals in a subsequent model. We used the same model structure and approach, but this model did not include trial as a fixed effect or individual ID as a random effect since there was only one trial per individual.

In the common garden model, we included habitat type as a fixed effect so we could determine whether birds from urban and forest origins differed in the number of errors during the task. We included trial number (1-3, categorical) and sex (female vs. male) as fixed effects, and their interaction with habitat type to evaluate differential effects across habitat of origin. We also accounted for time of day (continuous format) as a fixed effect. We included the following random effects : nest of origin ID accounted for variance among origin nests (V_{NO}), Individual ID accounted for variance among individuals (V_{ID}), foster nest ID accounted for variance among social groups (V_{AV}).

We determined the significance of fixed effects using type II (non-interacting terms) and type III ANOVAs (interacting terms) in the car package (Fox et al., 2010); we used Wald chi-square tests as recommended for non-gaussian models (Bolker et al., 2009). We determined the significance of random effects using likelihood ratio tests by comparing models with and without the effect of interest. If interactions were not significant in the full model, we refit the model to exclude them so we could evaluate non-interacting effects independently. In an additional model, we substituted the habitat type effect with the proportion ISA so we could evaluate how the number of errors changed across the continuous urbanization gradient. We verified model fit by visually inspecting histograms and QQ plots of model residuals, and the relationship between the residuals and fitted values with the DHARMa package (Hartig, 2019).

5.2.7 Statistical approach : latency to escape

The latency to escape the task was also right skewed with most individuals taking less than 50 seconds to escape and a few taking more time (Figure S5.1B&D). Since some individuals did not escape the task and were given a ceiling value of 180 seconds, there was also a small peak in observations on the right extreme of the distribution (especially for common garden birds). To include all successful trials of birds, whether they reached the goal or not during the study period, we applied cox proportional hazards mixed-effect models (using package 'coxme' and 'survival'; Therneau, 2024). These models do not make assumptions about the underlying distribution of the response variable and are used for semi-parametric survival analysis where an event of interest may not occur during a defined study period (Therneau & Grambsch, 2000). We do not know whether individuals would have eventually escaped the task if given more time and so this approach allowed us to include all individuals whether they completed the task or not. This approach has previously been applied to evaluate latency to the reward in problem-solving tasks in other species (Cook et al., 2017; Papp et al., 2015; Prasher et al., 2019; Sol et al., 2011).

We used the latency to escape the task as the response variable and informed the model of whether these values were censored (max 180 seconds) or not. The model estimates the probability of not escaping the cage as a function of time, with positive effect sizes indicating a higher probability of not escaping. In the

wild and common garden models, we included the same fixed and random effect structures used to evaluate the number of errors in both respective contexts. We also followed a similar approach where interactions were tested first and dropped if non-significant, and we replaced the habitat type effect with the proportion ISA in subsequent models. We also examined the latency to escape in the wild using data only from an individual's first trial to evaluate the robustness of our results. We evaluated model fit by testing that the global model and each predictor followed the assumption of proportional hazards (i.e. random non-significant pattern of Schoenfeld residuals across time; function 'cox.zph') and by visually inspecting the DFBETA residual plots (all residuals <1; Xue & Schifano, 2017).

5.3 Results

5.3.1 Number of errors

In the wild populations, there was a non-significant tendency for urban birds to make fewer errors than forest birds (Figure 5.2A; Table 5.1.1A; P = 0.09). The effect of urbanization became significant when examining how the number of errors changed along the urbanization gradient where birds made significantly fewer errors with increasing proportion ISA (Figure 5.2B; Table S5.1.1A, but note significant ISA*trial effect). These results were qualitatively similar when evaluating the number of errors made during the task in an individual's first trial only (Table S5.2A). The number of errors did not significantly differ across trials (Table 5.1.1A; Figure 5.3A). Individual ID explained significant variation in the number of errors, but variance among sites was negligible (Table 5.1.1A, Table S5.1.1A).

In the common garden, the number of errors during the task was not affected by the origin habitat or level of urbanization (i.e., non-significant habitat or ISA effects; Figure 5.2A&B; Table 5.1.2A; Table S5.1.2A). The number of errors changed over trials (Table 5.1.2A) where individuals made significantly fewer errors as the trials progressed. Although there was no significant difference in performance over trials by habitat of origin (i.e., non-significant habitat*trial effect), the decline in errors over trials tended to be more apparent in urban than forest birds (Figure 5.3A). Apart from individual ID, which explained a marginally non-significant amount of variation (P = 0.07), random effects explained negligible variation in the number of errors (Table 5.1.1A, Table S5.1.2A).

Table 5.1: Model estimates (effect sizes and variance), standard error (SE), and P-values (P) for fixed and random effects from separate 1) wild and 2) common garden contexts evaluating the A) number of errors and B) latency to escape in a motor detour task. The number of errors was fit with negative binomial generalized linear mixed-effect models and the latency to escape was fit with cox proportional hazards mixed-effect models. Model estimates in B represent the probability of not escaping the cage as a function of time, with positive effect sizes indicating a higher probability of not escaping. The number of observations (obs), individuals (ind), and repeated individual measures are shown for each context and trait. The latency to escape the task included all individuals tested while we only included individuals that successfully escaped the task to evaluate the number of errors, so differences in observations and individuals between A and B reflect this.

1) WILD	A) ERRC N = 442 obs, 38	PRS 30 ind		B) LATENCY N = 456 obs, 393 ind			
	(50 ind–2 trials, 6 ind–3 trials) ((63 ind-2 trials, 10 ind-3 trials)			
Fixed effects	Estimate	SE	Р	Estimate	SE	Р	
Intercept	2.06	0.89					
Habitat (urban)	-0.21	0.12	0.09	-0.20	0.11	0.08	
Sex (male)	0.10	0.12	0.41	-0.13	0.10	0.20	
Age (juvenile)	0.08	0.12	0.52	-0.08	0.11	0.46	
Julian date	0.00	0.01	0.38	0.01	0.00	0.19	
Time of day	-0.03	0.03	0.32	0.07	0.03	0.02	
Year (2022)	-0.10	0.12	0.45	0.28	0.12	0.02	
Year (2023)	-0.09	0.15	0.54	0.12	0.14	0.39	
Blood (yes)	-0.30	0.17	0.07	0.11	0.16	0.47	
Trial (2)	0.21	0.17	0.23	-0.02	0.17	0.90	
Trial (3)	-0.33	0.37	0.37	0.47	0.35	0.18	
Random effects							
Individual ID ($N = 393$)	0.42		< 0.001	< 0.001		0.97	
Site ID $(N = 9)$	0.014		0.99	< 0.001		0.96	
2) COMMON GARDEN	N = 153 obs, 72	2 ind		N = 203 obs, 7	72 ind		
2) COMMON GARDEN	N = 153 obs, 72 (70 ind-2 trials)	2 ind 61 ind-3	trials)	N = 203 obs, (54 ind-2 tria	72 ind ls, 32 ind—	3 trials)	
2) COMMON GARDEN Fixed effects	N = 153 obs, 72 $(70 ind-2 trials,)$ Estimate	2 ind 61 ind–3 SE	trials) P	N = 203 obs, (54 ind-2 tria Estimate	72 ind ls, 32 ind– SE	3 trials) P	
2) COMMON GARDEN Fixed effects Intercept	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01	2 ind 61 ind–3 SE 0.91	trials) P	N = 203 obs, (54 ind–2 tria Estimate	72 ind ls, 32 ind– SE	3 trials) P	
2) COMMON GARDEN <i>Fixed effects</i> Intercept Habitat (urban)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11	2 ind 61 ind-3 SE 0.91 0.22	trials) P 0.61	N = 203 obs, (54 ind-2 tria Estimate -0.07	72 ind ls, 32 ind– SE 0.19	3 trials) <u>P</u> 0.72	
2) COMMON GARDEN <i>Fixed effects</i> Intercept Habitat (urban) Sex (male)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19	2 ind 61 ind–3 SE 0.91 0.22 0.20	trials) P 0.61 0.36	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25	72 ind ls, 32 ind– SE 0.19 0.19	3 trials) P 0.72 0.19	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06	2 ind 61 ind–3 SE 0.91 0.22 0.20 0.09	trials) P 0.61 0.36 0.49	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25 0.09	72 ind ls, 32 ind– SE 0.19 0.19 0.11	3 trials) P 0.72 0.19 0.39	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day Trial (2)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06 -0.26	2 ind 61 ind–3 SE 0.91 0.22 0.20 0.09 0.21	trials) P 0.61 0.36 0.49 0.23	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25 0.09 0.31	72 ind ls, 32 ind– SE 0.19 0.19 0.11 0.21	3 trials) P 0.72 0.19 0.39 0.14	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day Trial (2) Trial (3)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06 -0.26 -0.26 -0.44	2 ind 61 ind-3 SE 0.91 0.22 0.20 0.09 0.21 0.22	trials) P 0.61 0.36 0.49 0.23 0.04	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25 0.09 0.31 1.14	72 ind ls, 32 ind– SE 0.19 0.19 0.11 0.21 0.25	3 trials) P 0.72 0.19 0.39 0.14 < 0.001	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day Trial (2) Trial (3) Random effects	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06 -0.26 -0.44	2 ind 61 ind-3 SE 0.91 0.22 0.20 0.09 0.21 0.22 0.22	trials) P 0.61 0.36 0.49 0.23 0.04	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25 0.09 0.31 1.14	72 ind ls, 32 ind– SE 0.19 0.19 0.11 0.21 0.25	3 trials) P 0.72 0.19 0.39 0.14 < 0.001	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day Trial (2) Trial (3) Random effects Individual ID (N = 72)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06 -0.26 -0.26 -0.44	2 ind 61 ind–3 SE 0.91 0.22 0.20 0.09 0.21 0.22	trials) P 0.61 0.36 0.49 0.23 0.04 0.07	N = 203 obs, 7 (54 ind-2 tria Estimate -0.07 -0.25 0.09 0.31 1.14 0.17	72 ind 1s, 32 ind– SE 0.19 0.19 0.11 0.21 0.25	3 trials) P 0.72 0.19 0.39 0.14 < 0.001 0.14	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day Trial (2) Trial (3) Random effects Individual ID (N = 72) Origin nest ID (N = 23)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06 -0.26 -0.26 -0.44 0.21 <0.001	2 ind 61 ind–3 SE 0.91 0.22 0.20 0.09 0.21 0.22 0.21 0.22	trials) P 0.61 0.36 0.49 0.23 0.04 0.07 0.99	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25 0.09 0.31 1.14 0.17 0.008	72 ind ls, 32 ind– SE 0.19 0.19 0.11 0.21 0.25	3 trials) P 0.72 0.19 0.39 0.14 <0.001 0.14 0.97	
2) COMMON GARDEN Fixed effects Intercept Habitat (urban) Sex (male) Time of day Trial (2) Trial (3) Random effects Individual ID (N = 72) Origin nest ID (N = 23) Foster nest ID (N = 11)	N = 153 obs, 72 (70 ind-2 trials, Estimate 1.01 -0.11 0.19 0.06 -0.26 -0.26 -0.44 0.21 <0.001 <0.001	2 ind 61 ind–3 SE 0.91 0.22 0.20 0.09 0.21 0.22	trials) P 0.61 0.36 0.49 0.23 0.04 0.07 0.99 0.99	N = 203 obs, (54 ind-2 tria Estimate -0.07 -0.25 0.09 0.31 1.14 0.17 0.008 0.004	72 ind ls, 32 ind– SE 0.19 0.19 0.11 0.21 0.25	3 trials) P 0.72 0.19 0.39 0.14 < 0.001 0.14 0.97 0.98	


Figure 5.2: The effect of urbanization on the number of errors (top panel: A & B) and the latency to escape the cage (bottom panel: C & D) in wild and common garden contexts (statistical models run separately by context). For the number of errors, the effect of habitat type (forest vs. urban; A) and proportion ISA (impervious surface area; B) are shown in wild (purple) and common garden contexts (red). For latency, we show the effect of habitat type on the probability of an individual escaping the cage over time separately for the wild (C) and common garden (D). Probability of not escaping estimates from the cox proportional hazards models (Table 5.1.1B&2B) were subtracted from 1 so that plots show the probability of escaping. Figures show model estimates and associated 95% confidence intervals when continuous predictors held at their means and categorical predictor held at the level included in the intercept.



Figure 5.3: The number of errors (A) and latency to escape (B) the detour task over trials (individuals assayed annually in the wild and every three months in common garden). A) the number of errors over trials separately for habitat type (urban vs forest) and context (common garden trials: purple to pink; wild trials: red to orange). B) the probability of escaping over trials (purple to pink) in the common garden experiment. Estimates from the cox proportional hazards models (Table 5.1.2B) were subtracted from 1 so that plots show the probability of escaping. Figures show model estimates and associated 95% confidence intervals when continuous predictors held at their means and categorical predictor held at the level included in the intercept.

5.3.2 Latency to excape

In the wild, there was a non-significant tendency for urban birds to have a higher probability of not escaping the detour task over time than forest birds (Figure 5.2C; Table 5.1.1B; P = 0.08), but the probability of escaping did not change significantly along the urbanization gradient (ISA effect; Table S5.1.1B). These results were qualitatively similar when using data from only the first trial of individuals (Table S5.2B). There was no significant evidence that the probability of escaping changed over repeated trials (Table 5.1.1B). Individual and site ID explained neglibible variation in the latency to escape in the wild.

In the common garden, the probability of escaping the task was not significantly affected by the origin habitat type or urbanization level (i.e., non-significant habitat or ISA effects; Figure 5.2D; Table 5.1.2B; Table S5.1.2B). There was a significant effect of trial (Table 5.1.2B) where the probability of not escaping significantly decreased with trials in the common garden (Figure 5.3B). The individual ID random effect (i.e., individual-specific permanent and early environmental variance) explained low and non-significant

variation in the probability of escaping, while all other random effects considered explained negligible variation in this trait (Table 5.1.2B).

5.4 Discussion

We addressed two major unknowns in the literature about whether urban and forest individuals differ in their inhibitory control performance on a detour task, and whether genetic variation underlies cognitive variation observed along an urban gradient. Despite growing interest in the evolutionary potential of cognitive traits and the relevance of inhibitory control for adjusting to urban conditions, no studies have yet examined this cognitive trait in wild urban populations. We found that birds in more urbanized habitats (high ISA) made significantly fewer errors during an adapted motor detour task than birds in less urbanized habitats (Table S5.1) and a non-significant trend for urban birds to make fewer errors than forest ones (Table 5.1). However, we found no statistically clear difference between urban and forest birds or no statistically significant effect of urbanisation level in the latency to escape the task. The difference in errors observed in the wild was not maintained in the common garden experiment; birds from urban and forest origins reared under common conditions did not differ in the number of errors or the latency to escape when administering the motor detour task in captivity. Together our results show that wild urban birds may have higher performance related to inhibiting recurring predominant responses in a detour task, but that this cognitive shift in urban birds is likely driven by exposure to environmental conditions in urban habitats.

Birds in more urbanized habitats hit the transparent barrier less than those in less urbanized habitats (i.e., significantly fewer errors with increasing ISA) suggesting that urbanization affects performance during a cognitive task related to inhibiting predominant responses (i.e., inhibitory control). However, the effect of urbanization on the number of errors during the task was statistically non-significant when comparing errors across habitat type (forest vs. urban; P = 0.09). We expected that urban birds would also take less time to escape than forest birds (i.e., have higher inhibitory control; van Horik et al., 2018), but we did not find differences between wild urban and forest birds in the latency to escape the task. A previous study using individual pheasants showed that the number of errors or pecks on a transparent barrier in a motor and cylinder detour task, but not the latency to the food reward, correlated with the number of errors and the latency to the reward in detour tasks may not measure the same cognitive processes, and the number of errors during these tasks may be more representative of individual measures of persistence (see also Prasher et al., 2019). Other studies employing detour tasks in the wild view contacts with the barrier as failures and evaluate inhibitory control by examining the number of successful consecutive trials where individuals

make the detour around the barrier without touching it (e.g., Davidson et al., 2022; McCallum & Shaw, 2023). Our assay may not exclusively measure inhibitory control and other cognitive processes (and noncognitive processes, discussed below) could impact the number of errors and the latency to escape the cage during our task, meaning our results may indicate that urban birds are less persistent than forest birds. The latency to escape our task and the latency to a reward in a classic cylinder detour task were positively correlated in a different subset of birds, suggesting that our task is measuring processes related to inhibitory control, but in future we will need to validate our cognitive measures by comparing individual performance across different inhibitory control tasks (i.e., convergent validity; Völter et al., 2018). Previous work found that individual wild great tits performed similarly on a detour task that was presented at nest boxes in the wild to the classic cylinder task presented in captivity, and demonstrated convergent validity of their field task in measuring inhibitory control in the wild (Davidson et al., 2022). Using a similar cognitive assay to Davidson et al. (2018) in the Montpellier urban and forest populations could further evaluate how inhibitory control differs in urban populations and determine whether our detour task measures cognitive processes related to inhibitory control.

Despite high heterogeneity among studies, urban animals tend to have higher performance on cognitive tasks (e.g., problem solving; Vincze & Kovács, 2022), but the underlying drivers of cognitive performance in urban organisms are unknown (Sol et al., 2020). Assuming the number of errors during our assay represents a cognitive process related to inhibitory control, our results provide some support for higher cognitive performance in urban animals. This difference in errors was not maintained between urban and forest individuals reared in a common garden experiment suggesting that plasticity to urban conditions was the main driver behind the phenotypic difference in the wild, rather than genetic change between populations. Similarly in wild New Zealand toutouwai (North Island robins; Petroica longipes), a quantitative genetic study revealed that individual variation in inhibitory control was mainly environmentally determined and had little genetic basis (McCallum & Shaw, 2023). Cognitive traits like learning, problem solving, or inhibitory control can buffer wild populations from environmental changes and have important evolutionary consequences for urban populations. For instance, the cognitive abilities of urban populations could move them closer to new adaptive peaks, which would slow evolutionary processes by weakening the strength of selection on phenotypes (Sol et al., 2020). This could "buy time" for newly established populations to adapt to urban conditions (Caspi et al., 2022) but, if these cognitive abilities do not contain underlying genetic variation, then urban populations may not be able to adapt to further environmental change. Determining whether inhibitory control aids adjustments to urban habitats by evaluating its relationship with fitness in the wild (Morand-Ferron et al., 2016) would be an obvious next step to explore how this cognitive ability could facilitate or impede evolution in wild urban populations.

Experience with urban environmental conditions could also explain the difference in errors observed along the Montpellier urban gradient during the detour task. Indeed, non-cognitive processes including experience, motivation, and non-cognitive traits can explain performance on a variety of cognitive tasks (Dougherty & Guillette, 2018; Kabadayi et al., 2018; Prasher et al., 2019; Shaw, 2016; van Horik et al., 2018). Cognitive studies attempt to reduce the influence of these non-cognitive processes by standardizing conditions before assays (e.g., food deprivation or training) or by controlling for these factors statistically (e.g., experience on similar tasks); approaches that are more difficult in wild populations (Shaw, 2016). We do not have information on the past experience of each individual but, as urban animals may be exposed to more non-natural surfaces like windows in their environments, experience with transparent barriers could play a role in how urban animals respond to detour tasks (van Horik et al., 2018). Prior experience and comprehension of transparent barriers could thus explain why urban great tits made fewer errors during the task than forest tits. As the population difference in errors did not persist in the common garden where all individuals were exposed to the same artificial materials, this further supports the role of experience in shaping the habitat difference in performance in the wild.

Performance in the detour task differed across wild and common garden contexts where birds in the common garden on average made fewer errors and took more time to escape the cage than birds in the wild. Differences in motivation between the wild and common garden could explain why performance differed between these contexts. Individuals in the common garden were reared for part of their life in cages and were manipulated several times by humans. Therefore, common garden birds may have been less motivated initially than wild birds to escape the detour task (i.e., the goal) which on average reduced the number errors and increased the time to escape the cage. Another difference between contexts related to the improvement in performance over repeated trials of the task. We did not find clear evidence that performance in the wild improved over trials, but we did see statistically significant declines in the number of errors and the latency to escape the cage in the common garden experiment. In the common garden, we assayed most individuals every three months, whereas in the wild we administered the task annually and had fewer repeated individual measures. Improved performance in the common garden could indicate that individuals learned the task or that their cognitive performance increased with age between 74 and 264 days old. In the wild, however, a lack of improvement suggests that individuals lacked long-term memory of the task over a one year period but, due to limited repeated measures, further testing would be required to evaluate this conclusion. Future common garden experiments exploring urban and nonurban differences across a variety of cognitive dimensions (e.g., learning or problem solving) would be useful for informing how genetic and environmental contributions affect cognitive shifts in populations more broadly.

In conclusion, our results suggest that phenotypic differences observed between urban and nonurban populations during cognitive tasks are driven mainly by exposure to environmental conditions in urban habitats. However, as we present the first examination of shifts in inhibitory control and its genetic basis in an urban population, more studies will be needed to corroborate our results. Previous work highlights that urban organisms may have higher cognitive performance than their nonurban counterparts (Sol et al., 2020; Vincze & Kovács, 2022), but manipulated environmental conditions related to urban contexts (i.e., traffic noise and heat stress) have been shown to decrease cognitive performance (Blackburn et al., 2022; Templeton et al., 2023). This discrepancy highlights that more work is needed to understand how urban conditions shape individual variation in cognition and multi-treatment common garden experiments would be a useful approach to address these gaps. The cognitive abilities of urban individuals could initially buffer populations from environmental change but, if these abilities lack a genetic basis, the evolutionary potential of urban populations may be limited. Evaluating the role of cognition in urban evolution will be especially important for determining how cognitive traits facilitating rapid adjustments to novel urban conditions can affect the long-term persistence of populations in urban environments.

5.5 Acknowledgements

We would like to thank everyone who participated in field monitoring and the common garden including Segoléne Deltaire, Amélie Fargevieille, Christophe de Franceschi, Sam Perret from the CEFE and Baptiste Chenet, Marc Romans, Vivian Espinasse, Thibault Pujol, Flavien Daunis, Cathie Troussier, Jérôme Brière, Laetitia Boscardin, Sébastien Pouvreau, Lucas Boussioux, Charlotte Gay, Marion Darde from the Montpellier Zoo. MJT was supported by a Canadian Graduate Scholarship from the Natural Sciences and Engineering Research Council (NSERC) of Canada, a Fonds de recherche du Québec Nature et technologies PhD scholarship, and a PhD mobility grant from le Centre Méditerranéen de l'Environnement et de la Biodiversité (CeMEB). This project was funded by the Agence Nationale de la Recherche (URBANTIT grant ANR-19-CE34-0008-05 to AC), the OSU-OREME, and a Fonds de Recherche du Quebec Nature et Technologie to DR.

CHAPITRE 6

General Discussion

6.1 Summary of results

I addressed two main questions in my thesis. My first question was: How does urbanization affect phenotypic variation? My literature review synthesized how multiple processes in cities can increase phenotypic variation and my complementary meta-analysis showed that urbanization increases morphological variation in European great and blue tit populations (Chapter 2). A collaborative meta-analysis similarily showed that urbanization increases phenological variation in bird populations globally (appendix E). Although there is an emerging trend that urbanization increases trait variation, this was not a universal result across behavioural traits in great tits (appendix F). Applying a mega-analysis approach in Chapter 3 allowed a more exhaustive examination of how urbanization increases patterns of phenotypic variation between and within subpopulations of tits along urban gradients. I found that urbanization increases tarsus length and lay date variation within subpopulations at local spatial scales. I also showed that urbanization can increase diversity among individuals at both local and larger spatial scales (i.e., both between and within subpopulations), but this was specific to tarsus length. Overall, my thesis demonstrates that urbanization increases phenotypic variation, at least for the morphological and phenological traits examined, and that this individual diversity exists at local spatial scales in cities.

My second question was: How do genetic and plastic changes shape urban phenotypes? To address this question we reared urban and forest great tits under common environmental conditions to determine whether phenotypic divergences observed in the wild were a result of genetic differentiation between populations or plasticity to urban conditions. My synthesis of previous urban common garden experiments showed that both genetic and environmental effects can shape urban phenotypes. Similarily, my common garden results highlighted contributions from both genetic change and plasticity in shaping urban phenotypic shifts since some of the examined trait differences were maintained in the experiment, while others were not. Specifically, we found evidence that genetic change (or early maternal investment in eggs) contributes to faster breath rates under constraint and lighter body mass in urban tits compared with forest tits (Chapter 4). Conversely, no clear differences in exploration and aggression behaviours (Chapter 4), or cognitive abilities related to inhibitory control (Chapter 5), were observed between birds from urban and forest origins in the experiment, indicating that behavioural and cognitive differences observed in the wild may be a result of plasticity to urban conditions. Overall, I found support that both genetic and plastic changes can drive urban phenotypic shifts and that their relative contributions are trait-specific.

6.2 Hypothesized drivers of increased urban phenotypic variation

Multiple processes can shape phenotypic variation in urban populations (synthesized in Chapter 2) and evaluating how phenotypic variation was distributed at different scales along replicated urban gradients allowed me to identify specific and testable hypotheses for these patterns in wild tit species (Chapter 3). For example, environmental heterogeneity in nestling food sources (environmental variation) or genetic differences between individuals (genetic variation) could both increase tarsus length variation between and within subpopulations of tits in cities, and limited or non-random dispersal likely plays a meaningful role in the spatial distribution of urban phentoypes. My common garden results suggest that a combination of environmental and genetic effects shape urban tarsus length in great tits (Chapter 4), meaning that environmental heterogeneity and genetic differences between individuals together could increase tarsus variation in urban environments. As discussed in Chapter 3, dispersal dynamics are still poorly understood in many urban systems, but especially in urban bird species, and so efforts examining individual movements across the urban matrix are needed to evaluate the role of dispersal (and other potential neutral evolutionary processes like genetic drift) in spatial distributions of phenotypic variation.

I found that urbanization increased individual differences in lay date in urban subpopulations at fine local scales (Chapter 3). Lay date in tits has been previously been shown to associate with environmental variation at fine spatial scales in woodland habitats (Cole et al., 2021; Hinks et al., 2015), and so this result corroborates these findings in an urban context. Female tits use environmental cues like temperature, light, or tree phenology to plastically adjust their lay dates to annual spring conditions (Bonamour et al., 2019), which implies that higher urban lay date variation could be environmentally-induced if individuals respond to high heterogeneity in urban environmental cues (e.g., heat island effects, artificial light, or non-native trees). My common garden results show that the behavioural and cognitive traits of urban tits are likely plastic responses to urban conditions (Chapter 4 and 5) and, since behavioural and cognitive traits are also labile and represent individual responses to environmental variation (i.e., plastic; Dingemanse & Wolf, 2013; Stirling et al., 2002), this could suggest that higher urban lay date variation is similarly environmentally-induced. However, lay date in tits is heritable (Garant et al., 2008; Thorley & Lord, 2015; Van Noordwijk et al., 1981) and individual plasticity in lay date can also be heritable and under selection (Nussey et al., 2005), so deciphering the genetic or environmental influences driving higher urban lay date variation remains a future research objective. Multi-treatment common garden approaches where individual tits are reared under different temperatures could be useful for comparing lay date plasticity between urban and nonurban populations, and evaluating whether population-level plastic responses may have a genetic basis. On average, urban tit populations lay earlier (Chapter 3) and have lower reproductive success than

forest populations (Caizergues et al., 2018; Charmantier et al., 2017; Vaugoyeau et al., 2016), but previous work in the Montpellier great tit system did not find that lay date was associated with reproductive success at the population level (Caizergues et al., 2018). Since urbanization increases lay date variation within subpopulations and it is unclear how this variation associates with fitness at this scale, further work examining the fitness consequences of diverse urban lay date strategies at fine spatial scales may be better placed to determine how selection may shape lay date diversity in urban systems.

6.3 Future directions

My thesis highlights mutli-population and mega-analysis approaches as beneficial tools that can be used to move urban research forward. Urban systems present an oppurtunity to study how predictable environmental changes via urbanization (e.g., heat island effects, pollution, or artificial light) affect replicated populations globally (Szulkin et al., 2020). Utilizing this level of spatial and temporal replication by combining long-term urban studies will not only allow highly generalizable conclusions concerning urbanization's impact on wildlife, but can also be used to test eco-evolutionary theory more generally. The mega-analysis presented in Chapter 3 also shows that European cities differ considerably in how they impact the traits of wild populations (i.e. high variance across study systems), and so there is also considerable scope to use these mega-analyses as tools to further explore which characteristics across city replicates (e.g., human density, green space, connectivity, pollution) have differential impacts on wild populations (Magle et al., 2019).

A major limitation currently is that most urban research is being conducted across European and North American cities, and so it is unknown whether major findings in Urban Evolutionary Ecology also apply to areas where urban wildlife is less well studied like the Global South (Verrelli et al., 2022). There have been recent initiatives that are attempting to rectify this by extending urban research networks and collaborations globally. For example, I utilized the SPI-Birds Network during my thesis (Culina et al., 2020), an initative that aims to create visibility for studies on individually-marked bird populations around the world in hopes that new global collaborations can be initiated. There is also the Global Urban Evolution project which studies parallel urban evolution in white clover (*Trifolium repens*) across an impressive 160 cities and has initiated new urban studies through collaborations (Santangelo et al., 2022). Citizen science programs have great potential in further extending research into diverse urban areas (Rivkin et al., 2019) and, as these programs benefit researchers and increase community engagement, their use in urban research has previously been effective (e.g., Kerstes et al., 2019; Wang Wei et al., 2016). Taking advantage of replicated

research programs globally by iniating new research collaborations or by establishing citizen science initiatives will help advance the field of Urban Evolutionary Ecology.

My results from Chapter 3 indicate that urbanization is associated with increased individual differences at fine spatial scales, suggesting that urban eco-evolutionary processes should also be studied at these local scales. Mega-analyses rely on standardized data collection procedures across study systems and so may not always be able to address questions that require finer scale data or experiments (e.g., common garden experiments; Chapter 4 and 5). Therefore, single population research can make important contributions alongside these larger collaborative efforts to, for instance, determine how fine-scale environmental variation shapes urban phenotypic variation. To address this the quantification of environmental variables, individual phenotypes, and fitness at small spatial scales would be useful for contructing fitness landscapes to evaluate whether the spatial distribution of individual diversity in urban habitats is non-random (Camacho & Hendry, 2020; Edelaar & Bolnick, 2012) as well as identifying which urban environmental axes drive selective processes (Appendix G). Establishing whether genetically-driven urban phenotypic changes are adaptive will be especially challenging as evaluating adaptation through reciprocal transplant experiments is not always feasible in wild populations, and in some cases impossible. In these cases, future work could integrate phenotypic and genomic studies (i.e. long-term field studies, common garden experiments, molecular approaches), ideally across multiple population replicates, to determine whether urban phenotypic shifts are consistent with adaptation (Lambert et al., 2021).

There is emerging concern about how multiple processes of global anthropogenic environmental change can interact to affect natural systems, and so recent studies have emphasized a need to evaluate how climate warming affects urban populations differently to nonurban ones (Sumasgutner et al., 2023; Urban et al., 2024; Verrelli et al., 2022). Together, my synthesis of urban common garden studies (Table 4.1) and finding that genetic change could drive smaller urban body sizes in tits (Chapter 4) imply that organisms may be adapting to heat island effects in urban habitats. However, the extent to which urban populations can continue to adapt to warming temperatures under climate change is largely unknown and research in this area will be imperative for future urban planning and conservation. Specifically, I found that urbanization increases lay date variation at local scales (Chapter 3) and my thesis highlights that urbanization changes the phenological distribution of urban populations (see also appendix E). Lay date phenology in tit species is plastic to environmental cues like spring temperatures and climate change has advanced the phenological timing of population lay dates with increasingly warmer springs over time (Bailey et al., 2022; Both et al., 2009). Urban habitats dramatically alter the abiotic (e.g., heat island effects) and biotic conditions (e.g. species compositions) that wild populations are exposed to and so urbanization may affect the

transmissibility and reliability of environmental cues (Jochner & Menzel, 2015; Schlaepfer et al., 2002). Variation in environmental cues or their weakened saliency in urban habitats could drive the observed variation in urban lay dates or may even disrupt phenological responses. Examining how tit species plastically adjust annual lay date to fine-scale environmental variation related to temperature or artificial light could help demonstrate how urban environmental heterogeneity can directly drive fine-scale phenotypic variation in cities. Further, comparing lay date plasticity and optimal lay date timing (de Villemereuil et al., 2020; Macphie et al., 2023) between urban and forest populations would establish whether urban populations are more vulnerable than nonurban populations to projected climate warming scenarios (Simmonds et al., 2020).

My thesis shows that urbanization can increase individual differences (Chapter 2 and 3), and so an obvious future direction would be to explore the consequences of this urban-modified phenotypic variation. Individual differences can shape processes at population, community, and ecosystem levels (Bolnick et al., 2011; Violle et al., 2012), and so evaluating how variation affects processes at these ecological levels could make crucial fundamental and applied advances in urban research (Des Roches et al., 2021; Mimura et al., 2017; Moran et al., 2016). Conducting research at multiple trophic levels in urban environments would enable examinations into the consequences of urban variation (El-Sabaawi, 2018; Faeth et al., 2005). For example, tit species make annual adjustments in lay date timing to match the spring peak availability of caterpillars; an important food source that tits rely on during reproduction to feed their nestlings (Visser et al., 2012). Further, caterpillars time their spring hatching phenology to match the peak availability of their food source - bud burst in trees (Both et al., 2009). Phenological synchrony across this woodland foodweb is crucial for maintaining reproduction and population persistence in tits (Radchuk et al., 2019; Samplonius et al., 2020; Thackeray et al., 2016), and it is currently unknown how urbanization may disrupt phenological synchrony. Urbanization has been shown to affect the phenology of trees (Jochner & Menzel, 2015), but it is less known how urbanization affects the abundance or phenology of caterpillars (Jensen et al., 2022; Seress et al., 2018), so examining phenology across urban trophic levels could be informative for determing the causes and consequences of the more variable urban lay date strategies reported here. There are also now common garden experiements that examine the traits and interactions of multiple species (Brans et al., 2022), which are powerful approaches for showing how trophic interactions can impact focal species evolution.

6.4 The significance of urban research

The amount of research being conducted in Urban Evolutionary Ecology has grown immensely over the last several decades (Diamond & Martin, 2021; Johnson & Munshi-South, 2017; Szulkin et al., 2020). Long-term studies in natural populations have served as a major inspiration for the development of similar research into urban areas. My thesis is an example of how long-term studies have generated new research topics and motivated the establishment of parallel research in urban populations. For instance, the monitoring of great tits along the Montpellier gradient initated in La Rouvière forest 33 years ago and the rise of urban research encouraged parallel monitoring in the city of Montpellier 20 years later. Monitoring of tits in the city of Montpellier has continued for 13 years with findings from this system and other long running urban systems (e.g., Veszprem) inspiring recently initated monitoring programs in other European cities like Strasbourg and Budapest. The ongoing "colonization" of research in urban areas not only has immense fundamental value but also has potential conservation applications.

Ongoing research in Urban Evolutionary Ecology will be important for three main reasons. First, urban systems can be used as labs to study eco-evolutionary processes in wild populations. The ease of monitoring wildlife where we live, the growing research applications of citizen science programs (e.g., iNaturalist and eBird), and the replication of urban gradients globally make urban systems incredibly powerful contexts to test and generalize fundamental concepts in Ecology and Evolution. Second, urbanization is a global and increasing process of environmental change that is only expected to expand in the coming decades (United Nations, 2019). Therefore, more and more natural systems will be exposed to urbanization with time, meaning a broad comprehension of how urbanization affects the evolution of wild populations will be needed to develop conservation initiatives that preserve species, populations, and individual diversity into the future (Forsman, 2014; Mimura et al., 2017; Moran et al., 2016). Third, urbanization has wide impacts on animal health and the ecosystem services that natural systems provide to human communities. For instance, urban environments may increase the risk of zoonotic (and reverse zoonotic) spillover of infectious diseases given that the diversity of zoonotic hosts is often higher in cities and non-human animal populations are less healthy (Gibb et al., 2020; Murray et al., 2019). Altered urban species compositions and reductions in functional diversity can also impact important processes like seed dispersal, pollination, or nutrient cycling (Snell et al., 2019; Sol et al., 2020; Theodorou et al., 2021). Incorporating community ecology themes and research across multiple species will be especially valuable in determining how urban ecoevolutionary processes can impact the functioning of urban systems and the ecosystem services they provide (El-Sabaawi, 2018; Faeth et al., 2005).

6.5 General conclusion

So why do wild populations and the indviduals that comprise them differ? My thesis demonstrates that environmental change via urbanization can drive diversity in wild populations, both through genetic and plastic changes. Thus, my thesis highlights that phenotypic variation can play an important role in urban evolution. These conclusions are especially significant considering the extensive transformation of environments to urban systems worldwide. My thesis addresses important unknowns in the literature about urbanization's impact on genetic, environmental, and total phenotypic variation, but also identifies new research avenues. Specifically, my results indicate that urbanization is associated with increased individual differences at multiple spatial scales in wild tit populations, and that both genetic and plastic change can drive urban phenotypic divergences. My thesis is an example of how both multi and single population studies can make important contributions in urban research, and highlights that integrating climate and community ecology themes into Urban Evolutionary Ecology will be especially timely research objectives moving forward.

ANNEXE A

Supplementary materials for Chapter 2

Box S1 Supplementary Methods

We obtained means, standard deviations, and sample sizes directly from multiple research systems for great tits and blue tits separately by sex and morphological traits (see Table S2.2; Figure S2.2 & S2.3). The number of urban and nonurban sites, and the number of years, varied by city (Table S2.2). Analyses were completed in R v.4.0.4 (R Core Team, 2024) using both the metafor (Viechtbauer, 2010) and orchaRd packages (Nakagawa et al., 2021). We used collated summary statistics to calculate the natural logarithm of the ratio of the coefficient of variation (lnCVR) and the natural logarithm of the ratio between the two means (lnRR; Nakagawa et al., 2015) using the *escalc* function with the urban group as the numerator. Positive and negative estimates then indicate that urbanization increases or decreases effect sizes. We then evaluated how urbanization influenced the mean and variance effect sizes separately in the following models using the *rma.mv* function (eight models total): 1) an overall model that included no fixed effects, 2) a model that included species (great tit and blue tit) as a fixed effect, 3) a model that included sex (male and female) as a fixed effect, and 4) a model that included morphological traits (wing length, tarsus length, and mass) as a fixed effect. All models included random intercept effects for research group and observations (i.e., residual variance; Sánchez-Tójar et al., 2020). In one case, we received multiple city comparisons from a research group (Göteborg, Helsingborg, and Malmö). Instead of including "city" as a random effect, we decided that "research group" was more appropriate for explaining variation. This was because cities from the same research group were from the same country, spatially close to each other, and likely followed similar methodological protocol for measuring morphological traits. Furthermore, our results were qualitatively the same when using city instead of research group as a random effect.

Overall, urbanization tended to decrease the mean (lnRR), but increase the variance (lnCVR), in blue tit and great tit morphology (Box 3; Table S2.4a). Trends differed across morphological traits (Box 3; Table S2.4d), where higher variation among urban groups was mainly driven by tarsus length. The morphological traits examined have different developmental trajectories which may explain this pattern. The tarsus develops early and remains fixed over the lifetime, wing metrics can vary annually with moulting events, and body mass may fluctuate continuously. Therefore, the fact that we see stronger urban and nonurban differences in variation for tarsus length may be attributable to tarsus length being a more constant trait. We also examined the contributions of species and sex. We found that differences in mean and variance estimates were similar between great and blue tit species (Table S2.4b; Figure S2.3), but found our estimates to be slightly stronger in females than males (Table S2.4c; Figure S2.3). The computed mean and variance effect sizes were not significantly correlated (all P > 0.35) with the number of years, the number of urban sites, or the number of nonurban sites contributed (Table S2.3).

Table S2.1 : Related to main text. List of studies (non-exhaustive) that compare intraspecific phenotypic variation between urban and nonurban populations. Information concerning the species, environmental comparison, types of traits, and direction of trend are shown for each study. The first letter of each environmental comparison is used to represent the trend reported (higher: >, lower: <, and no difference: =). For example, if higher phenotypic variation was found in the urban compared to the rural population the trend would be denoted as U > R.

Species	Comparison	Traits	Trend	Note	Ref
Common ragweed (<i>Abrosia</i>	Urban vs. rural	Fitness	U > R	Significant at some sites	(Gorton et al.,
artemisiifolia)		Phenology	U > R	Not significant	2018)
Bumblebees (Bombus pascuorum, Bombus lapidarius)	Urban vs. rural	Functional	U > R	Significant for both species	(Eggenberger et al., 2019)
Bumblebees (Bombus pascuorum, Bombus lapidaries, Bombus terrestris)	Urban vs. rural	Morphology	U = R	Higher variation associated with higher temperature	(Theodorou et al., 2021)
Mosquito (Anopheles cruzii)	Urban vs. peri- urban vs. sylvatic	Morphology	$\begin{array}{l} U > P \\ U > S \end{array}$	Higher urban variation over time	(Multini et al., 2019)
Anolis lizards (A. Sagrei, A. lineatopus, A.grahami, A. cybotes, A. cristatellus)	Urban vs. forest	Morphology	U > F	Significant for all species	(Falvey et al., 2020)
Common wall lizard (<i>Podarcis muralis</i>)	Urban vs. rural	Morphology	U > R		(Lazić et al., 2015)
Eastern water dragon (Intellagama lesuerii)	City vs. nonurban	Morphology	C > N	Differentiation among city populations	(Littleford- Colquhoun et al., 2017)
Whiptail lizard (Aspidoscelis costatus costatus)	Urban vs. wildland	Morphology	$\mathbf{U} = \mathbf{W}$		(Gómez-Benitez et al., 2021)
Blue-tailed skink (<i>Emoia impar</i>)	High vs. medium vs. low human disturbance	Behaviour	H < M < L		(Williams et al., 2019)
Northern cardinal (Cardinalis cardinalis)	Urban vs. rural	Fitness	U < R	Reproductive contribution among and within individuals	(Rodewald & Arcese, 2017)
House sparrow (Passer domesticus)	Urban vs. rural	Behaviour	U = R	Trends vary across assays and subpopulations	(Bókony et al., 2012)

Table S2.2: Related to Box 1. Examples demonstrating how environmental features may increase (black text) or decrease (grey text) environmental heterogeneity in urban habitats depending on the scale considered. We indicate for each example whether environmental heterogeneity is affected at small (local or home range level) or large (city or regional level) spatial scales and across short (within a day or week) or long (between seasons or years) temporal scales.

Environmental		Se	cale	
feature	Heterogeneity vs. V Heterogeneity	Spatial	Temporal	References
Human presence				
Pedestrians	Pedestrian abundance and distribution varies	Small		(Corsini et al., 2019)
	across the urban matrix.		Short	(Miranda-Moreno & Lahti, 2013)
	and seasonally		Long	(
Anthropogenic	Anthropogenic food availability fluctuates over a	Small	Short	(Stofberg et al., 2019)
food sources	week.			
	Anthropogenic food sources are more predictable		Long	(Tryjanowski et al., 2015)
	and stable over seasons or years.			
Roads and traffic	Variation in wildlife collisions within a day,	Small,	Short,	(Neumann et al., 2012)
	between seasons, and within a region.	Large	Long	(Khalid et al. 2018: 7hou &
	variation in traffic-related air pollutants is	Sman	Long	Levy, 2007)
	seasons			
Artificial light	Artificial light varies with land use and building	Small		(Hale et al., 2013)
	density within a city.			
	Higher light levels at night reduce light cycle		Short	(Leveau, 2018)
	transitions between day and night in urban areas.			
	Artificial light from cities causes the skyglow	Large		(Kyba & Hölker, 2013)
	phenomenon which increases light exposure over			
Sound pollution	Iarge areas.	Small	Short	(Kheirbek et al., 2014)
Naturalness	orban horse revers vary within a city and a week.	Sinan	bilott	
Species	Increased inter- and intra-specific competition in	Small	Long	(Shochat, 2004; Shochat et al.,
composition	urban environments.		Ū.	2004)
L	Declines in species diversity within urban areas.	Large	Long	(McKinney, 2006)
	Species composition more stable between seasons	Small,	Long	(Leveau, 2018; Pearse et al.,
	and years, and across local yards and regions.	Large	0	2018)
Predators	Novel predation events caused by pets.	Small	Long	(Krauze-Gryz et al., 2017; Loss & Marra 2017)
	Decision measure more releved in when we	Small	Long	(Eötyös et al., 2018: Vincze et
	nonurban areas	Sman	Long	al., 2017)
Land cover and	Land cover fragmentation in urban areas increases	Small,	Long	(Pickett et al., 2017)
vegetation	spatial heterogeneity.	Large		
	Urban trees have lower species and genetic	Large		(Lohr et al., 2014; Vanden
	diversity.	-		Broeck et al., 2018)
	Higher primary productivity in urban areas, which		Long	(Faeth et al., 2005)
	is more seasonally and annually stable.			
	Local land cover types increase vegetative	Small	Long	(Qiu et al., 2017)
	growing seasons in urban areas.	C 11	<u>C1-</u>	(Kouchel et al. 2014; Kouchel
Hydrology	Variation in urban watershed functioning and	Small, Large	Snort, Long	(Kaushai et al., 2014; Kaushal et al., 2014)
	time		0	
	Hydrological characteristics including high-flow	Large		(Brown et al., 2009)
	events and toxicity vary between cities.	-		

Climate may contribute more variation to urban environments within a season through extreme weather and heat waves in cities, increased precipitation, and snow cover variation.	Large	Short	(Chapman et al., 2017; Collier, 2006)
At a microclimatic scale in urban environments, temperature increases at a faster rate during the day and changes more rapidly across space.	Small	Short	(Diamond et al., 2018)
Urban heat island effect reduces temperature variability between seasons and years, and the presence of building and other barriers reduces wind intensity in cities.	Large	Long	(Bang et al., 2010; Collier, 2006; Hall et al., 2016; Rizwan et al., 2008; Zhou et al., 2017)
	Climate may contribute more variation to urban environments within a season through extreme weather and heat waves in cities, increased precipitation, and snow cover variation. At a microclimatic scale in urban environments, temperature increases at a faster rate during the day and changes more rapidly across space. Urban heat island effect reduces temperature variability between seasons and years, and the presence of building and other barriers reduces wind intensity in cities.	Climate may contribute more variation to urban environments within a season through extreme weather and heat waves in cities, increased precipitation, and snow cover variation. At a microclimatic scale in urban environments, temperature increases at a faster rate during the day and changes more rapidly across space. Urban heat island effect reduces temperature variability between seasons and years, and the presence of building and other barriers reduces wind intensity in cities.	Climate may contribute more variation to urban environments within a season through extreme weather and heat waves in cities, increased precipitation, and snow cover variation. At a microclimatic scale in urban environments, temperature increases at a faster rate during the day and changes more rapidly across space. Urban heat island effect reduces temperature variability between seasons and years, and the presence of building and other barriers reduces wind intensity in cities.

Table S2.3 : Related to Figure 2.2. Overview of effect sizes (mean = $\ln RR$, variance = $\ln CVR$) averaged across species, sexes, and traits for each city (by decreasing latitude). Check marks indicate which species and morphological traits were included for each city system. We also include the maximum number of years and sites for each habitat type included. See Figure S2.1& S2.2 for overview of summary statistics for each city. Most of the study systems below have shared data through the SPI-birds data hub, but we also include example references where data from these systems have been described and published.

						# sites	Species	Traits				
References	City	Latitude	lnRR	lnCVR	Years	Urban	Nonurban	Great tit	Blue tit	Mass	Wing	Tarsus
(Eeva et al., 2014; Eeva & Lehikoinen, 1996)	Harjavalta	61.32	0.008	0.067	29	14	17	\checkmark	\checkmark	\checkmark	\checkmark	
(Isaksson & Andersson	Göteborg	57.72	- 0.006	0.110	2	2	2	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
2007)	Helsingborg	56.05	0.001	-0.007	2	2	3	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
(Capilla- Lasheras et al., 2017; Pollock et al., 2017)	Glasgow	55.87	0.021	0.047	4	4	3	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
(Isaksson et al., 2017)	Malmö	55.61	- 0.007	0.083	3	5	3	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
(Corsini et al., 2021)	Warsaw	52.25	- 0.009	0.150	5	7	1	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
(Casasole et al., 2017; Raap et al., 2016)	Antwerp	51.22	- 0.007	-0.016	9	1	1	\checkmark		\checkmark	\checkmark	\checkmark
(Biard et al., 2017)	Paris	48.86	0.020	-0.062	8	2	3	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
(Saulnier et al., 2023)	Strasbourg	48.58	- 0.026	0.026	5	5	1	\checkmark		\checkmark	\checkmark	\checkmark
(Sprau et al., 2017)	Munich	48.14	- 0.013	0.528	2	1	12	\checkmark		\checkmark		\checkmark
(Ágh et al., 2020; Seress et al., 2018)	Veszprém	47.10	0.014	0.095	10	4	1	\checkmark		\checkmark	\checkmark	\checkmark
(Caizergues et al., 2021; Charmantier et al., 2017)	Montpellier	43.61	0.017	0.084	2	8	1	\checkmark		\checkmark	\checkmark	\checkmark
(Björklund et al., 2010; Riyahi et al., 2017; Senar & Björklund, 2021)	Barcelona	41.40	0.031	0.420	5	5	1	\checkmark		\checkmark	\checkmark	\checkmark

		lnRR				lnCVR			
	Effect	Estimate	Cl	[Estimate	C	I	
II	Intercept	-0.014	-0.019	-0.009		0.120	0.025	0.215	
Overa	Researcher	0.020	< 0.0001		$I^2 = 0.20$	0.018			$I^2 = 0.22$
a)	Observation	0.028	0.0002		$I^2 = 0.72$	0.053			$I^2 = 0.67$
S	Blue tit	-0.013	-0.020	-0.006	$B^2 = 0.002$	0.137	0.006	0.268	$D^2 = 0.001$
ecie	Great tit	-0.014	-0.019	-0.009	$K^{2} = 0.002$	0.115	0.013	0.216	$K^2 = 0.001$
) Sp	Researcher	0.022	< 0.0001			0.019			
ą	Observation	0.029	0.0002			0.054			
	Female	-0.015	-0.021	-0.009	\mathbf{P}^{2} 0.004	0.136	0.029	0.243	\mathbf{P}^{2} 0.002
)ex	Male	-0.013	-0.019	-0.007	$R^2 = 0.004$	0.103	-0.005	0.210	$R^2 = 0.003$
c) (2	Researcher	0.020	< 0.0001			0.018			
	Observation	0.030	0.0002			0.054			
	Mass	-0.021	-0.027	-0.015		0.068	-0.042	0.177	
iit	Tarsus length	-0.016	-0.022	-0.010	$R^2 = 0.16$	0.277	0.163	0.390	$R^2 = 0.19$
Tra	Wing length	-0.006	-0.012	0.000		0.032	-0.080	0.144	
(p	Researcher	0.021	< 0.0001			0.017			
	Observation	0.024	0.0001			0.042			

Table S2.4 : related to Figure 2.2. Mean estimates and 95% confidence intervals of urban *versus* nonurban differences in mean (lnRR) and variance (lnCVR) effect sizes for models evaluating a) the overall effect, b) species, c) sex, and d) morphological traits. We show the relative heterogeneity (I^2) explained by each random effect in the overall model (a). As well, we show the estimated amount of variance explained by the fixed effects (marginal R^2) in models b-d. Model intercepts have been stripped from models b-d.



Figure S2.1 : Related to Figure 2.2. Means \pm standard deviations of morphological traits (wing length, tarsus length, and body mass) from each comparison in order of decreasing latitude for urban (blue) and nonurban (green) great tits separated by sex (male = dark shade, female = light shade).



Figure S2.2 : Related to Figure 2.2. Means \pm standard deviations of morphological traits (wing length, tarsus length, and body mass) from each comparison in order of decreasing latitude for urban (blue) and nonurban (green) blue tits separated by sex (male = dark shade, female = light shade).



Figure S2.3: Related to Figure 2.2. Estimates, 95% confidence (thick lines), and prediction (thin lines) intervals of mean (lnRR) and variance (lnCVR) effect sizes for models evaluating contributions from species and sex. Individual effect sizes are shown and scaled by their sample size. See Box 3 of main text for overall results and trends across morphological traits.

ANNEXE B

Supplementary materials for Chapter 3

Table S3.1 : Summary of datasets used to examine patterns of phenotypic variation along urban gradients comprising urban (U) and forest (F) habitats across Europe in two species (GT = great tits, BT = blue tits) and across three traits (adult tarsus length = tars, nestling tarsus length = ntars, and lay date).

Study system	Country	ISA tile	# individuals tars, ntars, lay date	Years (U/F) ¹	(#) Urban sites / locations ²	# Forest sites / locations ³	# Nest boxes (U/F)	Tarsus method
Harjavalta	Finland	E49N42 E49N43	GT = NA, NA, 524 BT = NA, NA, 126	1991-2018/ 1991-2018	(12) 01, 02, 03, 04, 05, 17, 18, 19, 20, 21, 22, 25 / (8) 1:5, 17:19	(12) 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 23 / (12) 6:16, 20	181 / 257	Alternative
Göteborg	Sweden	E44N38	GT = 83, 39, 70 BT = 7, 4, 26	2017-2020/ 2017-2019	(2) RY, SL / (2) 81, 84	(2) GR, VO / (2) 73, 89,	46 / 41	Alternative
Helsingborg	Sweden	E44N36	GT = 148,107, 76 BT = 78, 50, 40	2018-2020/ 2017-2020	(2) AK, JB / (2) 71, 74,	(3) KB, SM, SB / (4) 76:77, 82, 85	66 / 51	Alternative
Glasgow	Scotland	E34N37	GT = 72, 136, 77 BT = 178, 306, 292	2015-2020/ 2016-2020	(2) GAR, KEL / (2) 58:59	(3) CAS, SAL, SCE / (3) 55:57	87 / 186	Alternative
Malmö	Sweden	E45N36	GT = 897, 624, 482 BT = 378, 284, 198	2013-2020/ 2013-2020	(5) S, K, P, R, RS / (4) 75,78:80	(5) SK, DN, SW, TO, V / (5) 72,83,86,88	232 / 340	Alternative
Warsaw	Poland	E50N32 E50N33	GT = 450, 250, 198 BT = 524, 197, 261	2016-2020/ 2016-2020	(7) BIB, CMZ, LOL, MUR, OLO, POL, UNI / (5) 90:91, 93, 95:96	(2) KPN, PAL / (2) 92, 94	262 / 103	Alternative
Antwerp	Belgium	E39N31	GT = 5960, 1974, 3106 BT = 4400, 74, 2339	1997-2022/ 1994-2018	(1) UA / (1) 50	(2) BOS, PEE / (8) 42:49	216 / 636	Alternative & Standard
Strasbourg	France	E41N28	GT = 303, 341, 170	2016-2022/ 2016-2022	(11) ALPE, CAMP, CITA, CNRS, ESPL, HEYR, JARD, OBSE, QUIN, ROBES, ROUG / (8) 111:118	(1) WANT / (1) 119	88 / 66	Alternative
Paris	France	E37N28	GT = 871, 664, 430 BT = 835, 488, 429	2012-2021/ 2010-2021	(2) PAR, RUE / (9) 102:110	(3) FOL, FON, COM / (5) 97:101	82 / 252	Oxford
Munich	Germany	E44N27	GT = 2509, 1950, 1177	2014-2015/ 2010-2019	(1) MUC / (1) 200 ⁴	(12) 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 / (12) 30:41	54 / 549	Alternative
Budapest	Hungary	E49N27 E50N27	GT = 133, 107, 71	2019-2021/ 2020-2021	(1) BUD / (3) 60:61, 65	(1) ORD / (3) 62:64	49 / 32	Alternative
Veszprém	Hungary	E49N26	GT = 445, 529, 268	2013-2020/ 2013-2020	(1) VES / (3) 66:67, 70	(2) VIL, GUL / (2) 68:69	84 / 88	Alternative
Montpellier	France	E38N22 E38N23	GT = 1082, 950, 465 BT = 2076, 1502, 1060	2013-2018/ 1991-2018	(8) BOT, CEF, FAC, FON, GRA, MAS, MOS, ZOO / (8) 21:27, 29	(1) ROU / (1) 28	144 / 281	Alternative
Barcelona	Spain	_010m_E36N20	GT = 645, NA, NA BT = 157, NA, NA	1992-2018/ 1998-2021	(5) LAB, SET, SAR, ZOO, CIT / (3) 51:53	(1) CAC / (1) 54	28 / 178	Alternative

¹ Urban (U) and forest (F) year overlap not the same across systems so this is max range, ²Number of urban sites defined by data owners vs. number of urban locations, ³Same as ² but for forest sites and locations, ⁴ MUC city did not location together as points in city are too far apart, so this location is defined after to test whether results change

Table S3.2 : Summary of cluster characteristics across each study system for urban and forest clusters. ISA represents the proportion of impervious surface area and heterogeneity is the Shannon diversity index of the number of land cover types¹.

Study System	Habitat	# clusters	Mean ISA (1000m)	Range ISA (1000m)	Mean ISA (100m)	Range ISA (100m)	Mean Heterogeneity	Range heterogeneity	Mean area (m ²)	Mean years of data collection
Hariavalta	urban	8	0.40	0.04-	0.35	0-1	1.44	1.05-1.82	455737.27	24.32
Haijavalta	forest	12	0.04	0-0.26	0.01	0-0.37	1.00	0.16-1.56	178081.42	23.87
Cätabora	urban	2	0.64	0.55- 0.77	0.18	0-0.65	1.26	0.89-1.7	95621.73	3.60
Goleborg	forest	2	0.05	0.03- 0.07	0.01	0-0.16	1.60	1.41-1.8	148195.74	3.70
Helsingborg	urban	2	0.71	0.59- 0.82	0.14	0-0.53	1.16	0.75-1.36	72709.29	3.51
00	forest	4	0.04	0-0.11	0.00	0-0.03	0.98	0.62-1.3	133695.72	3.22
Glasgow	urban	2	0.77	0.51-	0.49	0.02-1	0.96	0.68-1.34	487630.29	7.00
Ū.	forest	3	0.00	0-0.01	0.01	0-0.13	1.12	0.75-1.54	247665.30	8.82
Malmö	urban	4	0.76	0.68- 0.94	0.26	0-0.89	1.09	0.17-1.5	359894.48	7.96
	forest	5	0.01	0-0.11	0.00	0-0.09	0.84	0-1.39	1819914.87	3.57
Warsaw	urban	5	0.64	0.13- 0.92	0.38	0-1	0.89	0-1.6	350636.23	4.44
	forest	2	0.03	0-0.16	0.06	0-0.57	0.94	0.54-1.52	385092.60	4.65
	urban	1	0.46	0.28- 0.61	0.29	0-1	1.11	0.68-1.65	1595673.00	26.00
Antwerp	forest	8	0.16	0.05- 0.39	0.02	0-0.32	1.44	1.17-1.82	141088.22	21.67
G/ 1	urban	8	0.69	0.04- 0.95	0.48	0-1	1.03	0.26-1.51	334457.37	6.93
Strasbourg	forest	1	0.10	0.01- 0.38	0.00	0-0.16	1.27	1.02-1.4	634267.30	9.00
Paris	urban	9	0.86	0.41- 0.99	0.64	0.05-1	0.70	0-1.36	57649.88	9.47
	forest	5	0.05	0-0.24	0.01	0-0.15	0.85	0.04-1.71	495775.74	11.22
Munich	urban	1	0.80	0.29- 0.97	0.88	0.11-1	0.91	0.02-1.52	110806.06	2.00
	forest	12	0.07	0-0.3	0.00	0-0.33	1.25	0.29-1.79	70248.00	9.81
Budapest	urban	3	0.80	0.71- 0.86	0.44	0.07- 0.94	0.87	0.68-1.06	34868.44	2.88
1	forest	3	0.29	0.1-0.58	0.06	0-0.23	0.88	0.74-0.99	419612.46	1.72
Veszprém	urban	3	0.75	0.55- 0.87	0.59	0.25- 0.98	0.83	0.54-1.33	221298.71	7.81
1	forest	2	0.02	0-0.17	0.00	0-0.03	1.24	0.7-2.02	384684.49	6.82
Montpellier	urban	8	0.53	0.13- 0.94	0.49	0-1	1.08	0.2-1.75	2865311.15	7.74
	forest	1	0.00	0-0	0.00	0-0	0.68	0-1.25	455737.27	28.00
Barcelona	urban	3	0.56	0.24- 0.87	0.24	0-0.65	1.17	0.7-1.5	101870.3	25.02
	forest	1	0.00	0-0.03	0.00	0-0	0.08	0-0.25	690549.5	24

¹Of the 45 possible Corine land cover types, the spatial extent of the combined dataset included 29 land cover types including continuous urban fabric, discontinuous urban fabric, industrial or commercial units, road and rail networks, port areas, mineral extraction sites, dump sites, construction sites, green urban areas, sport and leisure facilities, non-irrigated arable land, vineyards, pastures, complex cultivation patterns, agricultural areas with significant natural vegetation, agro-forestry areas, broad-leaved forest, coniferous forest, mixed forest, natural grasslands, moors and heathland, sclerophyllous vegetation, transitional woodland shrub, sparsely vegetated area, inland marshes, peat bog, water courses, water bodies, estuaries, and sea and ocean.

Table S3.3: Model estimates when examining the effect of continuous urbanization (i.e., impervious surface area; ISA) at 1000 meters instead of the categorical effect of habitat (i.e., urban vs. forest). Effects highlighted in bold have changed conclusions (i.e., credible interval overlaps or not zero) for A) adult tarsus length and B) nestling tarsus length, and C) female lay dates from Table 3.1 in main text, but most conclusions remain the same.

Mean model Great tit Blue tit Great tit Blue tit Great tit Blue tit Litercept (Au0) 0.088 0.165 -1.453 -0.875 0.177 0.242 Intercept (Au0) [-0.379.0.563] [-0.731.1085] [-1.976-0.926] [-1.805.0.124] [-0.076-0.431] [-0.222.0.746] ISA 1000 m 0.067 0.066 -0.0168 -0.0021 [-0.077-0.023] [-0.072-0.029] [-1.31-0.073] [-0.072-0.029] Latitude 0.0554 0.502 0.609 0.57 [0.665 0.718 Sex (male) [Chick age] 0.522 0.588 0.108 0.064 0.380-0991 Rendom Effects 0.597 [0.084-0.541] [0.382-0.632] [0.083-0.155] [0.094-0.191] [0.370-0.516] [0.395-0.668] Study system 0.677 1.216 0.759 10.483 0.225 0.136 0.225 Cluster 0.093 0.067 0.488 0.223 0.136 0.225 0.2284.0996] Lottery (Mw_emp) [0.053-0.126] [A) Adul	t tarsus	B) Nestli	ng tarsus	C) Lay date		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Mean model	Great tit	Blue tit	Great tit	Blue tit	Great tit	Blue tit	
$\begin{array}{llllllllllllllllllllllllllllllllllll$	<u>Fixed effects</u>							
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Intercept (β_{m0})	0.088	0.165	-1.453	-0.875	0.177	0.242	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	10 4 1000	[-0.39/-0.563]	[-0.751-1.085]	[-1.9/60.926]	[-1.805-0.124]	[-0.076-0.431]	[-0.222-0.746]	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	ISA 1000 m	-0.107	-0.06	-0.168	-0.092	-0.102	-0.021	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Latituda	[-0.1280.085]	[-0.0800.030]	[-0.210.120]	[-0.1/20.02]	[-0.1310.0/3]	[-0.072-0.029]	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Latitude	0.034	0.302	0.009	0.37	0.005	0.710	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Sey (male) Chick age	0.522	0.588	0 108	0.064	[0.465-0.641]	[0.38-0.999]	
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	NA	0.522	[0 563-0 613]	[0 09-0 125]	[0 037-0 091]			
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Random Effects	[0.501 0.511]	[0.505 0.015]	[0.09 0.123]	[0.057 0.071]			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Breeding Season	0.039	0.246	0.113	0.136	0.396	0.507	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	5	[0.025-0.058]	[0.188-0.327]	[0.083-0.155]	[0.094-0.191]	[0.307-0.516]	[0.395-0.668]	
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Study system	0.877	1.216	0.759	1.038	0.37	0.536	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		[0.582-1.341]	[0.742-1.969]	[0.488-1.2]	[0.613-1.718]	[0.24-0.582]	[0.298-0.996]	
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Cluster	0.093	0.067	0.18	0.253	0.136	0.225	
Dispersion model $Fixed effects$ -0.582 -0.512 -0.405 -0.433 -0.708 -0.485 Intercept ($\beta \nu_{0,exp}$) -0.582 -0.512 -0.405 -0.433 -0.708 -0.485 ISA 1000 m 0.011 0.011 0.068 0.081 0.043 0.013 $[-0.009-0.031]$ $[-0.019-0.044]$ $[0.03-0.107]$ $[0.011-0.155]$ $[0.008-0.076]$ $[-0.03-0.054]$ Heterogeneity 1000 m -0.01 0.001 0.005 0.001 0.024 -0.004 $[-0.035-0.015]$ $[-0.041-0.051]$ $[-0.034-0.045]$ $[-0.069-0.074]$ $[-0.016-0.063]$ $[-0.056-0.055]$ Latitude 0.045 0.275 0.083 0.1 -0.032 -0.024 $[-0.054-0.151]$ $[0.01-0.543]$ $[-0.043-0.213]$ $[-0.069-0.673]$ $[-0.105-0.043]$ $[-0.17-0.122]$ Cluster area 0.033 0.03 0.006 0.216 -0.014 -0.011 $[0.017-0.049]$ $[-0.123-0.193]$ $[-0.026-0.039]$ $[-0.023-0.452]$ $[-0.073-0.045]$ $[-0.163-0.127]$ Cluster years -0.012 0.026 0.013 -0.151 0.021 0.079 $Age (1)$ $[0.061-0.037]$ $[-0.037-0.028]$ $[-0.054-0.032]$ $[-0.043-0.088]$ $[-0.041-0.18]$ Sex (male) NA 0.245 0.414 0.248 0.111 0.13 0.208 Study system $[0.257-0.421]$ $[-0.66-0.525]$ $[0.669-0.383]$ $[-0.755-0.711]$ $[-0.22-0.818]$ $[-0.359-0.858]$ Study		[0.065-0.126]	[0.034-0.11]	[0.135-0.233]	[0.173-0.346]	[0.109-0.167]	[0.165-0.294]	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Dispersion model							
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	<u>Fixed effects</u>							
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Intercept ($\beta v_{0,exp}$)	-0.582	-0.512	-0.405	-0.433	-0.708	-0.485	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		[-0.7330.431]	[-0.8910.068]	[-0.570.224]	[-0.6570.205]	[-0.8130.596]	[-0.7380.267]	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	ISA 1000 m	0.011	0.011	0.068	0.081	0.043	0.013	
Heterogeneity 1000 m -0.01 0.001 0.005 0.001 0.024 -0.004 Latiude $[-0.035-0.015]$ $[-0.041-0.051]$ $[-0.034-0.045]$ $[-0.069-0.074]$ $[-0.016-0.063]$ $[-0.065-0.055]$ Latiude 0.045 0.275 0.083 0.1 -0.032 -0.024 $[-0.054-0.151]$ $[0.01-0.543]$ $[-0.043-0.213]$ $[-0.096-0.263]$ $[-0.05-0.043]$ $[-0.17-0.122]$ Cluster area 0.033 0.03 0.006 0.216 -0.014 -0.011 $[0.017-0.049]$ $[-0.123-0.193]$ $[-0.026-0.039]$ $[-0.023-0.452]$ $[-0.073-0.045]$ $[-0.163-0.127]$ Cluster years -0.012 0.026 0.013 -0.151 0.021 0.079 $[-0.061-0.037]$ $[-0.088-0.137]$ $[-0.066-0.091]$ $[-0.356-0.032]$ $[-0.043-0.088]$ $[-0.041-0.18]$ Sex (male) NA 0.026 -0.005 -0.032 -0.035 $[-0.043-0.088]$ $[-0.041-0.18]$ Study system (intercept) 0.245 0.414 0.248 0.111 0.13 0.208 $[0.149-0.4]$ $[0.182-0.873]$ $[0.142-0.425]$ $[0.004-0.367]$ $[0.059-0.235]$ $[0.065-0.469]$ Study system -0.098 -0.098 -0.098 -0.174 -0.062 0.383 0.327 $(r_{mean,dispersion})$ $[-0.567-0.421]$ $[-0.66-0.525]$ $[-0.669-0.383]$ $[-0.765-0.711]$ $[-0.22-0.818]$ $[-0.369-0.858]$ Cluster (intercept) 0.066 0.057 0.144 0.192 0.1	11 1000	[-0.009-0.031]	[-0.019-0.044]	[0.03-0.107]	[0.011-0.155]	[0.008-0.076]	[-0.03-0.054]	
Latitude $[-0.035-0.015]$ $[-0.041-0.051]$ $[-0.034-0.045]$ $[-0.069-0.074]$ $[-0.018-0.063]$ $[-0.050-0.053]$ Latitude 0.045 0.275 0.083 0.1 -0.032 -0.032 -0.024 $[-0.054-0.151]$ $[0.01-0.543]$ $[-0.043-0.213]$ $[-0.096-0.263]$ $[-0.105-0.043]$ $[-0.17-0.122]$ Cluster area 0.033 0.03 0.006 0.216 -0.014 -0.011 $[0.017-0.049]$ $[-0.123-0.193]$ $[-0.026-0.039]$ $[-0.023-0.452]$ $[-0.073-0.045]$ $[-0.163-0.127]$ Cluster years -0.012 0.026 0.013 -0.151 0.021 0.079 $[-0.061-0.037]$ $[-0.088-0.137]$ $[-0.066-0.091]$ $[-0.356-0.032]$ $[-0.043-0.088]$ $[-0.041-0.18]$ Sex (male) NA 0.026 -0.005 -0.032 -0.035 -0.032 -0.035 Age (1) $[0-0.051]$ $[-0.037-0.028]$ $[-0.066-0.091]$ $[-0.356-0.032]$ $[-0.043-0.088]$ $[-0.041-0.18]$ Study system (intercept) 0.245 0.414 0.248 0.111 0.13 0.208 $[0.149-0.4]$ $[0.182-0.873]$ $[0.142-0.425]$ $[0.004-0.367]$ $[0.059-0.235]$ $[0.065-0.469]$ Study system -0.098 -0.098 -0.174 -0.062 0.383 0.327 $(r_{mean,dispersion})$ $[-0.567-0.421]$ $[-0.66-0.525]$ $[-0.669-0.383]$ $[-0.765-0.711]$ $[-0.22-0.818]$ $[-0.369-0.858]$ Cluster (intercept) 0.066 0.057 0.144 <t< td=""><td>Heterogeneity 1000 m</td><td>-0.01</td><td>0.001</td><td>0.005</td><td>0.001</td><td>0.024</td><td>-0.004</td></t<>	Heterogeneity 1000 m	-0.01	0.001	0.005	0.001	0.024	-0.004	
Latitude 0.045 0.275 0.07 0.035 0.1 -0.052 -0.024 Cluster area $[-0.054-0.151]$ $[0.01-0.543]$ $[-0.043-0.213]$ $[-0.096-0.263]$ $[-0.105-0.043]$ $[-0.7024]$ Cluster area 0.033 0.03 0.006 0.216 -0.014 -0.011 $[0.017-0.049]$ $[-0.123-0.193]$ $[-0.026-0.039]$ $[-0.023-0.452]$ $[-0.073-0.045]$ $[-0.163-0.127]$ Cluster years -0.012 0.026 0.013 -0.151 0.021 0.079 $[-0.061-0.037]$ $[-0.088-0.137]$ $[-0.066-0.091]$ $[-0.356-0.032]$ $[-0.043-0.088]$ $[-0.041-0.18]$ Sex (male) NA 0.026 -0.005 -0.035 -0.032 -0.035 Age (1) $[0-0.051]$ $[-0.037-0.028]$ $[-0.043-0.087]$ $[-0.075-0.009]$ $[-0.088-0.016]$ Study system (intercept) 0.245 0.414 0.248 0.111 0.13 0.208 $[0.149-0.4]$ $[0.182-0.873]$ $[0.142-0.425]$ $[0.004-0.367]$ $[0.059-0.235]$ $[0.065-0.469]$ Study system -0.098 -0.098 -0.174 -0.062 0.383 0.327 $(r_{mean,dispersion})$ $[-0.567-0.421]$ $[-0.66-0.525]$ $[-0.669-0.383]$ $[-0.765-0.711]$ $[-0.22-0.818]$ $[-0.369-0.858]$ Cluster (intercept) 0.066 0.057 0.144 0.192 0.131 0.114 $[0.039-0.098]$ $[0.01-0.11]$ $[0.17-0.188]$ $[0.107-0.29]$ $[0.092-0.174]$ $(0.031-0.204]$ </td <td>Latituda</td> <td>[-0.035-0.015]</td> <td>[-0.041-0.051]</td> <td>[-0.034-0.045]</td> <td>[-0.069-0.074]</td> <td>[-0.010-0.003]</td> <td>[-0.065-0.055]</td>	Latituda	[-0.035-0.015]	[-0.041-0.051]	[-0.034-0.045]	[-0.069-0.074]	[-0.010-0.003]	[-0.065-0.055]	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Latitude	0.045 [-0.054-0.151]	0.275 [0.01_0.543]	[_0.043_0.213]	[_0.096_0.263]	-0.032 [_0.105_0.043]	-0.024	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Cluster area	0.033	0.03	0.006	0.216	-0.014	-0.011	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Cluster area	[0 017-0 049]	[-0.123-0.193]	[-0.026-0.039]	[-0.023-0.452]	[-0.073-0.045]	[-0.163-0.127]	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Cluster years	-0.012	0.026	0.013	-0.151	0.021	0.079	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	<u> </u>	[-0.061-0.037]	[-0.088-0.137]	[-0.066-0.091]	[-0.356-0.032]	[-0.043-0.088]	[-0.041-0.18]	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Sex (male) NA	0.026	-0.005			-0.032	-0.035	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Age (1)	[0-0.051]	[-0.037-0.028]			[-0.075-0.009]	[-0.088-0.016]	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	<u>Random effects</u>							
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Study system (intercept)	0.245	0.414	0.248	0.111	0.13	0.208	
Study system -0.098 -0.098 -0.174 -0.062 0.383 0.327 $(r_{mean,dispersion})$ $[-0.567-0.421]$ $[-0.66-0.525]$ $[-0.669-0.383]$ $[-0.765-0.711]$ $[-0.22-0.818]$ $[-0.369-0.858]$ Cluster (intercept) 0.066 0.057 0.144 0.192 0.131 0.114 $[0.039-0.098]$ $[0.01-0.11]$ $[0.107-0.188]$ $[0.107-0.29]$ $[0.092-0.174]$ $[0.031-0.204]$		[0.149-0.4]	[0.182-0.873]	[0.142-0.425]	[0.004-0.367]	[0.059-0.235]	[0.065-0.469]	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Study system	-0.098	-0.098	-0.174	-0.062	0.383	0.327	
Cluster (intercept) 0.066 0.057 0.144 0.192 0.131 0.114 $[0.039-0.098]$ $[0.01-0.11]$ $[0.107-0.188]$ $[0.107-0.29]$ $[0.092-0.174]$ $[0.031-0.204]$	(<i>r</i> _{mean,dispersion})	[-0.567-0.421]	[-0.66-0.525]	[-0.669-0.383]	[-0.765-0.711]	[-0.22-0.818]	[-0.369-0.858]	
[0.039-0.098] [0.01-0.11] [0.107-0.188] [0.107-0.29] [0.092-0.174] [0.031-0.204]	Cluster (intercept)	0.066	0.05/	0.144	0.192	0.131	0.114	
(1) (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Chuster (n	[0.039-0.098]	[0.01-0.11]	[0.10/-0.188]	[0.107-0.29]	[0.092-0.1/4]	[0.031-0.204]	
$\begin{array}{c} \textbf{-0.009} & \textbf{-0.009} & \textbf{-0.001} \\ \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} \\ \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} \\ \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} & \textbf{-0.004} \\ \textbf{-0.004} & \textbf{-0.004} &$	Cluster (<i>r</i> mean, dispersion)	-0.040	-0.329 [-0.86-0.414]	-0.009	-0.301	[-0.34-0.348]	[-0.353-0.646]	

Table S3.4 : Model estimates when examining the effect of continuous urbanization (i.e., impervious surface area; ISA) at 100 meters instead of the categorical effect of habitat (i.e., urban vs. forest). Effects highlighted in bold have changed conclusions (i.e., credible interval overlaps or not zero) for A) adult tarsus length and B) nestling tarsus length, and C) female lay dates from Table 3.1 in main text, but most conclusions remain the same.

	A) Adult tarsus		B) Nestli	ng tarsus	C) Lay date		
Mean model	Great tit	Blue tit	Great tit	Blue tit	Great tit	Blue tit	
Fixed effects							
Intercept (β_{m0})	0.077	0.118	-1.472	-0.909	0.175	0.256	
77 4 400	[-0.413-0.568]	[-0.722-1.014]	[-1.9910.96]	[-1.848-0.091]	[-0.079-0.442]	[-0.215-0.768]	
ISA 100 m	-0.085	-0.053	-0.14	-0.077	-0.098	-0.037	
T	[-0.1120.057]	[-0.0/50.03]	[-0.1850.094]	[-0.1390.017]	[-0.1260.0/1]	[-0.082-0.008]	
Latitude	0.619	0.551	0.601	0.564	0.655	0.718	
Say (mala) Chialt aga	[0.295-0.924]	[-0.018-1.089]	[0.269-0.931]	[-0.1/2-1.25/]	[0.465-0.828]	[0.393-1]	
Sex (male) Chick age	0.325	0.300	0.109	0.005			
NA Baudom Efforts	[0.504-0.54]	[0.505-0.015]	[0.092-0.127]	[0.038-0.092]			
Random Effects	0.020	0.247	0.114	0.127	0.205	0.505	
Breeding Season	[0.039	[0.191_0.324]	[0.082_0.156]	[0.096_0.192]	0.395 [0.308_0.513]	[0.301_0.652]	
Study system	0.859	1 179	0.746	1 021	0.385	0.527	
Study system	[0 568-1 314]	[0 709-1 931]	[0 479-1 1821	[0 597-1 736]	[0 246-0 626]	[0 297-0 947]	
Cluster	0 142	0.061	0 224	0 249	0 134	0 223	
Clubter	[0 113-0 179]	[0 027-0 103]	[0 175-0 282]	[0 169-0 342]	[0 108-0 165]	[0 164-0 299]	
Dispersion model	[0.115 0.175]	[0.027 0.105]	[0.175 0.202]	[0.107 0.5 12]	[0.100 0.100]	[0.101 0.299]	
Fixed effects							
Intercept $(\beta v_{0, exp})$	-0.581	-0.501	-0.411	-0.439	-0.719	-0.48	
1 () (),(),(),()	[-0.7250.433]	[-0.8770.076]	[-0.5740.246]	[-0.6420.23]	[-0.8230.605]	[-0.6980.27]	
ISA 100 m	0.009	0.007	0.06	0.072	0.035	0.022	
	[-0.012-0.03]	[-0.019-0.035]	[0.02-0.099]	[0.022-0.123]	[0.004-0.067]	[-0.013-0.057]	
Heterogeneity 100m	-0.007	-0.016	0.009	0.002	0.006	-0.032	
	[-0.029-0.014]	[-0.047-0.014]	[-0.027-0.046]	[-0.055-0.055]	[-0.031-0.044]	[-0.081-0.014]	
Latitude	0.042	0.276	0.084	0.127	-0.027	-0.022	
	[-0.055-0.151]	[-0.003-0.539]	[-0.034-0.209]	[-0.051-0.283]	[-0.103-0.048]	[-0.156-0.103]	
Cluster area	0.033	0.004	0.002	0.219	-0.025	-0.018	
	[0.016-0.05]	[-0.145-0.151]	[-0.031-0.036]	[0.005-0.421]	[-0.08-0.031]	[-0.153-0.117]	
Cluster years	-0.009	0.039	0.007	-0.142	0.015	0.094	
	[-0.059-0.042]	[-0.064-0.146]	[-0.076-0.086]	[-0.329-0.028]	[-0.047-0.081]	[-0.012-0.187]	
Sex (male) NA	0.026	-0.003			-0.032	-0.034	
Age (1)	[-0.001-0.051]	[-0.037-0.03]			[-0.0/4-0.01]	[-0.086-0.016]	
Random effects	0.044	0.422	0.040	0.1	0.122	0.10	
Study system (intercept)	0.244	0.433	0.242	0.1	0.133	0.18	
Study system	[0.147-0.402]	[0.191-0.883]	[0.138-0.415]	[0.004-0.341]	[0.039-0.243]	[0.048-0.432]	
(r	-0.103	-0.098	-0.1/1	0.007	0.330	0.376 0.9041	
(I mean, dispersion)	0.065	0.056	[-0.008-0.387]	0.177	0 120	0.11	
Clusier (Intercept)	[0.038-0.096]	[0.030]	0.149 [0 111_0 1071	[0 093_0 275]	[0 09_0 172]	[0.034-0.101]	
Cluster $(r \dots)$	-0 514	_0.461	-0.654	-0 587	0.041	0 139	
('mean,dispersion/	[-0.830.074]	[-0.923-0.298]	[-0.8540.379]	[-0.9060.111]	[-0.31-0.376]	[-0.423-0.601]	

	A) Adult tarsus	B) Nestling tarsus
Mean model	Great tit	Great tit
Fixed effects		
Intercept (β_{m0})	0.157 [-0.304-0.626]	-2.215 [-2.7381.706]
Habitat (urban)	-0.296 [-0.3790.213]	-0.618 [-0.7710.467]
Latitude	0.589 [0.238-0.912]	0.179 [-0.153-0.492]
Sex (male) Chick age -	0.522 [0.504-0.541]	0.169 [0.143-0.195]
Random Effects		
Breeding Season	0.039 [0.025-0.058]	0.154 [0.108-0.219]
Study system	0.871 [0.58-1.329]	0.46 [0.238-0.888]
Cluster:		
Forest	0.052 [0.031-0.079]	0.153 [0.091-0.237]
Urban	0.223 [0.16-0.304]	0.206 [0.09-0.344]
Dispersion model		
Fixed effects		
Intercept ($\beta v_{0,exp}$)	-0.586 [-0.7390.429]	-0.113 [-0.421-0.179]
Habitat (urban)	0.044 [-0.023-0.111]	0.19 [0.054-0.331]
Heterogeneity 1000m	-0.007 [-0.037-0.02]	0.001 [-0.039-0.042]
Latitude	0.042 [-0.051-0.146]	0.105 [-0.116-0.336]
Cluster area	0.232 [-0.492-0.935]	0.511 [-1.319-2.282]
Cluster years	-0.013 [-0.069-0.042]	0.01 [-0.09-0.109]
Sex (male) - Age (1)	0.026 [0.001-0.051]	
Random effects		
Study system (intercept)	0.239 [0.146-0.394]	0.302 [0.152-0.605]
Study system ($r_{\text{mean,dispersion}}$)	-0.121 [-0.602-0.398]	-0.281 [-0.8-0.396]
Cluster:		
Forest (intercept)	0.064 [0.031-0.105]	0.115 [0.072-0.174]
Forest (<i>r</i> _{mean,dispersion})	-0.446 [-0.864-0.172]	-0.687 [-0.9460.235]
Urban (intercept)	0.065 [0.006-0.131]	0.152 [0.069-0.259]
Urban (<i>r</i> mean, dispersion)	-0.3 [-0.851-0.45]	-0.605 [-0.957-0.015]

Table S3.5 : Model estimates when not including the city of Munich location. Effects highlighted in bold have changed conclusions (i.e., credible interval now overlaps or not zero) for A) adult tarsus length and B) nestling tarsus length from Table 3.1 in main text, but most conclusions remain the same.



Figure S3.1 : Number of individuals for adult tarsus length for each cluster (or groups of individuals) across study systems (ANT = Antwerp, BUD = Budapest, GLA = Glasgow, GOT = Göteborg, HAR = Harjavalta, HEL = Helsingborg, Malmo = Malmö, MON = Montpellier, MUN = Munich, PAR = Paris, STR = Strasbourg, VES = Veszprém, WAR = Warsaw). Top panel shows range of observations between 0 and 20 while bottom panel shows full range of observations. Habitat type (forest = green, urban = blue), species (light colour = blue tit, dark colour = great tit), and the cluster ID (number above each bar; see also Table S1) are shown.



Figure S3.2 : Number of individuals for nestling tarsus length for each cluster (or groups of individuals) across study systems (ANT = Antwerp, BUD = Budapest, GLA = Glasgow, GOT = Göteborg, HAR = Harjavalta, HEL = Helsingborg, Malmo = Malmö, MON = Montpellier, MUN = Munich, PAR = Paris, STR = Strasbourg, VES = Veszprém, WAR = Warsaw). Top panel shows range of observations between 0 and 20 while bottom panel shows full range of observations. Habitat type (forest = green, urban = blue), species (light colour = blue tit, dark colour = great tit), and the cluster ID (number above each bar; see also Table S1) are shown.



Figure S3.3: Number of individuals for female lay date for each cluster (or groups of individuals) across study systems (ANT = Antwerp, BUD = Budapest, GLA = Glasgow, GOT = Göteborg, HAR = Harjavalta, HEL = Helsingborg, Malmo = Malmö, MON = Montpellier, MUN = Munich, PAR = Paris, STR = Strasbourg, VES = Veszprém, WAR = Warsaw). Top panel shows range of observations between 0 and 20 while bottom panel shows full range of observations. Habitat type (forest = green, urban = blue), species (light colour = blue tit, dark colour = great tit), and the cluster ID (number above each bar; see also Table S1) are shown.



Figure S3.4: Relationship between the Shannon diversity index of land cover heterogeneity (calculated using the number of different land cover types, see Chapter 3 methods) and the proportion of impervious surface area (ISA or urbanization) at 1000m for: (top panel) each nest box (N = 7210) in the combined dataset and (bottom panel) the averages for each study location (N = 119) in the dataset.



Figure S3.5 : The effect of proportion of impervious surface area (ISA; higher values indicate higher urbanization) at 1000 meters on the mean adult tarsus length (left panel), mean nestling tarsus length (middle panel), and mean lay date (right panel; in Julian days since Jan 1) in great and blue tits (orange and blue, respectively).

Supplementary Adult tarsus model equations : Summary and descriptions of model equation terms used to evaluate changes in mean and variation of adult tarsus length in response to urbanization using double hierarchical linear mixed models where fixed and random effects are fit in the mean and dispersion (i.e., to explain residual variation) parts of the model. Models presented for nestling tarsus and lay date have similar model equations.

	Model notation	Rand	om effects	F	ixed effects (X_{ij})	Type*
Yijkln	Phenotypic value of trait y for the <i>l</i> th system, the <i>n</i> th year, the <i>k</i> th cluster, and the <i>j</i> th habitat at individual (instance) <i>i</i>	CLU	clusters (k)	HABITAT	Habitat type (j) of cluster k where $X_k = 0$ for forest and 1 for urban	CAT
β_m	Estimate from mean model where β_{m0} is population intercept	SYST	Study system (1)	LAT	Latitude of cluster k where $X_k = 0$ is mean latitude	CONT
$\beta_{v,exp}$	Estimate (on ln scale) from dispersion model where $\beta v_{0,exp}$ is population intercept	YEAR	Year of each breeding season (m)	SEX	Sex of system <i>l</i> , for year <i>n</i> , at cluster <i>k</i> , of individual <i>i</i> where X _{ikln} = 0 for females and 1 for males	CAT
e _{ikln}	Residual error or difference between predicted and fitted value <i>l</i> th system, the <i>n</i> th year, and the <i>k</i> th cluster at individual (instance) <i>i</i>			HET	Land cover heterogeneity of cluster k where $X_k = 0$ is mean heterogeneity	CONT
ρ(a,b)	Correlation between two random effect variables			AREA	Area (m ²) of cluster k where $X_k = 0$ is mean area	CONT
				YEARS	Number of years of data collection of cluster k where $X_k = 0$ is mean number of years	CONT

$$y_{ijkln} = (\beta_{m0} + CLU_{m0kj} + SYST_{m0l} + YEAR_{m0n}) + \beta_{m1}HABITAT_k + \beta_{m2}LAT_k$$

$$+ \beta_{m3}SEX_{ikln} + e_{ikln}$$
[Eq.S3.1]

$$ln(\sigma_{e_{ikln}}^{2}) = (\beta_{v0,exp} + CLU_{v0kj,exp} + SYST_{v0l,exp} + YEAR_{v0n,exp}) + \beta_{v1,exp}HABITAT_{k}$$

$$+ \beta_{v2,exp}HET_{k} + \beta_{v3,exp}LAT_{k} + \beta_{v4,exp}AREA_{k} + \beta_{v5,exp}YEARS_{k}$$

$$+ \beta_{v6,exp}SEX_{kln}$$

$$(Eq.S3.2)$$

$$e_{ikln} \sim N(0, \sigma_{e_{ikln}}^2)$$
 [Eq.S3.3]

$$YEAR_n \sim N(0, \sigma_{YEAR_n}^2)$$
 [Eq.S3.4]

$$SYST_{l} \sim N_{2} \left(0, \frac{\sigma^{2}_{SYST_{m0}} \quad \rho\left(SYST_{m0j}, SYST_{v0j,exp}\right) \sigma_{SYST_{v0j,exp}} \sigma_{SYST_{v0j,exp}} \right)$$
[Eq.S3.5]

	/	$\sigma^2_{CLU^{urban}_{m0}}$	$ hoig({\it CLU}_{m0j}^{urban}$, ${\it CLU}_{v0j,exp}^{urban}ig) \sigma_{{\it CLU}_{m0}}^{urban} \sigma_{{\it CLU}_{v0,exp}}^{urban}$	0	0	[Eq.S6]
$CLU_k \sim N_4$			$\sigma^2_{_{CLU_{v0,exp}^{urban}}}$	0	0	
	0,	,		$\sigma^2_{CLU_{m0}^{forest}}$	$ ho(CLU_{m0j}^{forest}, CLU_{v0j,exp}^{forest})\sigma_{CLU_{m0}}^{forest}\sigma_{CLU_v}^{fores}$	
					$\sigma^2{}_{\scriptscriptstyle CLU^{forest}_{v0,exp}}$	

ANNEXE C

Supplementary materials for Chapter 4

Supplementary methods :

Common garden animal models: We built upon the common garden models presented in Table 4.2.2 of the main text by additionally fitting a genetic relatedness matrix (GRM) as random effect. This approach allowed us to partition variance between the GRM (individual genetic relatedness; V_A), individual ID (includes individual-specific environmental variation; V_{ID}), and origin nest (includes differences in maternal investment; V_{NO}) random effects, otherwise model structures remained the same. For these animal models, we used weakly informative inverse-Gamma priors (V = 1, nu = 0.002) for fixed effects and parameter-expanded priors (i.e., V = 1, nu = 1, alpha.mu = 0, alpha.V = 1000) for random effects as we expected quantitative genetic parameters to be small (de Villemereuil, 2018). We ran all models with MCMCglmm for 1000000 iterations (except tarsus which we ran for 2000000), with a thinning of 500 and a burn-in period of 10000, which achieved effective sample sizes > 1000 across all estimates.

Heritability and Q_{ST} / F_{ST} *comparisons* : We computed the heritability (h^2) of each trait in the common garden animal models as:

$$h^2 = \frac{V_A}{V_P} \tag{1}$$

where
$$V_P = V_A + V_{ID} + V_{NO} + V_{NF} + V_{AV} + V_F$$
 (2)

where V_A is the individual genetic variance (i.e., additive genetic variance) and V_P is the total phenotypic variance and comprises variance across individual genetic relatedness (V_A), individuals (V_{ID}), origin nests (V_{NO}), foster nests (V_{NF}), aviaries (V_{AV}), and residuals (V_R). We also included variance generated by nonexperimental fixed effects (V_F) in the model (de Villemereuil et al., 2018), which included habitat and sex effects. For the Poisson animal model (exploration), we used the QCglmm package (de Villemereuil et al., 2016) to convert the variance components and heritability estimate from the latent scale to the data scale.

We also computed Q_{ST} values for the common garden traits where habitat differences were clearly maintained (i.e., breath rate and body mass) so we could make Q_{ST} - F_{ST} comparisons and evaluate whether processes other than adaptive evolution, specifically genetic drift, can partly explain phenotypic and genetic

differentiation observed between populations (Leinonen et al., 2013). Q_{ST} , measured at the phenotypic level in the common garden, quantifies the additive genetic variation between populations relative to the total genetic variance in a phenotype. As we use animal models that directly estimated the additive genetic variation for each trait in the common garden, we estimated Q_{ST} as:

$$Q_{ST} = \frac{V_B}{V_B + 2V_A} \tag{3}$$

where V_B is the between-population genetic variance (habitat fixed effect variance) and V_A is the withinpopulation genetic variance. We additionally corrected V_B by the uncertainty around estimating this effect (product of the beta and design covariance matrices) due to our sample size. We compared the Q_{ST} value to F_{ST} , measured at the molecular level across wild populations, which quantifies neutral molecular variance and represents a null expectation that the observed differentiation between populations is a result of genetic drift and migration. We compared to previous estimates of F_{ST} for the study populations where all urban and forest comparisons were between 0.006-0.009 (Perrier et al., 2018). $Q_{ST} > F_{ST}$ (here 0.11 and 0.12 for breath rate and body mass, respectively, Table S4.3) reveals opposite directional selection pressures in the two populations favouring local adaptation and a stronger divergence in the trait between these populations than expected with genetic drift alone (Leinonen et al., 2013).

Table S4.1: Summary of the transfer of eggs to foster nests and nestlings transferred to the nursery under common garden conditions from urban and rural origin habitats in Montpellier, France. Origin nests were spread across four urban sites (FON, MAS, FAC, CEF) and one forest site (ROU), and were transferred to wild forest nests at a single site at the Montpellier Zoo (ZOO). We could not identify the exact origin nest for foster nest ZOO46 as we were missing parental identity and genotyping revealed that these origin nests (FAC2, CEF7) included 3 pairs of siblings.

Origin habitat	Origin nest ID	Origin lay date (days since Jan 1)	Eggs	Foster nest ID	Average egg weight (g)	Nestlings	Nursery date (days since Jan 1)
Urban	FON16	91	4	ZOO42	NA	4	119
	MAS31	90	4	ZOO42	NA	4	
	FON18	91	4	ZOO63	NA	4	123
	MAS28	91	4	ZOO63	NA	3	
	FAC7	94	3	ZOO69	1.49	1	123
	FAC17	94	3	ZOO69	1.63	3	
	FAC4	98	3	ZOO32	1.56	1	126
	CEF9	100	3	ZOO32	1.33	3	
	FON3	91	4	ZOO40	1.48	3	126
	MAS40	90	3	ZOO40	1.57	1	
	FAC2	101	3	ZOO46	1.66	2 or 4	131
	CEF7	100	4	ZOO46	1.43	2 or 4	151
	MAS37	107	4	ZOO30	1.70	4	135
	FON5	108	4	ZOO30	1.74	4	155
Total/ Average	14	97.08	50	7	1.56	41	126.33
Forest	ROU13s	98	4	ZOO19	1.83	4	128
	ROU334	99	4	ZOO19	1.64	4	
	ROU12s	105	4	ZOO67	1.66	4	133
	ROU17s	104	4	ZOO67	1.75	4	
	ROU15s	105	4	ZOO35	1.81	4	131
	ROU9s	104	4	ZOO35	1.67	4	
	ROU324	108	4	ZOO65	1.69	0	NA
	ROU7s	108	4	ZOO65	1.62	0	
	ROU3s	109	4	ZOO47	1.65	4	126
	ROU10s	107	4	ZOO47	1.67	4	130
Total/ Average	10	104.70	40	5	1.70	32	131.86
Table S4.2: Overview of phenotyping in the common garden experiment including number of observations, individuals, and mean and range of number of repeated measures per individual. The age when individuals were measured is shown as well as repeatability across wild and common garden contexts. Note that genotypic data was only available for 72 individuals and so animal models will exclude the one individual that wasn't sequenced.

Phenotype	Observations	Individuals	Mean number of repeated measures	Range of number of repeated measures	Mean age when assayed (days old)
Tarsus	211	72	2.92	1-3	44.67, 159.25, 264.83
Mass	210	71	2.92	1-3	44.67, 159.25, 264.83
Aggression	282	73	3.89	1-4	44.67, 74.38, 159.25, 264.83
Breath rate	284	73	3.89	1-4	44.67, 74.38, 159.25, 264.83
Exploration	203	70	2.89	1-3	74.38, 159.25, 264.83

Table S4.3 : Common garden animal model comparison to Table 4.3 when including the genetic relatedness matrix (GRM) used to additionally estimate individual genetic variance (V_A). Fixed and random model estimates and 95% credible intervals (CI) across phenotypic traits (A: Aggression in hand, B: Breath rate index, C: Exploration, D: Tarsus length, and E: body mass). Exploration estimates are from a Poisson generalized mixed-effect model, while all other traits were fit with Gaussian mixed-effect models. Interactions between sex and habitat were not significant across traits and dropped from the model. The number of observations (obs) and individuals (ind) for each trait and context are shown in the top panel. We were missing genetic data on one individual and so our sample sizes and observations differ slightly from those reported in Table 4.3. We also report estimated heritability across all traits and Q_{ST} values for traits where we see a habitat difference (breath rate index and body mass).

	A) .	Aggression	B) Breath rate		C) Ex	ploration	D) Tarsus length		E) Body mass	
	N = 23	80 obs, 72 ind	N = 2	79 obs, 72 ind	N = 200) obs, 69 ind	N = 20	8 obs, 71 ind	N = 20	07 obs, 70 ind
Fixed effects	Est	CI	Est	CI	Est	CI	Est	CI	Est	CI
Intercept	2.20	0.99-3.55	9.41	7.14-11.84	4.03	1.89-6.11	19.41	19.04-19.8	15.93	14.76-17.13
Habitat (urban)	0.15	-0.23-0.53	-1.11	-2.54-0.46	-0.05	-1.12-1.23	-0.22	-0.75-0.25	-0.51	-1.14-0.03
Sex (male)	-0.22	-0.48-0.05	0.76	-0.05-1.51	-0.41	-0.97-0.08	0.51	0.28-0.71	1.03	0.77-1.31
Time of day	-0.04	-0.16-0.08	0.47	0.28-0.68	-0.06	-0.26-0.11			-0.11	-0.22-0
Measurement	-0.12	-0.42-0.17	0.88	0.42-1.32	-0.19	-0.62-0.27			0.12	-0.02-0.32
(2)										
Measurement	0.34	-0.18-0.82	0.44	0.03-0.87	-0.09	-0.53-0.35			0.51	0.36-0.66
(3)										
Measurement	0.23	-0.26-0.72	-0.40	-0.78-0.02						
(4)										
Observer (2)	-0.67	-1.090.23					0.07	0.04-0.1		
Random effects										
GRM	0.04	0-0.17	1.46	0-3.35	0.43	0-1.08	0.16	0-0.34	0.26	0-0.49
(V_A)										
Individual ID	0.17	0-0.29	1.18	0-2.48	0.28	0-0.79	0.09	0-0.21	0.09	0-0.26
(V _{ID})										
Origin nest ID	0.02	0-0.08	0.51	0-1.68	0.22	0-0.74	0.08	0-0.24	0.07	0-0.25
(V _{ON})										
Foster nest ID	0.02	0-0.08	0.39	0-1.53	0.47	0-1.68	0.05	0-0.19	0.06	0-0.25
(V _{NF})										
Aviary ID	0.02	0-0.08	0.47	0-1.62	0.13	0-0.51				
(V_{AV})										
Residual	0.52	0.42-0.62	1.54	1.25-1.83	1.59	1.16-2	0.01	0.01-0.02	0.18	0.14-0.23
variance (V _R)										
h^2	0.02	0.00006-	0.24	0.002-0.59	0.02	0-0.06	0.33	0.003-0.72	0.28	0.01-0.49
		0.24								
Q _{ST}			0.11	0.0008-0.94					0.12	0.001-0.82

Table S4.4 : Model comparisons to Table 4.3 when replacing the habitat effect with the proportion ISA (impervious surface area at 100m; continuous urbanization). Fixed and random model estimates and 95% credible intervals (CI) for 1) wild and 2) common garden contexts across phenotypic traits (A: Aggression in hand, B: Breath rate index, C: Exploration, D: Tarsus length, and E: Body mass). Exploration estimates are from Poisson generalized mixed-effects model, while all other traits were fit with Gaussian mixed-effects models. The number of observations (obs) and individuals (ind) for each trait and context are shown in the top panel.

1) WILD										
	A) A	Aggression	B)]	Breath rate	C) E	Exploration	D) (Farsus length	E)	Body mass
	N = 1	308 obs, 773	N = 70	2 obs, 531 ind	N = 5	581 obs, 472	N = 14	74 obs, 861 ind	N = 13	91 obs, 817 ind
Eined offecte	Eat	ind	Eat	CI	Eat	ind	Eat	CI	Eat	CI
<u>Fixed effects</u>	Est	1 21 2 06	Est 12.91	10.4.14.90	2 97	1.05.6.71	ESt 10.22	10 17 10 47	ESt	15 20 16 2
Intercept	2.50	0.20.0.22	12.81	10.4-14.69	3.07	0.07.2.15	19.55	0.24 0.06	0.25	15.59-10.5
ISA Say (mala)	0.01	-0.29-0.32	-0.80	-1.380.05	1.25	-0.07-2.13	-0.20	-0.340.00	-0.55	-0.620.02
Sex (male)	0.17	-0.03-0.33	0.00	-0.3-0.41	-0.04	-0.43-0.30	0.55	0.47-0.62	0.02	0.31-0.72
Age (yearing)	-0.08	-0.18-0.03	0.00	-0.31-0.5	0.11	-0.25-0.45			-0.32	-0.390.23
Data of	-0.04	-0.060.01	0.00	0.02.0.01	-0.01	-0.1-0.07			0.04	0.02-0.00
Date 01	0.00	0-0	0.00	-0.02-0.01	-0.01	-0.03-0.01			0.00	0-0
Protocol (old)			0.18	0 35 0 73	0.17	0 30 0 71				
Temperature			0.18	-0.35-0.73	0.17	-0.39-0.71				
ISA * Sex	0.26	0.0.57	0.09	0.00-0.15						
Bandom affacts	0.20	0-0.57								
Individual ID	0.45	0.36.0.54	2.63	2 07 3 18	283	2 08 3 67	0.28	0.25.0.31	0.38	0 33 0 45
(Vnr)	0.45	0.30-0.34	2.03	2.07-5.10	2.05	2.08-3.07	0.20	0.25-0.51	0.36	0.33-0.45
Site ID	0.02	0-0.08	0.24	0-0.76	0.21	0-1.08	0.01	0-0.04	0.03	0-0.13
(V _{SITE})	0.02	0 0.00	0.21	0 0.70	0.21	0 1.00	0.01	0 0.01	0.05	0 0.15
Year ID	0.02	0-0.07	0.05	0-0.18	0.09	0-0.33	0.00	0-0	0.06	0.01-0.14
(V _{YEAR})										
Observer ID	0.04	0.01-0.09	0.57	0.08-1.39			0.01	0-0.01		
(V _{OBS})										
Residual	0.54	0.48-0.61	1.75	1.41-2.14	1.61	1.12-2.16	0.02	0.02-0.02	0.28	0.24-0.31
variance										
2) COMMON GA	ARDEN									
2) COMMON GA	ARDEN A) A	Aggression	B) I	Breath rate	C) E	Exploration	D) 7	Farsus length	E)	Body mass
2) COMMON GA	ARDEN A) A N = 28	Aggression 80 obs, 72 ind	B) I N = 27	Breath rate 9 obs, 72 ind	C) E N = 20	Exploration 00 obs, 69 ind	D) 7 N = 2	Farsus length 08 obs, 71 ind	E) N = 20	Body mass 07 obs, 70 ind
2) COMMON GA	ARDEN A) A N = 28 Est	Aggression 30 obs, 72 ind CI	B) I N = 27 Est	Breath rate 79 obs, 72 ind CI	C) E N = 20 Est	Exploration 00 obs, 69 ind CI	D) $T = 2$ Est	Farsus length 08 obs, 71 ind CI	E) N = 20 Est	Body mass 07 obs, 70 ind CI
2) COMMON GA	ARDEN A) A N = 28 Est 2.13	Aggression 30 obs, 72 ind CI 0.93-3.25	B) I $N = 27$ Est 9.06	Breath rate 79 obs, 72 ind CI 6.85-11.44	C) E N = 20 Est 3.86	Exploration 00 obs, 69 ind CI 1.91-6.02	D) 7 N = 2 Est 19.44	Farsus length 08 obs, 71 ind CI 19.14-19.73	E) N = 20 Est 15.81	Body mass 07 obs, 70 ind CI 14.47-16.91
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61	B) I = 27 Est 9.06 -0.58	Breath rate 79 obs, 72 ind CI 6.85-11.44 -2.14-1.01	C) E N = 20 Est 3.86 0.47	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41	D) $T = 2$ Est 19.44 -0.29	Carsus length .08 obs, 71 ind CI 19.14-19.73 -0.75-0.13	E) N = 20 Est 15.81 -0.54	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18	Aggression 80 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12	B) I = 27 Est 9.06 -0.58 0.80	Breath rate 79 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58	C) E N = 20 Est 3.86 0.47 -0.35	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10	Aggression 80 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.46	Breath rate '9 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67	C) E N = 20 Est 3.86 0.47 -0.35 -0.06	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 -0.10	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10	Aggression 80 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18	$B) 1 \\ N = 27 \\ Est \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ ext{}$	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37	C) = 0 $N = 20$ Est 3.86 0.47 -0.35 -0.06 -0.19	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 -0.26	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.40 000 000 000 0000000000	Breath rate /9 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 0.12	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27
2) COMMON GA	ARDEN ARDEN N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88	$B) 1 \\ N = 27 \\ Est \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ 0.49$	Breath rate /9 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95	$\begin{array}{c} C) \ E \\ N = 20 \\ \hline Est \\ 3.86 \\ 0.47 \\ -0.35 \\ -0.06 \\ -0.19 \\ -0.13 \end{array}$	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) / N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 0.18.0.76	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 0.70.0.04	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 0.60	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 1.11 0.2	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27	$\begin{array}{c} \text{E)} \\ \text{N} = 2t \\ \text{Est} \\ 15.81 \\ -0.54 \\ 1.02 \\ -0.10 \\ 0.12 \\ 0.50 \\ \end{array}$	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05	B) 1 N = 27 Est 9.06 -0.58 0.80 0.46 0.94 0.49 -0.38 2.11 0.85	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13 0.49 0.19	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97 0-0.97	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45 0.07 0.18 0.10	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27 0-0 24	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0 29
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05	$\begin{array}{c} \text{B) 1} \\ \text{N} = 27 \\ \text{Est} \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ -0.38 \\ 2.11 \\ 0.85 \end{array}$	Breath rate '9 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16	$\begin{array}{c} \text{C) E} \\ \text{N} = 20 \\ \text{Est} \\ 3.86 \\ 0.47 \\ -0.35 \\ -0.06 \\ -0.19 \\ -0.13 \\ \end{array}$	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97 0-0.66	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45 0.07 0.18 0.10	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27 0-0.24	$\begin{array}{c} \text{E)} \\ \text{N} = 2t \\ \text{Est} \\ 15.81 \\ -0.54 \\ 1.02 \\ -0.10 \\ 0.12 \\ 0.50 \\ \end{array}$	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0.29
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01 0.01	Aggression 0 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05 0-0.05	$\begin{array}{c} \text{B) 1} \\ \text{N} = 27 \\ \text{Est} \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ -0.38 \\ 2.11 \\ 0.85 \\ 0.24 \end{array}$	Breath rate 29 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16 0-1.12	$\begin{array}{c} \text{C) E} \\ \text{N} = 20 \\ \text{Est} \\ 3.86 \\ 0.47 \\ -0.35 \\ -0.06 \\ -0.19 \\ -0.13 \\ \end{array}$	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97 0-0.97 0-0.66 0-0.74	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45 0.07 0.18 0.10 0.02	Farsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27 0-0.24 0-0.09	$\begin{array}{c} \text{E)} \\ \text{N} = 2t \\ \text{Est} \\ 15.81 \\ -0.54 \\ 1.02 \\ -0.10 \\ 0.12 \\ 0.50 \\ \end{array}$	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0.29 0-0.14
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05 0-0.05	$\begin{array}{c} \text{B) I} \\ \text{N} = 27 \\ \hline \text{Est} \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ -0.38 \\ \hline 2.11 \\ 0.85 \\ 0.24 \end{array}$	Breath rate 79 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16 0-1.12	$\begin{array}{c} \text{C) E} \\ \text{N} = 20 \\ \text{Est} \\ 3.86 \\ 0.47 \\ -0.35 \\ -0.06 \\ -0.19 \\ -0.13 \\ \end{array}$	Output Output<	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45 0.07 0.18 0.10 0.02	Orarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27 0-0.24 0-0.09	E) = 20 $Est = 15.81$ -0.54 1.02 -0.10 0.12 0.50 0.27 0.11 0.04	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0.29 0-0.14
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01 0.01	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05 0-0.05 0-0.04	$\begin{array}{c} \text{B) 1} \\ \text{N} = 27 \\ \hline \text{Est} \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ -0.38 \\ \hline 2.11 \\ 0.85 \\ 0.24 \\ 0.14 \end{array}$	Breath rate 79 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16 0-1.12 0-0.66	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13 0.49 0.19 0.18 0.06	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97 0-0.66 0-0.74 0-0.24	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45 0.07 0.18 0.10 0.02	Output Output<	$\begin{array}{c} E)\\ N=20\\ Est\\ 15.81\\ -0.54\\ 1.02\\ -0.10\\ 0.12\\ 0.50\\ \end{array}$	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0.29 0-0.14
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01 0.01	Aggression 30 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05 0-0.05 0-0.04	$\begin{array}{c} \text{B) I} \\ \text{N} = 27 \\ \hline \text{Est} \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ -0.38 \\ \hline 2.11 \\ 0.85 \\ 0.24 \\ 0.14 \end{array}$	Breath rate 79 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16 0-1.12 0-0.66	C) E N = 20 Est 3.86 0.47 -0.35 -0.06 -0.19 -0.13 0.49 0.19 0.18 0.06	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97 0-0.66 0-0.74 0-0.24	D) 7 N = 2 Est 19.44 -0.29 0.45 0.45 0.07 0.18 0.10 0.02	Output Output<	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50 0.27 0.11 0.04	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0.29 0-0.14
2) COMMON GA	ARDEN A) A N = 28 Est 2.13 0.20 -0.18 -0.03 -0.10 0.36 0.26 -0.69 0.20 0.01 0.01 0.51	Aggression 00 obs, 72 ind CI 0.93-3.25 -0.15-0.61 -0.44-0.12 -0.14-0.08 -0.38-0.18 -0.09-0.88 -0.18-0.76 -1.110.3 0.08-0.32 0-0.05 0-0.05 0-0.04 0.41-0.61	$\begin{array}{c} \text{B) 1} \\ \text{N} = 27 \\ \hline \text{Est} \\ 9.06 \\ -0.58 \\ 0.80 \\ 0.46 \\ 0.94 \\ 0.49 \\ -0.38 \\ \hline 2.11 \\ 0.85 \\ 0.24 \\ 0.14 \\ 1.58 \end{array}$	Breath rate 79 obs, 72 ind CI 6.85-11.44 -2.14-1.01 0.03-1.58 0.26-0.67 0.48-1.37 0.08-0.95 -0.79-0.04 1.11-3.25 0-2.16 0-1.12 0-0.66 1.3-1.91	$\begin{array}{c} \text{C) E} \\ \text{N} = 20 \\ \text{Est} \\ 3.86 \\ 0.47 \\ -0.35 \\ -0.06 \\ -0.19 \\ -0.13 \\ \end{array}$ $\begin{array}{c} 0.49 \\ 0.19 \\ 0.18 \\ 0.06 \\ 1.65 \end{array}$	Exploration 00 obs, 69 ind CI 1.91-6.02 -0.44-1.41 -0.86-0.18 -0.25-0.14 -0.62-0.31 -0.57-0.34 0-0.97 0-0.66 0-0.74 0-0.24 1.18-2.1	$\begin{array}{c} D) & \\ N = 2 \\ \hline N = 2 \\ \hline 19.44 \\ -0.29 \\ 0.45 \\ \end{array}$	Tarsus length 08 obs, 71 ind CI 19.14-19.73 -0.75-0.13 0.23-0.67 0.04-0.11 0.11-0.27 0-0.24 0-0.09 0.01-0.02	E) N = 20 Est 15.81 -0.54 1.02 -0.10 0.12 0.50 0.27 0.11 0.04 0.18	Body mass 07 obs, 70 ind CI 14.47-16.91 -1.110.05 0.78-1.34 -0.22-0.01 -0.07-0.27 0.37-0.66 0.14-0.42 0-0.29 0-0.14 0.14-0.22

ANNEXE D

Supplementary materials for Chapter 5

Table S5.1: Model comparison to Table 5.1 in main text when examining the site-level proportion ISA effect (impervious surface area; 100m) instead of the habitat type (forest vs. urban) effect. There was one case where the interaction between ISA and trial was significant (wild errors model) where the number of errors decreased with increasing ISA in trial 1 and 3, but not trial 2. Otherwise, results are qualitatively similar.

1) WILD	A) ERRORS			B) LATENCY			
	N = 442 obs, 3	80 ind		N = 456 obs, 39	93 ind		
	(50 ind-2 trials	, 6 ind-3 tri	als)	(63 ind-2 trials,	, 10 ind-3 tria	als)	
Fixed effects	Estimate	SE	Р	Estimate	SE	Р	
Intercept	2.36	0.88					
ISA	-0.45	0.19	0.02	-0.10	0.19	0.59	
Sex (male)	0.09	0.12	0.44	-0.12	0.10	0.23	
Age (juvenile)	0.06	0.12	0.64	-0.07	0.11	0.56	
Julian date	0.00	0.01	0.78	0.01	0.00	0.15	
Time of day	-0.03	0.03	0.39	0.07	0.03	0.02	
Year (2022)	-0.06	0.12	0.63	0.27	0.12	0.02	
Year (2023)	-0.07	0.15	0.62	0.11	0.14	0.43	
Blood (yes)	-0.22	0.17	0.19	0.13	0.16	0.42	
Trial (2)	-0.14	0.22	0.51	0.00	0.17	1.00	
Trial (3)	-0.27	0.48	0.57	0.48	0.35	0.17	
ISA* Trial (2)	1.18	0.46	0.009				
ISA * Trial (3)	-0.17	1.02	0.87				
Random effects							
Site ID $(N = 9)$	< 0.001		0.99	0.006		0.57	
Individual ID (N = 393)	0.43		< 0.001	< 0.001		0.97	
2) COMMON	N = 153 obs, 7	2 ind		N = 203 obs, 72	2 ind		
GARDEN	(54 ind-2 trials	, 32 ind-3 t	rials)	(70 ind-2 trials,	, 61 ind-3 tria	als)	
Fixed effects	Estimate	SE	P	Estimate	SE	Р	
Intercept	1.00	0.90					
ISA	-0.16	0.27	0.56	-0.10	0.23	0.68	
Sex (male)	0.19	0.21	0.36	-0.25	0.19	0.19	
Time of day	0.06	0.09	0.48	0.09	0.11	0.38	
Trial (2)	-0.25	0.21	0.23	0.31	0.21	0.14	
Trial (3)	-0.44	0.22	0.04	1.13	0.25	<0.001	
Random effects							
Individual ID $(N = 72)$	0.21		0.07	0.17		0.14	
Origin nest ID ($N = 23$)	< 0.001		0.99	0.008		0.96	
Foster nest ID $(N = 11)$	< 0.001		0.99	0.004		0.97	
Aviary ID $(N = 8)$	0.09		0.15	0.003		0.98	

Table S5.2: Model comparison to Table 5.1 in main text when including data from only the first wild trial. Model results showing habitat type and site-level proportion ISA (impervious surface area) effect are shown. Results are qualitatively similar with this reduced dataset.

WILD	A) ERRORS (N = 376 obs / ind)				B) LATENCY (N = $390 \text{ obs} / \text{ ind}$)							
	Habitat ty	pe		ISA			Habitat type	e		ISA		
Fixed effects	Estimate	SE	Р	Estimate	SE	Р	Estimate	SE	Р	Estimate	SE	Р
Intercept	1.89	1.03		2.24	0.96							
Habitat (urban) / ISA	-0.24	0.18	0.19	-0.47	0.18	0.01	-0.18	0.13	0.15	-0.06	0.20	0.76
Sex (male)	0.09	0.12	0.46	0.08	0.12	0.48	-0.17	0.11	0.12	-0.17	0.11	0.13
Age (juvenile)	0.03	0.12	0.79	0.05	0.12	0.69	-0.09	0.11	0.46	-0.07	0.11	0.54
Julian date	0.01	0.01	0.22	0.01	0.01	0.41	0.01	0.01	0.35	0.01	0.01	0.22
Time of day	-0.02	0.03	0.57	-0.02	0.03	0.63	0.06	0.03	0.04	0.07	0.03	0.03
Year (2022)	-0.02	0.13	0.89	0.00	0.13	0.97	0.27	0.12	0.03	0.27	0.12	0.03
Year (2023)	-0.18	0.15	0.24	-0.16	0.15	0.30	0.07	0.15	0.62	0.05	0.15	0.71
Blood (yes)	-0.45	0.21	0.03	-0.46	0.20	0.02	0.13	0.19	0.51	0.15	0.19	0.44
Random effects												
Site ID $(N = 9)$	0.014		0.33	< 0.001		0.99	< 0.001		0.97	0.003		0.77



Figure S5.1 : Related to Figure 5.1.2. Means \pm standard deviations of morphological traits (wing length, tarsus length, and body mass) from each comparison in order of decreasing latitude for urban (blue) and nonurban (green) great tits separated by sex (male = dark shade, female = light shade).

APPENDICE E

Capilla-Lasheras et al. 2022 Ecology Letters

DOI: 10.1111/ele.14099

SYNTHESIS

ECOLOGY LETTERS WILEY

A global meta-analysis reveals higher variation in breeding phenology in urban birds than in their non-urban neighbours **D**C

Pablo Capilla-Lasheras¹ | Megan J. Thompson^{2,3} | Alfredo Sánchez-Tójar⁴ | Yacob Haddou¹ | Claire J. Branston¹ | Denis Réale² | Anne Charmantier³ | Davide M. Dominoni¹

¹School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK

²Département des Sciences Biologiques, Université du Québec à Montréal, Montreal, Canada

³Centre d'Ecologie Fonctionnelle et Evolutive, Université de Montpellier, CNRS, EPHE, IRD, Montpellier, France ⁴Department of Evolutionary Biology,

Bielefeld University, Bielefeld, Germany

Correspondence

Pablo Capilla-Lasheras, School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Graham Kerr Building, Glasgow G128QQ, UK. Email: pacapilla@gmail.com

Funding information

Natural Environment Research Council, Grant/Award Number: NE/S005773/1

Editor: Shinichi Nakagawa

Abstract

Cities pose a major ecological challenge for wildlife worldwide. Phenotypic variation, which can result from underlying genetic variation or plasticity, is an important metric to understand eco-evolutionary responses to environmental change. Recent work suggests that urban populations might have higher levels of phenotypic variation than non-urban counterparts. This prediction, however, has never been tested across species nor over a broad geographical range. Here, we conducted a meta-analysis of the avian literature to compare urban versus nonurban means and variation in phenology (i.e. lay date) and reproductive effort (i.e. clutch size, number of fledglings). First, we show that urban populations reproduce earlier and have smaller broods than non-urban conspecifics. Second, we show that urban populations have higher phenotypic variation in laying date than non-urban populations. This result arises from differences between populations within breeding seasons, conceivably due to higher landscape heterogeneity in urban habitats. These findings reveal a novel effect of urbanisation on animal life histories with potential implications for species adaptation to urban environments (which will require further investigation). The higher variation in phenology in birds subjected to urban disturbance could result from plastic responses to a heterogeneous environment, or from higher genetic variation in phenology, possibly linked to higher evolutionary potential.

KEYWORDS

global change, habitat heterogeneity, life-history traits, meta-analysis, phenotypic variation, synthesis, urban ecology, urbanisation

INTRODUCTION

Humans have drastically changed environmental conditions on Earth, particularly since the invention of agriculture during the Neolithic Revolution. The footprint of human activity is most pronounced in urban environments, where microclimatic conditions, biogeochemical cycles and sensory landscapes are considerably different from those in non-urban habitats (Grimm et al., 2008). Perhaps not surprisingly, multiple shifts in animal and plant phenotypes have been associated with the novel conditions and selective

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited. © 2022 The Authors. *Ecology Letters* published by John Wiley & Sons Ltd. pressures found in cities (Hendry et al., 2017). Indeed, numerous studies have reported divergent phenotypes between urban and non-urban populations in phenological, morphological, behavioural and reproductive traits (e.g. Alberti et al., 2017; Diamond et al., 2018; Campbell-Staton et al., 2020; reviewed in Johnson & Munshi-South, 2017; Lambert et al., 2020; Diamond & Martin, 2021). Most studies in urban ecology and evolution to date have focused on urban effects on mean phenotypes, and no study has explicitly investigated how urbanisation affects phenotypic variation. The extent to which populations can adapt to urban environments could be partly associated with how urbanisation affects their phenotypic variation (Thompson et al., 2022). Phenotypic variation is tightly linked to eco-evolutionary processes (Fusco, 2001; Pavlicev et al., 2011): it is an essential condition for current selection, it results from past selection pressures, and it depends on gene flow and phenotypic plasticity. As such, assessing how urbanisation affects phenotypic variation can help us understand the potential for future phenotypic changes in urban environments and the eco-evolutionary implications of such changes (Thompson et al., 2022).

Recent single-species studies suggest that phenotypic variation could be affected by urbanisation (Caizergues et al., 2018; Gorton et al., 2018; Thompson et al., 2022). For example in species with limited dispersal ability (i.e. whose dispersal occurs at a smaller scale than the scale at which the urban habitat varies), adaptation to local conditions could increase phenotypic variation within the urban matrix in heterogeneous urban environments. Findings from urban and non-urban meta-populations of the common ragweed (Ambrosia artemisiifolia) are consistent with this prediction as inter-population variation in several fitness proxies was greater in urban compared to non-urban environments (Gorton et al., 2018). A meta-analysis of selection strength found weaker selection occurring in human-disturbed populations (Fugère & Hendry, 2018; note that this analysis did not specifically test the effect of urbanisation on selection strength and only included one study directly associated with urbanisation), which if extrapolated to the urban context, could lead to higher phenotypic variation in urban populations compared to their non-urban counterparts. Overall, these studies converge with the notion that urban populations could display higher levels of phenotypic variation due to several eco-evolutionary processes. These findings also highlight that the extent to which urbanisation might impact phenotypic variation likely depends on the interplay between the temporal and spatial scale at which environmental conditions fluctuate in the urban habitat, as well as on the species' longevity and dispersal ability (Thompson et al., 2022).

The temporal scale at which differences in phenotypic variation between urban and non-urban habitats manifest can help us evaluate their ecological causes, and is likely to determine the eco-evolutionary implications of increased phenotypic variation in urban habitats (Thompson et al., 2022). First, urban populations could display higher phenotypic variation than non-urban populations within a given breeding season (i.e. intra-annual variation; as a result, for example of consistent differences in landscape heterogeneity between habitats; Pickett et al., 2017). Second, urban populations could display higher phenotypic variation than non-urban populations due to larger yearly fluctuations in environmental conditions (i.e. inter-annual variation; if, e.g. urban populations are more sensitive to changes in weather conditions), with or without intra-annual differences in phenotypic variation between urban and non-urban populations. In the latter scenario, similar levels of phenotypic variation would be exposed to natural selection in short-lived species (e.g. annual species).

Urban environments have been referred to as spatially more heterogeneous than non-urban habitats of the same geographical area (Pickett et al., 2017). High urban habitat heterogeneity could increase phenotypic variation compared to adjacent non-urban habitats if, for example urban organisms change their phenotype according to local environmental conditions (e.g. through either developmental or later-life phenotypic plasticity). The empirical assessment of this idea, however, largely depends on the scale at which urban habitat heterogeneity is measured, the spatial scale at which the organism of interest operates and the heterogeneity of the non-urban habitat of reference (Pickett et al., 2017; Uchida et al., 2021). For example a megacity could be spatially heterogeneous, containing a diverse array of habitats (e.g. multiple urban parks with different ecological conditions, a varying level of impervious surface, etc.), and, thus, be overall vastly more heterogeneous than a neighbouring non-urban habitat. However, species could reduce the range of environmental conditions that they experience through matching habitat choice (e.g. Muñoz et al., 2014), limiting the potential effect of urban habitat heterogeneity on phenotypic variation. Therefore, measuring habitat heterogeneity at different spatial scales will be paramount to understand the potential association between habitat heterogeneity and increased phenotypic variation in urban areas.

Here, we investigate how urbanisation impacts mean phenotypic values and phenotypic variation using a meta-analysis of 399 paired urban and non-urban comparisons of avian life-history traits (laying date, clutch size and number of fledglings) published between 1958 and 2020 including 35 bird species (Figure 1). We use paired within species urban-non-urban comparisons to investigate the following questions: (i) Is urbanisation associated with shifts in mean life-history traits? (ii) Is urbanisation associated with changes in variation



FIGURE 1 Phylogenetic and geographical breadth of the meta-analytic data. (a) Phylogenetic tree of the 35 avian species included in the meta-analysis along with the number of effect sizes (i.e. urban-non-urban comparisons) included per species ('k'; which may encompass multiple years of study from the same publication) and the proportion of observations for each life-history trait (purple: Laying date; orange: Clutch size; Green: Number of fledglings). (b) Our meta-analysis included a broad range of species, as examples, left to right from top to bottom: *Sturnus vulgaris, Spinus tristis, Aphelocoma coerulescens, Athene cunicularia, Mycteria americana* and *Fulica atra*. All images are copyright free (CC—Public Domain Mark 1.0. Authors: Shenandoah National Park [first two images], Mike Carlo/U.S. Fish & Wildlife Service, Jennifer Soos, Susan Young and Ekaterina Chernetsova) and were extracted from www.flickr.com. (c) Global map (excluding Antarctica) showing the location of each study included in the meta-analysis. Each point represents one study area in which one or more urban-non-urban pairs of populations were sampled across a varying number of years.

in life-history traits? (iii) What is the temporal and spatial scale at which urbanisation correlates with changes in phenotypic variation? Based on previous research (Chamberlain et al., 2009; Sepp et al., 2018), we predict that urban bird populations display on average earlier phenology, smaller clutch size and lower number of fledglings than non-urban populations. We also predict increased phenotypic variation in urban populations compared to non-urban populations for all three traits examined (see above). We disentangle urban effects on phenotypic variation across different temporal and spatial scales, suggesting an ecological mechanism for the effects of urbanisation on avian phenotypic variation. This study provides, for the first time, meta-analytical evidence that urban conditions can magnify phenotypic variation in phenology and highlights the potential role of increased habitat heterogeneity in urban areas as an ecological mechanism underlying this effect.

MATERIAL AND METHODS

Literature review

We began our literature search by inspecting two published reviews on the impact of urbanisation on avian biology (Chamberlain et al., 2009; Sepp et al., 2018). As we were interested in how phenology and reproduction were affected by urbanisation, we identified studies cited in Chamberlain et al. (2009) (n = 37) and Sepp et al. (2018) (n = 32) that could contain either raw data, or mean and variance estimates for first clutch laying initiation (hereafter laying date), clutch size and number of nestlings fledged per breeding attempt (hereafter number of fledglings), for paired urban and non-urban populations (see details below). Then, we performed four searches of the Web of Science Core Collection on the 27th of October 2020 (databases covered: SCI-EXPANDED—1900-present, SSCI—1956-present,

A&HCI—1975-present, BKCI-S-2005-present, BKCI-SSH-2005-present and ESCI-2015-present) to recover studies published since 1900 and including all languages and all document types. We performed the following four searches on the Web of Science Core Collection: (1) TS = ("urban*" AND ("bird*" OR "aves" OR "avian" OR "ornithol*" OR "passerine*" OR "passeriform*" OR "songbird*" OR list of bird genera) AND ("laying date" OR "lay date" OR "first egg" OR "clutch size" OR "eggs laid" OR "number of eggs" OR "fledgling*" OR "fledging" OR "reproductive success" OR "fitness")); (2) TS = ("urban*" AND "bird" AND "laying date"); (3) TS = ("urban*" AND "bird" AND "clutch size"); (4) TS = ("urban*" AND "bird" AND "fledglings"). The list of avian genera in the first search string consisted of a list of all avian genera and can be found in Supplementary text D (see also acknowledgements). We complemented the search on the Web of Science Core Collection by searching Scopus using search string '(1)' above (Scopus field 'TITLE-ABS-KEY'). Both literature searches, on the Web of Science Core Collection and Scopus, included studies published before the 27th of October 2020. We used the literature search results in these two major search engines to assess the comprehensiveness of our search (see Supplementary Text A for details). These searches found 892, 71, 198, 167 (on the Web of Science Core Collection) and 735 (on Scopus) studies, respectively, which we combined with the studies identified from Chamberlain et al. (2009) and Sepp et al. (2018) to create a list of 2132 (non-unique) studies (Figure S1). We then de-duplicated this list using the R package 'revtools' (using exact matching of study titles in function 'find_duplicates', v0.4.1; Westgate, 2019) and by manually inspecting all titles and author lists. Our final list contained 1166 unique studies (Figure S1), which we screened by reading their title and abstract (this first screening step was made by P.C.-L., C.J.B. and D.M.D.). If the title and/or abstract indicated that the paper could fit our requirements for data collection (see below), we read the study fully, aiming to extract mean, standard deviation (SD) and sample size (n) of our life-history traits of interest for urban and non-urban bird populations. If SD was not available but authors provided SE, the former was calculated as: $SD = SE \times \sqrt{n}$. Mean and SD were extracted from data quartiles and medians in four effect sizes from two studies following (Luo et al., 2016; Shi et al., 2020). When available, we extracted estimates per breeding season (i.e. papers sometimes reported mean, SD and *n* for urban and non-urban populations in multiple breeding seasons). If a study reported incomplete information for inclusion in our meta-analysis (e.g. mean was provided but not SD or SE), we contacted the authors to ask for this missing information (a complete list of authors that provided estimates can be found in the acknowledgements).

Criteria for inclusion

We were interested in investigating the effects of urbanisation on life-history traits, with an interest in testing the association between urbanisation and, mean and variation in trait values. Paired urban-nonurban designs, where an urban population is compared to an adjacent non-urban population, are a powerful approach to identify the effects of urban living while controlling for temporal and geographical variation, and large-scale genetic structure among populations (Caizergues et al., 2021; Salmón et al., 2021). Therefore, we included studies if they compared geographically close (i.e. paired) urban and non-urban populations and reported laying date of the first clutches of the season, clutch size or number of fledglings for the same breeding season across both habitats. When multiple populations were compared along a gradient of urbanisation, we extracted estimates for the two populations at the extremes of the gradient (i.e. most and least urbanised populations). When studies combined estimates across several breeding seasons, we included them in our meta-analysis if urban and non-urban populations had been sampled in the same breeding seasons. All effect sizes were extracted by one author (P.C.-L.). To validate data extraction, another author (M.J.T.) checked 15% of the studies included in the meta-analysis, comprising 55 effect sizes (17.80% of the final dataset; Supplementary Text B).

Initially, our dataset contained 443 paired urbannon-urban estimates from 40 bird species and 74 studies. Of these, three observations were removed due to missing sample sizes, 26 observations were removed due to missing SD and 11 observations were removed because their sample size was one (which precludes the calculation of mean and SD). Four observations were removed because they reported a SD of zero (these indeed had very low sample sizes: 3, 2, 7, 2 observations). Our final dataset included 399 comparisons between paired urban-non-urban populations from 35 bird species and 68 studies (Figure 1; refs.: Antonov & Atanasova, 2003; Bailly et al., 2016; Baldan & Ouyang, 2020; Beck & Heinsohn, 2006; Berardelli et al., 2010; Biard et al., 2017; Boal & Mannan, 1999; Bobek et al., 2018; Brahmia et al., 2013; Caizergues et al., 2018; Capilla-Lasheras et al., 2017; Cardilini et al., 2013; Charter et al., 2007; Conway et al., 2006; de Satgé et al., 2019; Dhondt et al., 1984; Eden, 1985; Evans & Gawlik, 2020; Gahbauer et al., 2015; Gladalski, Bańbura, Kaliński, Markowski, Skwarska, Wawrzyniak, Zieliński, & Bańbura, 2016; Glądalski, Bańbura, Kaliński, Markowski, Skwarska, Wawrzyniak, Zieliński, Cyżewska, & Bańbura, 2016; Gladalski et al., 2015, 2017, 2018; Gryz & Krauze-Gryz, 2018; Hajdasz et al., 2019; Hinsley et al., 2008; Ibáñez-Álamo & Soler, 2010; Isaksson et al., 2008; Isaksson & Andersson, 2007; Jarrett et al., 2020; Kelleher

HIGHER PHENOTYPIC VARIATION IN URBAN BIRDS

& O'Halloran, 2007; Kettel et al., 2019; Kopij, 2017; Lee et al., 2017; Lin et al., 2015; Liven-Schulman et al., 2004; Luna et al., 2020; Mcgowan, 2001; Mennechez & Clergeau, 2006; Middleton, 1979; Millsap et al., 2004; Minias, 2016; Morrissey et al., 2014; Newhouse et al., 2008; Partecke et al., 2020; Perlut et al., 2016; Pollock et al., 2017; Preiszner et al., 2017; Rollinson & Jones, 2003; Rosenfield et al., 2019; Schmidt & Steinbach, 1983; Schoech et al., 2007; Schoech & Bowman, 2001; Seress et al., 2012, 2018, 2020; Sharma et al., 2004; Shustack & Rodewald, 2011; Solonen, 2001, 2014; Solonen & Ursin, 2008; Stout et al., 1998; Stracey & Robinson, 2012; Sumasgutner et al., 2014; Thornton et al., 2017; Wawyrzyniak et al., 2015; Welch-Acosta et al., 2019). Of these 399 comparisons, 151 corresponded to comparisons of laying date (n = 32 studies), 119 were comparisons of clutch size (n = 42 studies) and 129 were comparisons of number of fledglings (n = 48studies) (Figure S2). Last, there were 363 comparisons for single years (n = 47 studies) and an additional 36 comparisons included estimates across multiple years (n = 21 studies).

Meta-analytic effect sizes

We standardised laying date across studies by coding it as the number of days after the 1st of January (January 1st = 1). Mean laying date estimates across habitats always fell within the same calendar year. For each of the three life-history traits, we computed the log response ratio (lnRR) and the log coefficient of variation ratio (lnCVR) to investigate differences in mean values and variability between urban and non-urban populations (Hedges et al., 1999; Nakagawa et al., 2015; Senior et al., 2020). We calculated lnRR and lnCVR along with their associated sampling variances (Nakagawa et al., 2015) using the R function 'escalc' in the 'metafor' R package (v3.4.0; Viechtbauer, 2010). Both lnRR and InCVR were calculated so that positive values meant higher estimates in urban populations compared to their non-urban counterparts. Often mean and variance values are positively associated (e.g. Taylor's Law; Cohen & Xu, 2015; Nakagawa & Schielzeth, 2013). Therefore, we chose lnCVR over lnVR (i.e. log total variation ratio; Nakagawa et al., 2015) as the former accounts for the mean-variance relationship (Nakagawa et al., 2015; Senior et al., 2020). However, we carried out sensitivity analysis using, among others, the log total variation ratio (Section 'Sensitivity analyses').

Quantifying habitat heterogeneity and urban index

We calculated habitat heterogeneity from the 3CS LC (Copernicus Climate Change Service Land Cover) and

the ESA-CCI LC (European Space Agency-Climate Change Initiative Land Cover) land cover products (ESA. Land Cover CCI Product User Guide, 2017; ESA. 3CS Land Cover Product User Guide 2020). These datasets provide methodologically consistent land cover per year and gridded maps from 1992 to 2019, with a global coverage and a spatial resolution of circa 300m per pixel (0.002778° or 10 arcseconds). Each pixel in the products is classified as one of the 22 land cover categories defined by the UN-FAO-LCCS (United Nations Food and Agriculture Organization Land Cover Classification System). From a subset of studies included in our main meta-analysis, we could extract the coordinates of their urban and non-urban populations (26 studies out of 68 provided accurate coordinates of their urban and non-urban populations). Then, we sampled the landscape of these studies by extracting the number of pixels belonging to each land cover category around each urban and non-urban location (i.e. within a circular buffer around each location). The extraction was performed for several buffer radii from 250 m to 5000 m in intervals of 250 m. Landscape heterogeneity was calculated as the effective number of land covers present in each buffer and computed as the exponential of the Shannon-Wiener diversity index (i.e. Hill's numbers for q = 1) (Chao et al., 2014; Hill, 1973), resulting in a measure that not only accounts for the absolute richness of land cover categories but also weights the relative abundance of each category. An urban index was calculated as the proportion of each buffer area categorised as an 'urban' land cover type. Land cover data were processed and analysed using R (v.4.2.0; R Core Team, 2022). Geospatial vectorial operations were conducted utilising the 'sf' R package (v.1.0-7; Pebesma, 2018) while raster extractions were performed with the 'raster' R package (v.3.5-15; Hijmans, 2020). All geospatial analyses were performed in the WSG 1984 projected Coordinate Reference Systems, EPSG: 6326. Additionally, we calculated the distance between each urban and non-urban pair of populations using the function 'pointDistance' in the R package 'raster'. We could retrieve location information for 232 urban versus non-urban comparisons for laying date, clutch size and number of fledglings, from 11 species and 26 studies between 1992 and 2017 (land cover data were not available before 1992; see above).

Meta-analyses

We handled the datasets, ran all analyses and produced visualisations using R (v.4.2.0; R Core Team, 2022). To evaluate the effect of urbanisation on bird life-history traits, we fitted phylogenetic multilevel (interceptonly) meta-analyses for each response term (i.e. lnRR [Model 1] and lnCVR [Model 3]; Table 1) combining the three life-history traits (i.e. laying date, clutch size and

Model ID	Response	Data	Moderators	Equations	Details
-	lnRR	All traits	Intercept		Overall meta-analysis. Univariate. Table SI. Figure S3
7	lnRR	All traits	Trait	Equation 1	Effect per trait. Trivariate. Tables S2 and S3. Figures 2 and 3, Figure S4
3	lnCVR	All traits	Intercept		Overall meta-analysis. Univariate. Table Sl. Figure S5
4	lnCVR	All traits	Trait	Equation 1	Effects per trait. Trivariate. Table 2, Tables S4 and S5. Figures 2 and 3, Figure S4
5	lnCVR	All traits	Trait	Equation 1	Comparison of intra-annual phenotypic variation. Table 2
9	lnCVR	All traits	Trait	Equations 1, 7 and 8	Comparison of inter-annual phenotypic. Table 2
7	lnCVR	All traits	Trait + Difference in urbanisation + Difference in habitat heterogeneity	Equation 1 (with additional moderators)	Trivariate. Fitted for different spatial scales. Figure 4
8	SDHM	All traits	Trait	Equation 1	Similar structure as Model 2
6	lnVR	All traits	Trait	Equation 1	Similar structure as Model 4
10	lnSD	Each trait individually	Intercept + Habitat + InMean	Equation 9	Armed-based model (Senior, Gosby, et al. 2016)

TABLE 1 Description of meta-models. Model IDs are given sequentially from 1 to 10 to facilitate understanding of methods and results. 'Data' refers to whether a given model contained

number of fledglings; we also fitted models that separated variation between these traits; see below; Table 1). Both meta-analytic models estimated four random intercept effects, publication identity (i.e. among-study variation), population identity (i.e. in several cases, we found multiple studies from the same urban-non-urban populations pairs), phylogeny (more details below), species identity (i.e. among-species variation not explained by phylogeny) and an observation ID term. For the intercept-only models, we estimated total heterogeneity (I^2) following Nakagawa and Santos (2012) and Senior, Grueber, et al. (2016) as implemented in the R function ' $i2_ml'$ ('orchaRd' R package v.0.0.0.9000; Nakagawa et al., 2021).

Phylogenies

Phylogenetic trees were extracted from the Open Tree of Life (Hinchliff et al., 2015; Rees & Cranston, 2017), using the interface provided by the R package 'rotl' (v3.0.12; Michonneau et al., 2016; OpenTreeOfLife et al., 2019). We calculated tree branch length (Grafen, 1989) and generated a phylogenetic correlation matrix to include in all our phylogenetic multilevel meta-analytic models (Figure 1). We assessed the phylogenetic signal in our meta-analysis based on the proportion of variation explained by the phylogeny; Cinar et al., 2022).

Modelling heterogeneous variances and correlations among traits

Laying date, clutch size and number of fledglings are often correlated in bird species (Dunn & Møller, 2014; Rowe et al., 1994). To assess whether urbanisation is associated with correlated responses across life-history traits and to test the robustness of our results to the existence of these correlations, we built trivariate metaanalytic models of lnRR and lnCVR that allowed us to simultaneously estimate trait-specific means (i.e. one intercept for each trait—Equation 1), trait-specific observation ID variances (i.e. one observation ID variance for each trait-Equations 1 and 2) and traitspecific among-study variances and correlation among traits (Equations 1 and 3). Including the random-effects detailed above, our model with heterogeneous variances and among-study correlations among traits can be written as: (we have omitted the term associated with sampling variance for simplicity—see Nakagawa et al., 2015 for more details)

$$y_{i} = \begin{bmatrix} \mu_{\rm LD} \\ \mu_{\rm CS} \\ \mu_{\rm NF} \end{bmatrix} + \begin{bmatrix} \varepsilon_{i-\rm LD} \\ \varepsilon_{i-\rm CS} \\ \varepsilon_{i-\rm NF} \end{bmatrix} + \begin{bmatrix} \tau_{t-\rm LD} \\ \tau_{t-\rm CS} \\ \tau_{t-\rm NF} \end{bmatrix} + v_{y} + a_{l} + h_{w}, \quad (1)$$

$$\sum_{\substack{\text{CS} \\ \text{NF}}} N \left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_{\epsilon-\text{LD}}^2 \\ \sigma_{\epsilon-\text{CS}}^2 \\ \sigma_{\epsilon-\text{NF}}^2 \end{bmatrix} \right),$$
(2)

$$\begin{aligned} \tau_{t-\text{LD}} & \\ \tau_{t-\text{CS}} & \sim N \left[\begin{array}{c} 0 \\ 0 \\ 0 \end{array} \right], \left[\begin{array}{c} \sigma_{t-\text{LD}}^2 & p_{\text{LD-CS}} \sigma_{t-\text{LD}} \sigma_{t-\text{CS}} & p_{\text{LD-NF}} \sigma_{t-\text{LD}} \sigma_{t-\text{NF}} \\ \sigma_{t-\text{CS}}^2 & p_{\text{CS-NF}} \sigma_{t-\text{CS}} \sigma_{t-\text{NF}} \\ \sigma_{t-\text{NF}}^2 & \sigma_{t-\text{NF}}^2 \end{array} \right], \end{aligned}$$

$$(3)$$

 ε_{i-}

 ε_{i-}

 ε_{i-}

$$v_{y}^{\sim} N(0, \sigma_{v}^{2}), \qquad (4)$$

$$a_l^{\sim} N(0, A\sigma_a^2), \tag{5}$$

$$h_w \sim N(0, \sigma_h^2), \tag{6}$$

where y_i is the statistic of interest (lnRR or lnCVR) for the *i*th effect size (i = 1, 2, 3, ..., k; where k is the number of the effect sizes included in the analysis-that is number of urban-non-urban paired comparisons). 'LD', 'CS' and 'NF' refer to overall means (μ), variances (σ^2) and correlations (ρ) involving effect sizes for laying date ('LD'), clutch size ('CS') and number of fledglings ('NF'). ε_i is the observation ID deviation for the *i*th observation, which is assumed to follow a normal distribution with mean zero and variance $\sigma_{\epsilon-\text{LD}}^2$, $\sigma_{\epsilon-\text{CS}}^2$, $\sigma_{\epsilon-\text{NF}}^2$ for laying date, clutch size and number of fledglings respectively. $\tau_{t-\text{LD}}$, $\tau_{t-\text{CS}}$ and τ_{t-NF} are the deviations from the mean associated with the tth study and trait ('LD', 'CS' or 'NF'), each following a multivariate normal distribution with mean of zero and variance–covariance structure detailed in Equation 5 (pprovides the correlations between $\tau_{t-\text{LD}}$, $\tau_{t-\text{CS}}$ and $\tau_{t-\text{NF}}$). $v_{\rm u}$ provides the deviation from the overall mean associated with the *y*th population (Equation 4). a_1 is the phylogenetic effect for the *l*th species, which follows a normal distribution with mean equal to zero and variance-covariance structure given by σ_a^2 , the variance of the phylogenetic effect, and A, a l by l matrix of distances between species calculated from a phylogenetic tree (Equation 5; details above). h_w captures among species variation not explained by the phylogenetic effect and follows a normal distribution around zero and variance σ_h^2 (Equation 6).

We compared models with different constraints in the parameters of the variance-covariance structure in Equation 3 to assess the strength of evidence for heterogeneous variances and correlations among traits (see results in Tables S2 and S4). We fitted these trivariate meta-analytic models in the 'metafor' R package ('*rma. mv*' function; v3.4.0; Viechtbauer, 2010) using maximum likelihood and compared models using AIC (Akaike Information Criterion; Burnham et al., 2011). We then calculated a Δ AIC value for each model (i.e. the difference in AIC between a given model and the model with the lowest AIC) and used this value to assess the strength of evidence for a given variance-covariance structure. We fitted models with the following constraints in the variance-covariance structure:

- Single variance across traits and zero covariances: (i)
- Single variance across traits and zero covariances: $\sigma_{t-\text{LD}}^2 = \sigma_{t-\text{CS}}^2 = \sigma_{t-\text{NF}}^2$; and all p = 0Compound symmetric variance-covariance matrix: $\sigma_{t-\text{LD}}^2 = \sigma_{t-\text{CS}}^2 = \sigma_{t-\text{NF}}^2$; and $p_{\text{LD}-\text{CS}} = p_{\text{LD}-\text{NF}} = p_{\text{CS}-\text{NF}}$ (ii)
- (iii) Heteroscedastic compound symmetric variance-covariance matrix: $\sigma_{t-\text{LD}}^2, \sigma_{t-\text{CS}}^2$ and $\sigma_{t-\text{NF}}^2$ can vary freely but $p_{\text{LD-CS}} = p_{\text{LD-NF}} = p_{\text{CS-NF}}$ Diagonal variance–covariance matrix:
- (iv) $\sigma_{t-LD}^2, \sigma_{t-CS}^2$ and σ_{t-NF}^2 can vary freely but all p = 0
- Unstructured variance-covariance matrix (v) $\sigma_{t-LD}^2, \sigma_{t-CS}^2, \sigma_{t-NF}^2, p_{LD-CS}, p_{LD-NF}$ and p_{CS-NF} can vary freely.

Within- and between-breeding season differences in phenology and life-history traits

Urban and non-urban populations may differ in both within- and between-breeding season variation in lifehistory traits. However, differences in variation for these two temporal scales are likely generated by contrasting ecological and evolutionary processes. To disentangle processes operating at these two temporal scales, we performed additional meta-analyses including (i) urbannon-urban comparisons within breeding seasons (k = 363) comparisons from 47 studies in the original dataset with effect sizes per year; Model 5) and (ii) urban-non-urban comparisons between breeding seasons (i.e. combining all within-breeding season estimates from a study; k = 36comparisons present in the original dataset, plus 67 additional comparison calculated from within-breeding season estimates; see below). When a given study reported estimates across multiple breeding seasons, we calculated between-breeding season mean and variance as:

$$\overline{x}_{\text{among-season}} = \sum_{i=1}^{g} \overline{x}_i \frac{n_i}{N}; \text{ where, } N = \sum_{i=1}^{g} n_i, \quad (7)$$

$$S_{\text{among-season}}^2 = \sum_{i=1}^g \frac{n_i}{N} s_i^2 + \sum_{i=1}^g \frac{n_i}{N} \left(\overline{x}_i - \overline{x}_{\text{among-season}}\right)^2 (8)$$

where, $\overline{x}_{among-season}$ and $S^2_{among-season}$ are mean and variance across multiple breeding seasons. g is the total number of breeding seasons reported by a given study; \overline{x}_i , s_i^2 , n_i , are mean, variance and sample size for each breeding season *i*. $\overline{x}_{\text{among-season}}$ for a given study is, therefore, the weighted average across breeding seasons (Equation 7); whereas $S^2_{\text{among-season}}$ for a given study is the weighted sum of within-season variances (first term in Equation 8)

and between-season mean variances (second term in Equation 8).

Assessing the effect of urbanisation and habitat heterogeneity on differences in phenotypic variation between habitats

We investigated the spatial drivers of differences in phenotypic variation between urban and non-urban populations using the subset of studies which allowed the quantification of an urban index in urban and non-urban populations (see above). We first verified that the urban index was indeed higher for urban than for non-urban populations. We compared the urban index in urban and non-urban populations at different spatial scales via linear models, with the difference in urban index between population as the response variable and an intercept term. Then, to assess whether the increase in phenotypic variation in urban habitats was predicted by habitat heterogeneity and/or urban index, we ran an additional meta-regression to explain differences in phenotypic variation between urban and non-urban populations (i.e. lnCVR), where the difference in habitat heterogeneity and urban index between urban and non-urban populations were included as continuous moderators. This meta-regression included 232 urban-non-urban comparisons from 11 species and 26 studies (i.e. the subset of observations after 1992 for which geolocations were available).

Sensitivity analyses

We assessed the robustness of our results with several complementary analyses. First, we re-ran the trivariate lnRR model (Model 2; Table 1) using Hedges' g (Hedges, 1981) with heteroscedastic population variances as the response variable (Model 8; Table 1; i.e. 'SMDH', calculated using the R function 'escalc' in the 'metafor' R package (v3.4.0; Viechtbauer, 2010)). In addition, we assessed the robustness of the lnCVR results by re-running the trivariate lnCVR model (Model 4; Table 1) using lnVR as the response variable (i.e. the logarithm of the total variation ration; Nakagawa et al., 2015; Model 9; Table 1). Last, we used an alternative approach that directly models the log of the phenotypic standard deviation (lnSD) to assess differences in phenotypic variation between urban and non-urban populations (equation 18 in Nakagawa et al., 2015; Model 10; Table 1). We followed the model specification shown in Senior, Gosby, et al. (2016), in short:

$$\ln SD_{j} = \beta_{0} + \beta_{1} Habitat_{j} + \beta_{2} ln \overline{Mean_{j}} + \tau_{i[j]} + v_{y} + a_{l} + h_{w},$$
(9)

where β_0 is the overall intercept, β_1 is the habitat effect on lnSD (i.e. a β_1 statistically different from zero would

indicate that urban and non-urban populations differ in their phenotypic variation) and β_2 is the slope of the regression of (log) mean values against (log) standard deviations, which is explicitly modelled. v_y , a_l and h_w are as per Equation 1. $\tau_i[j]$ is the random effect for the *j*th effect size in the *i*th study. Within each study, effect sizes across habitats are assumed to be correlated; this correlation is calculated by the model (Senior, Gosby, et al., 2016). We applied the model in Equation 9 for each trait independently (i.e. three univariate models, one per trait).

Publication bias

We assessed the evidence for the existence of two types of publication biases, small-study and decline effects (timelag effects), following Nakagawa et al. (2022). For that, we ran four additional uni-moderator multilevel metaanalytic models, two for lnRR and two lnCVR. Each of these models included as a single moderator either the square-root of the inverse of the effective sample size or the mean-centred year of study publication (Nakagawa et al., 2022; Trikalinos & Ioannidis, 2005). The variation explained by these moderators (i.e. $R^2_{marginal}$) was calculated using the R function 'r2_ml' ('orchaRd' R package v.0.0.0.9000; Nakagawa et al., 2021).

RESULTS

After systematically inspecting 1166 studies published between 1958 and 2020 (Figure S1), our meta-analysis

included 399 urban-non-urban comparisons for three bird life-history traits: laying date (k = 151 effect sizes, n = 32 studies), clutch size (k = 119 effect sizes, n = 42 studies) and number of fledglings (k = 129 effect sizes, n = 48studies) (Figure 1). This dataset included 35 bird species, with most studies located in the northern hemisphere (Figure 1c).

Is urbanisation associated with shifts in mean life-history traits?

We found that urban populations tended to have, on average, 3.6% lower mean values than their nonurban counterparts, but note that the 95% confidence interval (hereafter 'CI') for this estimate overlapped zero (Model 1: $\ln RR$ mean estimate [95% CI] = -0.035[-0.076, 0.005]; Figure S3; Table S1). Total heterogeneity was high $(I_{\text{total}}^2 = 97.8\%)$, with 17.2% of it explained by phylogenetic and species-specific effects combined $(I_{\text{phylogeny}}^2 = 1.7\%; I_{\text{species ID}}^2 = 15.5\%)$, while 8.4% was explained by differences among studies (Table S1). Further analyses calculating urban effects per trait and accounting for potential covariation in the response to urbanisation across the three focal traits (i.e. using a model with an unstructured variancecovariance matrix; see Methods and Table S2) confirmed that urban populations had indeed lower mean values in every life-history trait: urban populations laid their eggs earlier (Model 2: lnRR [95% CI] = -0.048 [-0.084, -0.012]; Figure 2a), laid smaller clutches (Model 2: $\ln RR [95\% CI] = -0.066 [-0.107]$,



FIGURE 2 Urban populations have earlier phenology, lower reproductive output and more variable life-history traits than non-urban populations. (a) Urban populations laid earlier and had smaller clutches, producing fewer fledglings, than their paired non-urban populations (illustrated by negative lnRR estimates; Model 2). (b) Our meta-analysis revealed that variation in life-history traits was higher in urban populations compared to non-urban counterparts, with a marked difference between populations in laying date (illustrated by positive estimates of lnCVR; Model 4). Model estimates for (a) lnRR and (b) lnCVR are shown along with their 95% confidence intervals per trait as calculated by our phylogenetic multilevel meta-analytic models accounting for correlated responses to urbanisation among traits (see Tables S3 and S5 for full model outputs and Figure S3 and S5 for overall meta-analyses of lnRR and lnCVR). Raw data and model estimates are presented in Figure S4. 'k' provides the number of urban–non-urban comparisons.

-0.025]; Figure 2a), and tended to produce fewer fledglings per clutch than non-urban populations (Model 2: $\ln RR [95\% CI] = -0.070 [-0.171, 0.032];$ Figure 2a). This meta-analytic model estimated different random effect intercepts per trait and allowed for correlations across traits (Model 2; see Methods for details). This model revealed correlations in the response to urbanisation across traits: studies reporting earlier laying date in urban populations also reported more similar clutch size and number of fledglings between populations (i.e. negative correlations between lnRR for laying dates and clutch size; Figure 3a,b). Likewise, studies reporting large differences in clutch size between urban and non-urban populations also reported large differences between both habitats in number of fledglings (Figure 3c; see 'Study ID (correlations)' in Table S3; i.e. correlations among studies in the values of lnRR for each trait).

Is urbanisation associated with changes in variation in life-history traits?

The coefficient of phenotypic variation in urban populations was, on average, 4.4% higher than in non-urban populations, but note that the 95%CI for this estimate overlapped zero (Model 3: lnCVR mean estimate [95% CI] = 0.043 [-0.092, 0.178]; I_{total}^2 = 74.3%; Figure S5 and Table S1). 9.1% of the heterogeneity in lnCVR was explained by phylogenetic and species-specific effects combined ($I_{phylogeny}^2$ = 5.8%; $I_{species ID}^2$ = 3.3%), while differences between studies explained no heterogeneity in lnCVR ($I_{study ID}^2$ = 0.0%; Table S1). A subsequent model of lnCVR separating urban effects on phenotypic variation per trait and accounting for potential covariation across the three investigated traits in the response to urbanisation (see Methods and Table S4) revealed that the overall effect of urbanisation on life-history trait variation



FIGURE 3 Life-history traits show a correlated response to urbanisation. Our meta-analysis investigated correlated responses to urbanisation across the three studied life-history traits, and revealed strong correlations in log response ratio (lnRR) but not log coefficient of variation ratio (lnCVR). (a) Earlier laying dates in urban populations compared to non-urban counterparts (i.e. negative values in the x axis) were associated with no differences in clutch size across habitats (i.e. y axis values close to zero), leading to a negative correlation between lnRR for these two traits. (b) A similar pattern was found between lnRR for laying dates and number of fledglings, while (c) lnRR for clutch size and number of fledglings were positively correlated (Tables S2 and S3; Model 2). (d–f) We found no strong statistical evidence for models including correlation in laying dates were not associated with differences between habitats in phenotypic variation in laying dates were not associated with differences between habitats in variation in clutch size were not associated with differences between habitats in variation in number of fledglings. Points represent mean raw values per study \pm SE. Regression lines (mean \pm SE) in a–c were fitted using linear regressions to illustrate the correlations revealed by our trivariate meta-analysis (Model 2; Table S3).

was driven by urban populations having a more variable phenology than their non-urban counterparts (Model 4: lnCVR mean for laying date [95% CI] = 0.176 [0.084, 0.268], that is 19.2% more variation, on average, in laying date in urban than non-urban populations). Although the 95%CIs overlapped zero, the direction of the average effects for clutch size and number of fledglings also reflected higher phenotypic variation in urban compared to non-urban populations (Model 4: lnCVR mean estimates [95% CI]: clutch size = 0.055 [-0.051, 0.160], number of fledglings = 0.037 [-0.096, 0.171]; Figure 2b). We did not find evidence for correlations in lnCVR between the three life-history traits (Figure 3; the model including correlations among traits scored more than 1.08 AIC points below the top model, which only included independent Study ID random intercepts per trait [Model 4]; Tables S4 and S5).

What is the temporal and spatial scale at which urbanisation affects phenotypic variation?

Differences in phenotypic variation in laying date between the urban and non-urban populations arose from differences in variation within breeding seasons (i.e. intra-annual) rather than between breeding seasons (i.e. inter-annual; Table 2). While laying dates in urban populations were more variable than in nonurban populations within breeding seasons (Model 5: $\ln CVR$ mean estimate [95% CI] = 0.177 [0.078, 0.281]; Table 2), a subsequent meta-analytic model isolating effects on phenotypic variation arising from between breeding season fluctuations revealed no difference between urban and non-urban populations (Model 6: $\ln CVR$ intercept mean [95% CI] = 0.074 [-0.014, 0.161]; Table 2). The sample size for this latter meta-analysis was almost four times smaller than for the metaanalysis of within breeding season differences in variation; however, the lnCVR estimates were very different between these models: the mean lnCVR within breeding seasons was more than 2.4 times larger than the mean lnCVR among breeding seasons (Table 2).

Furthermore, to assess whether urbanisation and/or habitat heterogeneity could explain increased phenotypic

variation in urban bird populations, we investigated the extent to which our quantification of urban index and habitat heterogeneity predicted differences in phenotypic variation across populations. First, we confirmed that the urban populations included in our meta-analysis showed higher levels of urbanisation than paired non-urban populations regardless of the spatial scale used (urban index in urban population \pm SE = 0.669 \pm 0.047; urban index in non-urban population \pm SE = 0.021 \pm 0.007; at a spatial scale of 2000m in both cases for reference; Figure 4a). Including the difference in urban index and habitat heterogeneity between paired urban and non-urban populations as a moderator in a meta-regression revealed that the more heterogeneous the urban habitat was, the larger the phenotypic variation in this habitat compared to the non-urban habitat; this effect was particularly strong at medium-large spatial scales (Figure 4c). Differences in urban index between populations did not strongly explain variation in lnCVR (Figure 4b). Urban and nonurban populations in each pair were located at a mean distance of 65.4 km (median = 33.1 km; range = [2.4 km,625.1 km]; n = 26 geo-referenced studies).

Sensitivity analyses and assessment of publication bias

In line with our main analysis of lnRR (Table S3), using SMDH as the effect size provided negative estimates (i.e. lower phenotypic means in urban populations) for laying dates (SMDH mean estimate [95% CI] = -0.298[-0.634, 0.039]), clutch size (SMDH mean estimate [95% CI] = -0.145 [-0.420, 0.130]) and number of fledglings (SMDH mean estimate [95% CI] = -0.022 [-0.298], 0.254]) (Model 8 in Table 1). Uncertainty around mean SMDH estimates was high and the 95%CIs overlapped zero. Analysing lnVR instead of lnCVR provided further evidence for increased phenotypic variation in urban populations, particularly for phenology (Model 9 in Table 1): the mean lnVR estimate for laying date was positive and statistically different from zero (InVR mean estimate for laying date [95% CI] = 0.158 [0.069, 0.247]). As in the lnCVR model, lnVR mean estimates for clutch size and number of fledglings were close to zero (lnVR

TABLE 2 Differences in variation (lnCVR) in life-history traits between urban and non-urban populations at different temporal scales. Urban–non-urban differences in variation (lnCVR) in laying date, clutch size and number of fledglings per clutch were meta-analysed to assess differences in variation between urban and non-urban populations within ('intra-annual') and among ('inter-annual') breeding seasons (e.g. different temporal scales). lnCVR estimates represent meta-analytic model intercepts following the model structure presented in Table S5; positive values indicate higher variation in urban populations than in non-urban populations and vice versa. 'CI' = confidence interval; 'k' = sample size. Terms in italic bold highlight lnCVR estimates whose 95%CIs do not overlap zero. See Table 1 for a description of model IDs.

	InCVR estimate [95% CI]					
Temporal scale	Laying date	Clutch size	Number of fledglings	k		
Overall [Model 4]	0.176 [0.084, 0.268]	0.055 [-0.051, 0.160]	0.037 [-0.096, 0.171]	399		
Intra-annual [Model 5]	0.177 [0.078, 0.282]	0.015 [-0.122, 0.152]	0.116 [-0.059, 0.291]	363		
Inter-annual [Model 6]	0.074 [-0.014, 0.161]	0.096 [-0.019, 0.211]	-0.006 [-0.147, 0.135]	103		



FIGURE 4 Effects of habitat heterogeneity on the difference in phenotypic variation between urban and non-urban bird populations (i.e. lnCVR). (a) After quantifying urban index and habitat heterogeneity, we verified that urban populations had higher urban index (i.e. the proportion of landcover at a given spatial scale categorised as 'urban' [see methods]). The y axis represents the difference in urban index between urban and non-urban populations. The positive values observed for all comparisons represent that urban populations had higher urban index than their non-urban neighbours. (b) Differences in urban index between urban and non-urban populations did not predict the magnitude of the difference in phenotypic variation between populations (i.e. lnCVR). This figure shows the estimated effect of differences in urban index between populations, the higher the lnCVR. Positive values indicate that the higher the difference in urban index between urban and non-urban populations compared to non-urban counterparts). (c) Differences in habitat heterogeneity between urban and non-urban populations did positively predict the magnitude of the difference in phenotypic variation between populations (i.e. lnCVR), particularly at large spatial scales. This figure shows the estimated effect of difference in habitat heterogeneity on lnCVR at different spatial scales. Positive values indicate that the higher the difference in habitat heterogeneity on lnCVR at different spatial scales. Positive values indicate that the higher the difference in habitat heterogeneity on lnCVR at different spatial scales. Positive values indicate that the higher the difference in habitat heterogeneity on uncVR at different spatial scales. Positive values indicate that the higher the difference in habitat heterogeneity on uncVR at different spatial scales. Positive values indicate that the higher the difference in habitat heterogeneity on uncVR at different spatial scales. Positive values indicate that the higher the difference in habitat hetero

mean estimate for clutch size [95% CI] = -0.012 [-0.110], 0.056]; lnVR mean estimate for number of fledglings [95% CI] = -0.034 [-0.120, 0.052]). Additionally, the arm-based model of lnSD for laying date (Model 10 in Table 1) revealed a positive 'urban' effect on lnSD: urban populations had lnSD values 0.197 higher than nonurban populations (i.e. β_1 in Equation 9; 95%CI = [0.122, 0.272]). Laying date (log) mean phenotypic values were positively correlated with lnSD (i.e. β_2 in Equation 9; estimate [95%CI] = 0.416 [0.068, 0.764]). The arm-based models of clutch size and number of fledglings confirmed correlations between lnMean and lnSD (β_2 in Equation 9 for clutch size, estimate [95%CI] = 0.326 [0.070, 0.582];for number of fledglings, estimate [95%CI] = 0.231[0.155, 0.307]), but did not provide evidence for urban effects on phenotypic variation in clutch size or number of fledglings (β_1 in Equation 9 for clutch size, estimate [95%CI] = 0.020 [-0.079, 0.119]; for number of fledglings, estimate [95%CI] = -0.017 [-0.099, 0.065]). We did not find evidence of publication bias in lnRR or lnCVR (Supplementary Text C).

DISCUSSION

We compiled a global dataset of bird life-history traits for paired urban and non-urban populations of the same species to assess how urban living is related to changes in phenotypic means and variation for breeding phenology, reproductive effort and reproductive success. A phylogenetically controlled multilevel meta-analysis of this dataset confirms a well-documented effect of urbanisation on mean phenotypes: urban bird populations lay earlier and smaller clutches than their non-urban counterparts. This model, however, also reveals correlated responses to urbanisation across life-history traits: for example, the earlier the laying date in urban populations, the smaller the difference in clutch sizes between habitats. Our study goes a step further than previous meta-analyses in urban ecology by explicitly investigating how urbanisation could impact phenotypic variation. Our findings highlight that urbanisation is associated with both a decrease in mean phenotypes, and an increase in phenotypic variation. Investigating the temporal and spatial

scale at which urban phenotypic variation increases revealed hints at the ecological causes and evolutionary consequences.

Urbanisation has been associated with shifts in mean phenotypic values across many organisms (Alberti et al., 2017; Merckx et al., 2018; Santangelo et al., 2022), including birds, which generally show smaller body sizes and lower life-history trait values in urban habitats (Chamberlain et al., 2009; Sepp et al., 2018; Thompson et al., 2022). Our analyses expand the spatial, temporal and phylogenetic coverage of previous meta-analyses of the avian literature (Chamberlain et al., 2009; Sepp et al., 2018), and agree on their findings. Our results indicate that urban bird populations lay their eggs earlier and produce smaller clutches, which results in a lower number of surviving nestlings, than their non-urban neighbouring populations. Note, that our analysis indicates a high total heterogeneity in $\ln RR (I_{total}^2 = 97.8\%)$. This finding indicates large variation (e.g. among studies and species) in how urbanisation associates with changes in mean phenotypes and suggests that additional ecological traits (e.g. diet or migratory strategy) may also affect how populations respond to urbanisation. Our results also indicate that the mean response to urbanisation is correlated among traits. Interestingly, we found that the earlier the laying dates were in urban versus non-urban populations, the smaller the difference in clutch size and in number of surviving nestlings between habitats. Many bird species show a negative phenotypic and genetic correlation between clutch size and lay date (Dunn & Møller, 2014; Rowe et al., 1994; Sheldon et al., 2003), and these two traits are often hypothesised to coevolve (Garant et al., 2008). All else being equal, urban conditions triggering an earlier onset of reproduction (because of e.g. light pollution (Dominoni et al., 2013) or increased resource availability during winter (Schoech et al., 2004)) could indirectly increase clutch size and, therefore, reduce differences in reproductive output between urban and non-urban populations that arise via other mechanisms (e.g. resource limitation in spring; Seress et al., 2018, 2020). The extent to which mean phenotypic shifts represent adaptive responses to urbanisation in birds, either via genetic changes or plasticity, or are maladaptive, is mostly unknown (Branston et al., 2021; Caizergues et al., 2022; Lambert et al., 2020; Santangelo et al., 2022). Our results, however, highlight that phenotypic shifts in urban populations are widespread and that the response to urbanisation of associated life-history traits should be investigated together.

Urbanisation has been recently hypothesised to increase phenotypic variation and, indeed, higher variation in morphological traits of urban great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) has been recently reported (Thompson et al., 2022). Our findings greatly expand the evidence for this emerging hypothesis showing that urbanisation is overall associated with increases in variation in laying date across many bird species. Previous studies have suggested that city characteristics, such as warmer temperatures in early spring due to the urban heat island effect, could allow birds to lay more clutches per season (Schoech et al., 2008; Yeh & Price, 2004), with thereby longer breeding seasons and hence higher phenotypic variation in urban laying dates (a similar result has also been reported in Lepidoptera; Merckx et al., 2021). This effect, however, does not necessarily explain our results as our meta-analysis only included first clutch laying dates per season. As such, our findings indicate that urban bird populations display more variation in the *onset* of reproduction than their non-urban neighbours.

Higher phenotypic variation in urban than in nonurban populations within breeding seasons could be explained by at least two, non-exclusive, eco-evolutionary mechanisms: differences in the underlying additive genetic variance in laying date, whereby urban birds have a wider range of breeding values for laying date; and / or differences in habitat heterogeneity influencing plasticity in laying date, whereby urban areas have larger environmental variation than non-urban habitats (Heisler & Brazel, 2018; Shochat et al., 2006; Strubbe et al., 2020; Thompson et al., 2022). No study to date has investigated whether urban birds show higher additive genetic variance than non-urban populations. However, genetic analyses of European great tits in urban and non-urban habitats generally suggest small differences in the magnitude of genetic variation between habitats (Björklund et al., 2010; Caizergues et al., 2021; Salmón et al., 2021). This is, perhaps, not surprising given the high mobility of birds and the fact that gene flow between urban and non-urban bird populations likely occurs at a large spatial scale (Salmón et al., 2021). Interestingly, some studies have reported weaker selection for laying date in urban areas than in non-urban habitats, suggesting relaxed selection on phenology in urban birds (Branston et al., 2021; Caizergues et al., 2018), which could increase genetic variation in phenology. Assessing differences in phenotypic variation between urban and non-urban populations of less mobile species will be important to evaluate how biological traits (e.g. dispersal ability) determine the evolutionary impact of urban ecological conditions. To this end, previous work in mammal and amphibian species that have a lower dispersal ability than birds suggests a similar level of (genetic) variation between urban and non-urban habitats (Fusco et al., 2021; Richardson et al., 2021).

Habitat complexity differs between urban and nonurban habitats (Arnfield, 2003; Pickett et al., 2017). Our analyses indicate that differences in urban versus non-urban habitat heterogeneity could indeed help explain the observed pattern of increased phenotypic variation in urban populations. Several ecological mechanisms could mediate this effect. Urban environments are characterised by an array of microhabitats with varying levels of human pressure, exotic plant species and resource availability. Thus, the intensity and timing of the environmental cues that birds use to time their reproduction could vary at a small local scale, increasing phenotypic variation in phenology in the presence of plasticity. The existence of plastic responses to urban habitat heterogeneity, which our results might indicate, do not preclude selection from acting on urban bird populations. First, plasticity is an important mechanism of adaptation, sometimes aligned in direction with adaptative genetic changes (De Lisle et al., 2022), and indeed is often involved in adaptation to urban environments (Campbell-Staton et al., 2021; Halfwerk et al., 2019). Second, plastic responses can aid adaptation to urban conditions in the presence of genetic-by-environment interactions by increasing genetic variation available for natural selection (Via & Lande, 1985). Addressing which evolutionary mechanisms cause the observed increase in phenotypic variation in urban bird populations is beyond the scope of this study and we acknowledge that these arguments are largely speculative at this point. However, our findings highlight that eco-evolutionary processes could largely differ between urban and nonurban bird populations and generate new avenues for future research in urban ecology and evolution.

In agreement with our initial predictions, habitat heterogeneity was associated with the magnitude of the difference in phenotypic variation between urban and non-urban bird populations. However, we acknowledge that this analysis has several limitations and that the results require cautious interpretation. First, only a subset of published studies provided coordinates for their urban and non-urban study populations (26 out of 68 published papers). When study site coordinates were provided, only one pair of coordinates per study location was provided, preventing an accurate assessment of the actual area over which a given breeding population was studied. Additionally, it is common in urban eco-evolutionary studies to monitor several populations within one single city. However, in most studies, spatial information was provided at the scale of the whole city (e.g. a single set of coordinates), preventing the accurate quantification of habitat heterogeneity for every sub-population within a given urban habitat. These limitations highlight that the ability to perform global meta-analyses on the effects of urban habitat heterogeneity on phenotypic variation would be greatly improved if individual studies provided accurate coordinates of the location of their study populations. Reporting such information would allow future research synthesis to quantify phenotypic variation within urban populations (e.g. across different sub-populations in the same city) and between urban and non-urban populations.

Taken together, our results show that urbanisation is associated with both a decrease in mean phenotypic values and increased phenotypic variation in bird populations. Our analyses also highlight a temporal and spatial mechanism that could generate such differences in phenotypic variation between urban and non-urban habitats. We show that urban bird populations have a more variable phenology than non-urban conspecifics within breeding seasons (i.e. differences in phenology across habitats are seemingly not due to between-year fluctuations) suggesting that the ecological conditions that generate such differences are constant across multiple years. Our coupled spatial analysis indicates habitat heterogeneity and plastic responses as potential ecoevolutionary drivers generating these results. The ecoevolutionary implications of higher phenotypic variation in urban environments will likely vary among species (Thompson et al., 2022) and our findings highlight the need for detailed investigation of these consequences. To this end, long-term studies of individually marked organisms in replicated paired urban and non-urban environments could be particularly fruitful to unravel whether differences in phenotypic variation between urban and non-urban populations are caused by differences in underlying genetic variation and/or plastic responses to the urban environment.

AUTHOR CONTRIBUTIONS

Pablo Capilla-Lasheras, Megan J. Thompson, Denis Réale, Anne Charmantier and Davide M. Dominoni conceived the study. Pablo Capilla-Lasheras, Alfredo Sánchez-Tójar, Claire J. Branston and Davide M. Dominoni performed the literature search. Pablo Capilla-Lasheras extracted effect sizes from suitable published papers. Megan J. Thompson validated effect size extraction. Pablo Capilla-Lasheras and Yacob Haddou performed all statistical analysis with advice from Alfredo Sánchez-Tójar. Pablo Capilla-Lasheras wrote the first draft of the manuscript with input from Megan J. Thompson, Alfredo Sánchez-Tójar, Denis Réale, Anne Charmantier and Davide M. Dominoni. All authors read and revised the manuscript.

ACKNOWLEDGEMENTS

We thank Paul Bellamy, Clint Boal, Peter Ferns, Michał Glądalski, Shelley A. Hinsley, Piotr Minias, Christy Morrissey, Matthew Reudink, Staffan Roos, Renaud Scheifler, Christine Stracey, Daniel Shustack and Jarosław Wawrzyniak for kindly replying to our data queries. We are grateful to Antje Girndt for developing the list of avian genera included in our search string.

FUNDING INFORMATION

P.C.-L., C.J.B. and D.M.D. were funded by a Highlight Topics grant from the Natural Environment Research Council awarded to D.M.D. (NE/S005773/1).

CONFLICT OF INTEREST

The authors declare no competing interests.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.14099.

OPEN RESEARCH BADGES

0 😳

This article has earned Open Data and Open Materials badges. Data and materials are available at https://ze-nodo.org/record/7010687.

DATA AVAILABILITY STATEMENT

All R scripts and datasets needed to reproduce the analyses presented in this paper are available at https://doi. org/10.5281/zenodo.7010687.

ORCID

Pablo Capilla-Lasheras b https://orcid. org/0000-0001-6091-7089 Megan J. Thompson b https://orcid. org/0000-0002-0279-5340 Alfredo Sánchez-Tójar b https://orcid. org/0000-0002-2886-0649 Yacob Haddou b https://orcid.org/0000-0003-0557-0339 Claire J. Branston b https://orcid. org/0000-0002-4416-0984 Denis Réale https://orcid.org/0000-0002-0419-7125 Davide M. Dominoni b https://orcid. org/0000-0003-2063-9955

REFERENCES

- Alberti, M., Correa, C., Marzluff, J.M., Hendry, A.P., Palkovacs, E.P., Gotanda, K.M. et al. (2017) Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Science of the United States of America*, 114, 8951–8956.
- Antonov, A. & Atanasova, D.Y. (2003) Small-scale differences in the breeding ecology of urban and rural Magpies Pica Pica. Ornis Fennica, 80, 21–30.
- Arnfield, A.J. (2003) Two decades of urban climate research: a review of turbulence, exchanges of energy and water, and the urban heat Island. *International Journal of Climatology*, 23, 1–26.
- Bailly, J., Scheifler, R., Berthe, S., Clément-Demange, V.A., Leblond, M., Pasteur, B. et al. (2016) From eggs to fledging: negative impact of urban habitat on reproduction in two tit species. *Journal* of Ornithology, 157, 377–392.
- Baldan, D. & Ouyang, J.Q. (2020) Urban resources limit pair coordination over offspring provisioning. *Scientific Reports*, 10, 15888.
- Beck, N.R. & Heinsohn, R. (2006) Group composition and reproductive success of cooperatively breeding white-winged choughs (*Corcorax melanorhamphos*) in urban and non-urban habitat. *Austral Ecology*, 31, 588–596.
- Berardelli, D., Desmond, M.J. & Murray, L. (2010) Reproductive success of burrowing owls in urban and grassland habitats in southern New Mexico. *Wilson Journal of Ornithology*, 122, 51–59.

- Biard, C., Brischoux, F., Meillère, A., Michaud, B., Nivière, M., Ruault, S. et al. (2017) Growing in cities: an urban penalty for wild birds? A study of phenotypic differences between urban and rural great tit chicks (*Parus major*). Frontiers in Ecology and Evolution, 5, 79.
- Björklund, M., Ruiz, I. & Senar, J.C. (2010) Genetic differentiation in the urban habitat: the great tits (*Parus major*) of the parks of Barcelona city. *Biological Journal of the Linnean Society*, 99, 9–19.
- Boal, C.W. & Mannan, R.W. (1999) Comparative breeding ecology of Cooper's hawks in urban and exurban areas of southeastern Arizona. *The Journal of Wildlife Management*, 63, 77–84.
- Bobek, O., Gal, A., Saltz, D. & Motro, U. (2018) Effect of nest-site microclimatic conditions on nesting success in the Lesser Kestrel *Falco naumanni. Bird Study*, 65, 444–450.
- Brahmia, Z., Scheifler, R., Crini, N., Maas, S., Giraudoux, P. & Benyacoub, S. (2013) Breeding performance of blue tits (*Cyanistes cæruleus ultramarinus*) in relation to lead pollution and nest failure rates in rural, intermediate, and urban sites in Algeria. *Environmental Pollution*, 174, 171–178.
- Branston, C.J., Capilla, P., Christopher, L., Griffiths, K., White, S. & Dominoni, D.M. (2021) Urbanisation weakens selection on the timing of breeding and clutch size in blue tits but not in great tits. *Behavioral Ecology and Sociobiology*, 75, 155.
- Burnham, K.P., Anderson, D.R. & Huyvaert, K.P. (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology* and Sociobiology, 65, 23–35.
- Caizergues, A.E., Charmantier, A. & Grégoire, A. (2018) Urban versus forest ecotypes are not explained by divergent reproductive selection. *Proceedings of the Royal Society B*, 285, 20180261.
- Caizergues, A.E., Grégoire, A., Choquet, R., Perret, S. & Charmantier, A. (2022) Are behaviour and stress-related phenotypes in urban birds adaptive? *Journal of Animal Ecology*, 00, 1–15.
- Caizergues, A.E., le Luyer, J., Grégoire, A., Szulkin, M., Senar, J., Charmantier, A. et al. (2021) Epigenetics and the city: nonparallel DNA methylation modifications across pairs of urbanrural Great tit populations. *Evolutionary Applications*, 15, 149–165.
- Campbell-Staton, S.C., Velotta, J.P. & Winchell, K.M. (2021) Selection on adaptive and maladaptive gene expression plasticity during thermal adaptation to urban heat islands. *Nature Communications*, 12, 6195.
- Campbell-Staton, S.C., Winchell, K.M., Rochette, N.C., Fredette, J., Maayan, I., Schweizer, R.M. et al. (2020) Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. *Nature Ecology & Evolution*, 4, 652–658.
- Capilla-Lasheras, P., Dominoni, D.M., Babayan, S.A., O'Shaughnessy, P.J., Mladenova, M., Woodford, L. et al. (2017) Elevated immune gene expression is associated with poor reproductive success of urban blue tits. *Frontiers in Ecology and Evolution*, 5, 64.
- Cardilini, A.P.A., Weston, M.A., Nimmo, D.G., Dann, P. & Sherman, C.D.H. (2013) Surviving in sprawling suburbs: suburban environments represent high quality breeding habitat for a widespread shorebird. *Landscape and Urban Planning*, 115, 72–80.
- Chamberlain, D.E., Cannon, A.R., Toms, M.P. & Leech, D.I. (2009) Avian productivity in urban landscapes: a review and metaanalysis. *Ibis*, 151, 1–18.
- Chao, A., Chiu, C. & Jost, L. (2014) Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through hill numbers. *Annual Review of Ecology, Evolution, and Systematics*, 45, 297–324.
- Charter, M., Izhaki, I., Bouskila, A. & Leshem, Y. (2007) Breeding success of the Eurasian Kestrel (*Falco tinnunculus*) nesting on buildings in Israel. *Journal of Raptor Research*, 41, 139–143.
- Cinar, O., Nakagawa, S. & Viechtbauer, W. (2022) Phylogenetic multilevel meta-analysis: a simulation study on the importance of

modeling the phylogeny. *Methods in Ecology and Evolution*, 13, 383–395.

- Cohen, J.E. & Xu, M. (2015) Random sampling of skewed distributions implies Taylor's power law of fluctuation scaling. *Proceedings of the National Academy of Science of the United States of America*, 112, 7749–7754.
- Conway, C.J., Garcia, V., Smith, M.D., Ellis, L.A. & Whitney, J.L. (2006) Comparative demography of Burrowing Owls in agricultural and urban landscapes in southeastern Washington. *Journal* of Field Ornithology, 77, 280–290.
- De Lisle, S.P., Mäenpää, M.I. & Svensson, E.I. (2022) Phenotypic plasticity is aligned with phenological adaptation on both microand macroevolutionary timescales. *Ecology Letters*, 25, 790–801.
- de Satgé, J., Strubbe, D., Elst, J., De Laet, J., Adriaensen, F. & Matthysen, E. (2019) Urbanisation lowers great tit Parus major breeding success at multiple spatial scales. *Journal of Avian Biology*, 50, e02108.
- Dhondt, A.A., Eyckerman, R., Moermans, R. & Hublé, J. (1984) Habitat and laying date of Great and Blue Tit *Parus major* and *P. caeruleus. Ibis*, 126, 388–397.
- Diamond, S.E., Chick, L.D., Perez, A., Strickler, S.A. & Martin, R.A. (2018) Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. *Proceedings of the Royal Society B*, 285, 20180036.
- Diamond, S.E. & Martin, R.A. (2021) Evolution in cities. Annual Review of Ecology, Evolution, and Systematics, 52, 519–540.
- Dominoni, D., Quetting, M. & Partecke, J. (2013) Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society B*, 280, 20123017.
- Dunn, P.O. & Møller, A.P. (2014) Changes in breeding phenology and population size of birds. *Journal of Animal Ecology*, 83, 729–739.
- Eden, S.F. (1985) The comparative breeding biology of magpies Pica Pica in an urban and a rural habitat (Aves: Corvidae). *Journal of Zoology*, 205, 325–334.
- ESA. (2017) Land Cover CCI Product User Guide Version 2. Technical Report. Available from: http://maps.elie.ucl.ac.be/CCI/viewe r/download/ESACCI-LC-Ph2-PUGv2_2.0.pdf [Accessed 20th June 2022].
- ESA. (2020) 3CS Land Cover Product User Guide. Belgium: European Space Agency. Available from: https://datastore.copernicusclimate.eu/documents/satellite-land-cover/D3.3.12-v1.3_PUGS_ ICDR_LC_v2.1.x_PRODUCTS_v1.3.pdf
- Evans, B.A. & Gawlik, D.E. (2020) Urban food subsidies reduce natural food limitations and reproductive costs for a wetland bird. *Scientific Reports*, 10, 14021.
- Fugère, V. & Hendry, A.P. (2018) Human influences on the strength of phenotypic selection. *Proceedings of the National Academy of Science of the United Science of America*, 115, 10070–10075.
- Fusco, G. (2001) How many processes are responsible for phenotypic evolution? *Evolution & Development*, 3, 279–286.
- Fusco, N.A., Pehek, E. & Munshi-South, J. (2021) Urbanization reduces gene flow but not genetic diversity of stream salamander populations in the New York City metropolitan area. *Evolutionary Applications*, 14, 99–116.
- Gahbauer, M.A., Bird, D.M., Clark, K.E., French, T., Brauning, D.W.
 & McMorris, F.A. (2015) Productivity, mortality, and management of urban peregrine falcons in northeastern North America. Journal of Wildlife Management, 79, 10–19.
- Garant, D., Hadfield, J.D., Kruuk, L.E.B. & Sheldon, B.C. (2008) Stability of genetic variance and covariance for reproductive characters in the face of climate change in a wild bird population. *Molecular Ecology*, 17, 179–188.
- Glądalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J. et al. (2016) Effects of extreme thermal conditions on plasticity in breeding phenology and doublebroodedness of Great Tits and Blue Tits in central Poland in

2013 and 2014. International Journal of Biometeorology, 60, 1795–1800.

- Glądalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J. et al. (2016) Effects of nest characteristics on reproductive performance in Blue Tits *Cyanistes caeruleus* and Great Tits *Parus major. Avian Biology Research*, 9, 37–43.
- Glądalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J. et al. (2017) Differences in the breeding success of blue tits *Cyanistes caeruleus* between a forest and an urban area: a long-term study. *Acta Ornithologica*, 52, 59–68.
- Glądalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J. et al. (2015) Inter-annual and inter-habitat variation in breeding performance of blue tits (*Cyanistes caeruleus*) in Central Poland. Ornis Fennica, 92, 34–42.
- Glądalski, M., Kaliński, A., Wawrzyniak, J., Bańbura, M., Markowski, M., Skwarska, J. et al. (2018) Physiological condition of nestling great tits *Parus major* in response to experimental reduction in nest micro- and macro-parasites. *Conservation Physiology*, 6, 1–9.
- Gorton, A.J., Moeller, D.A. & Tiffin, P. (2018) Little plant, big city: a test of adaptation to urban environments in common ragweed (*Ambrosia artemisiifolia*). Proceedings of the Royal Society B, 285, 20180968.
- Grafen, A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society of London*, 326, 119–157.
- Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X. et al. (2008) Global change and the ecology of cities. *Science*, 319, 756–760.
- Gryz, J. & Krauze-Gryz, D. (2018) Influence of habitat urbanisation on time of breeding and productivity of tawny owl (*Strix aluco*). *Polish Journal of Ecology*, 66, 153–161.
- Hajdasz, A.C., Otter, K.A., Baldwin, L.K. & Reudink, M.W. (2019) Caterpillar phenology predicts differences in timing of mountain chickadee breeding in urban and rural habitats. Urban Ecosystem, 22, 1113–1122.
- Halfwerk, W., Blaas, M., Kramer, L., Hijner, N., Trillo, P.A., Bernal, X.E. et al. (2019) Adaptive changes in sexual signalling in response to urbanization. *Nature Ecology and Evolution*, 3, 374–380.
- Hedges, L., Gurevitch, J. & Curtis, P. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150–1156.
- Hedges, L.V. (1981) Distribution theory for Glass's estimator of effect size and related estimators. *Journal of Educational Statistics*, 6, 107–128.
- Heisler, J.M. & Brazel, A.J. (2018) The urban physical environment: temperature and urban heat islands. In: Aitkenhead-Petersn, J. & Volder, A. (Eds.) Urban Ecosystem Ecology. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, pp. 29–56.
- Hendry, A.P., Gotanda, K.M. & Svensson, E.I. (2017) Human influences on evolution, and the ecological and societal consequences. *Philosophical Transactions of the Royal Society B*, 372, 20160028.
- Hijmans, R. (2020) raster: geographic data analysis and modeling. R package version 3.3-13. Available from: https://CRAN.R-proje ct.org/package=raster [Accessed 17th July 2020].
- Hill, M.O. (1973) Diversity and evenness: a unifying notation and its consequences. *Ecology*, 54, 427–432.
- Hinchliff, C.E., Smith, S.A., Allman, J.F., Burleigh, J.G., Chaudhary, R., Coghill, L.M. et al. (2015) Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceedings of the National Academy of Science of the United States of America*, 112, 12764–12769.
- Hinsley, S.A., Hill, R.A., Bellamy, P.E., Harrison, N.M., Speakman, J.R., Wilson, A.K. et al. (2008) Effects of structural and functional habitat gaps on breeding woodland birds: working harder for less. *Landscape Ecology*, 23, 615–626.

- Ibáñez-Álamo, J.D. & Soler, M. (2010) Does urbanization affect selective pressures and life-history strategies in the common blackbird (*Turdus merula* L.)? *Biological Journal of the Linnean Society*, 101, 759–766.
- Isaksson, C. & Andersson, S. (2007) Carotenoid diet and nestling provisioning in urban and rural great tits *Parus major. Journal of Avian Biology*, 38, 564–572.
- Isaksson, C., Johansson, A. & Andersson, S. (2008) Egg yolk carotenoids in relation to habitat and reproductive investment in the great tit *Parus major*. *Physiological and Biochemical Zoology*, 81, 112–118.
- Jarrett, C., Powell, L.L., McDevitt, H., Helm, B. & Welch, A.J. (2020) Bitter fruits of Hard Labour: diet metabarcoding and telemetry reveal that urban songbirds travel further for lower-quality food. *Oecologia*, 193, 377–388.
- Johnson, M.T.J. & Munshi-South, J. (2017) Evolution of life in urban environments. Science, 358, eaam8327.
- Kelleher, K.M. & O'Halloran, J. (2007) Influence of nesting habitat on breeding song thrushes *Turdus philomelos. Bird Study*, 54, 221–229.
- Kettel, E.F., Gentle, L.K., Yarnell, R.W. & Quinn, J.L. (2019) Breeding performance of an apex predator, the peregrine falcon, across urban and rural landscapes. *Urban Ecosystem*, 22, 117–125.
- Kopij, G. (2017) Changes in the number of nesting pairs and breeding success of the White Stork Ciconia in a large city and a neighbouring rural area in South-West Poland. *Ornis Hungarica*, 25, 109–115.
- Lambert, M.R., Brans, K.I., Des Roches, S., Donihue, C.M. & Diamond, S.E. (2020) Adaptive evolution in cities: Progress and misconceptions. *Trends in Ecology & Evolution*, 36, 239–257.
- Lee, S., Lee, H., Jablonski, P.G., Choe, J.C. & Husby, M. (2017) Microbial abundance on the eggs of a passerine bird and related fitness consequences between urban and rural habitats. *PLoS One*, 12, e0185411.
- Lin, W.L., Lin, S.M., Lin, J.W., Wang, Y. & Tseng, H.Y. (2015) Breeding performance of Crested Goshawk Accipiter trivirgatus in urban and rural environments of Taiwan. Bird Study, 62, 177–184.
- Liven-Schulman, I., Leshem, Y., Alon, D. & Yom-Tov, Y. (2004) Causes of population declines of the Lesser Kestrel Falco naumanni in Israel. *Ibis*, 146, 145–152.
- Luna, Á., Palma, A., Sanz-Aguilar, A., Tella, J.L. & Carrete, M. (2020) Sex, personality and conspecific density influence natal dispersal with lifetime fitness consequences in urban and rural burrowing owls. *PLoS One*, 15, 1–17.
- Luo, D., Wan, X., Liu, J. & Tong, T. (2016) Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Statistical Methods in Medical Research*, 27, 1785–1805.
- Mcgowan, K.J. (2001) Avian ecology and conservation in an urbanizing world. In: Marzluff, J.M., Bowman, R. & Donelly, R. (Eds.) *Avian ecology and conservation in an urbanizing world*. New York: Kluwer Academic Press, pp. 365–381.
- Mennechez, G. & Clergeau, P. (2006) Effect of urbanisation on habitat generalists: starlings not so flexible? Acta Oecologica, 30, 182–191.
- Merckx, T., Nielsen, M.E., Heliölä, J., Kuussaari, M., Pettersson, L.B., Pöyry, J. et al. (2021) Urbanization extends flight phenology and leads to local adaptation of seasonal plasticity in Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America, 118, e2106006118.
- Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F., Backeljau, T., Bonte, D. et al. (2018) Body-size shifts in aquatic and terrestrial urban communities. *Nature*, 558, 113–116.
- Michonneau, F., Brown, J.W. & Winter, D.J. (2016) Rotl: an R package to interact with the open tree of life data. *Methods in Ecology and Evolution*, 7, 1476–1481.
- Middleton, A. (1979) Influence of age and habitat on reproduction by the American goldfinch. *Ecology*, 60, 418–432.

- Millsap, B., Breen, T., McConnell, E., Steffer, T., Phillips, L., Douglass, N. et al. (2004) Comparative fecundity and survival of bald eagles fledged from suburban and rural natal areas in Florida. *Journal of Wildlife Management*, 68, 1018–1031.
- Minias, P. (2016) Reproduction and survival in the city: which fitness components drive urban colonization in a reed-nesting waterbird? *Current Zoology*, 62, 79–87.
- Morrissey, C.A., Stanton, D.W.G., Tyler, C.R., Pereira, M.G., Newton, J., Durance, I. et al. (2014) Developmental impairment in Eurasian dipper nestlings exposed to urban stream pollutants. *Environmental Toxicology and Chemistry*, 33, 1315–1323.
- Muñoz, M.M., Stimola, M.A., Algar, A.C., Conover, A., Rodriguez, A.J., Landestoy, M.A. et al. (2014) Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proceedings of the Royal Society B*, 281, 20132433.
- Nakagawa, S., Lagisz, M., Jennions, M.D., Koricheva, J., Noble, D.W.A., Parker, T.H. et al. (2022) Methods for testing publication bias in ecological and evolutionary meta-analyses. *Methods* in Ecology and Evolution, 13, 4–21.
- Nakagawa, S., Lagisz, M., O'Dea, R.E., Rutkowska, J., Yang, Y., Noble, D.W.A. et al. (2021) The orchard plot: cultivating a forest plot for use in ecology, evolution, and beyond. *Research Synthesis Methods*, 12, 4–12.
- Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M. et al. (2015) Meta-analysis of variation: ecological and evolutionary applications and beyond. *Methods in Ecology* and Evolution, 6, 143–152.
- Nakagawa, S. & Santos, E.S.A. (2012) Methodological issues and advances in biological meta-analysis. *Evolutionary Ecology*, 26, 1253–1274.
- Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142.
- Newhouse, M.J., Marra, P.P. & Johnson, L.S. (2008) Reproductive success of house wrens in suburban and rural landscapes. *The Wilson Journal of Ornithology*, 120, 99–104.
- OpenTreeOfLife, Redelings, B., Reyes, L.L.S., Cranston, K.A., Allman, J., Holder, M.T., et al. (2019). Open Tree of Life Synthetic Tree (12.3). Zenodo. Available from: https://doi.org/10.5281/ zenodo.3937742
- Partecke, J., Hegyi, G., Fitze, P.S., Gasparini, J. & Schwabl, H. (2020) Maternal effects and urbanization: variation of yolk androgens and immunoglobulin in city and forest blackbirds. *Ecology and Evolution*, 10, 2213–2224.
- Pavlicev, M., Cheverud, J.M. & Wagner, G.P. (2011) Evolution of adaptive phenotypic variation patterns by direct selection for evolvability. *Proceedings of the Royal Society B*, 278, 1903–1912.
- Pebesma, E. (2018) Simple features for R: standardized support for spatial vector data. *The R Journal*, 10, 439–446.
- Perlut, N.G., Bonter, D.N., Ellis, J.C. & Friar, M.S. (2016) Roof-top nesting in a declining population of herring gulls (*Larus argentatus*) in Portland, Maine, USA. *Waterbirds*, 39, 68–73.
- Pickett, S.T.A., Cadenasso, M.L., Rosi-Marshall, E.J., Belt, K.T., Groffman, P.M., Grove, J.M. et al. (2017) Dynamic heterogeneity: a framework to promote ecological integration and hypothesis generation in urban systems. *Urban Ecosystem*, 20, 1–14.
- Pollock, C.J., Capilla-Lasheras, P., McGill, R.A.R., Helm, B. & Dominoni, D.M. (2017) Integrated behavioural and stable isotope data reveal altered diet linked to low breeding success in urban-dwelling blue tits (*Cyanistes caeruleus*). Scientific Reports, 7, 5014.
- Preiszner, B., Papp, S., Pipoly, I., Seress, G., Vincze, E., Liker, A. et al. (2017) Problem-solving performance and reproductive success of great tits in urban and forest habitats. *Animal Cognition*, 20, 53–63.
- R Core Team. (2022) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

- Rees, J.A. & Cranston, K. (2017) Automated assembly of a reference taxonomy for phylogenetic data synthesis. *Biodiversity Data Journal*, 5, e12581.
- Richardson, J.L., Michaelides, S., Combs, M., Djan, M., Bisch, L., Barrett, K. et al. (2021) Dispersal ability predicts spatial genetic structure in native mammals persisting across an urbanization gradient. *Evolutionary Applications*, 14, 163–177.
- Rollinson, D.J. & Jones, D.N. (2003) Variation in breeding parameters of the Australian magpie *Gymnorhina tibicen* in suburban and rural environments. *Urban Ecosystem*, 6, 257–269.
- Rosenfield, R.N., Hardin, M.G., Taylor, J., Sobolik, L.E. & Frater, P.N. (2019) Nesting density and dispersal movements between urban and rural habitats of cooper's Hawks (*Accipiter cooperii*) in Wisconsin: are these source or sink habitats? *American Midland Naturalist*, 182, 36–51.
- Rowe, L., Ludwig, D. & Schluter, D. (1994) Time, condition, and the seasonal decline of avian clutch size. *American Naturalist*, 143, 698–772.
- Salmón, P., Jacobs, A., Ahrén, D., Biard, C., Dingemanse, N.J., Dominoni, D.M. et al. (2021) Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe. *Nature Communications*, 12, 2983.
- Santangelo, J.S., Ness, R.W., Cohan, B., Fitzpatrick, C.R., Innes, S.G., Koch, S. et al. (2022) Global urban environmental change drives adaptation in white clover. *Science*, 375, 1275–1281.
- Schmidt, V.K.-H. & Steinbach, J. (1983) Niedriger Bruterfolg der Kohlmeise (*Parus major*) in städtischen Parks und Friedhöfen. *Journal für Ornithologie*, 124, 81–83.
- Schoech, S.J. & Bowman, R. (2001) Variation in the timing of breeding between suburban and wildland Florida Scrub-Jays: do physiologic measures reflect different environments? In: Marzluff, J.M., Bowman, R. & Donelly, R. (Eds.) Avian ecology and conservation in an urbanizing world. New York: Kluwer Academic Press, pp. 289–306.
- Schoech, S.J., Bowman, R., Bridge, E.S. & Boughton, R.K. (2007) Baseline and acute levels of corticosterone in Florida Scrub-jays (*Aphelocoma coerulescens*): effects of food supplementation, suburban habitat, and year. *General and Comparative Endocrinology*, 154, 150–160.
- Schoech, S.J., Bowman, R. & Reynolds, S.J. (2004) Food supplementation and possible mechanisms underlying early breeding in the Florida Scrub-Jay (*Aphelocoma coerulescens*). *Hormones and Behavior*, 46, 565–573.
- Schoech, S.J., Bridge, E.S., Boughton, R.K., Reynolds, S.J., Atwell, J.W. & Bowman, R. (2008) Food supplementation: a tool to increase reproductive output? A case study in the threatened Florida Scrub-Jay. *Biological Conservation*, 141, 162–173.
- Senior, A.M., Gosby, A.K., Lu, J., Simpson, S.J. & Raubenheimer, D. (2016) Meta-analysis of variance: an illustration comparing the effects of two dietary interventions on variability in weight. *Evolution, Medicine and Public Health*, 2016, 244–255.
- Senior, A.M., Grueber, C.E., Kamiya, T., Lagisz, M., O'Dwyer, K., Santos, E.S.A. et al. (2016) Heterogeneity in ecological and evolutionary meta- analyses: its magnitude and implications. *Ecology*, 97, 3293–3299.
- Senior, A.M., Viechtbauer, W. & Nakagawa, S. (2020) Revisiting and expanding the meta-analysis of variation: the log coefficient of variation ratio. *Research Synthesis Methods*, 11, 553–567.
- Sepp, T., McGraw, K.J., Kaasik, A. & Giraudeau, M. (2018) A review of urban impacts on avian life-history evolution: does city living lead to slower pace of life? *Global Change Biology*, 24, 1452–1469.
- Seress, G., Bókony, V., Pipoly, I., Szép, T., Nagy, K. & Liker, A. (2012) Urbanization, nestling growth and reproductive success in a moderately declining house sparrow population. *Journal of Avian Biology*, 43, 403–414.
- Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., Pipoly, I. et al. (2018) Impact of urbanization on abundance and phenology

of caterpillars and consequences for breeding in an insectivorous bird. *Ecological Applications*, 28, 1143–1156.

- Seress, G., Sándor, K., Evans, K.L. & Liker, A. (2020) Food availability limits avian reproduction in the city: an experimental study on great tits *Parus major. Journal of Animal Ecology*, 89, 1570–1580.
- Sharma, R.C., Bhatt, D. & Sharma, R.K. (2004) Breeding success of the tropical spotted munia *Lonchura punctulata* in urbanized and forest habitats. *Ornithological Science*, 3, 113–117.
- Sheldon, B.C., Kruuk, L.E.B. & Merilä, J. (2003) Natural selection and inheritance of breeding time and clutch size in the collared flycatcher. *Evolution*, 57, 406–420.
- Shi, J., Luo, D., Weng, H., Zeng, X., Lin, L., Chu, H. et al. (2020) Optimally estimating the sample standard deviation from the five-number summary. *Research Synthesis Methods*, 11, 641–654.
- Shochat, E., Warren, P.S., Faeth, S.H., McIntyre, N.E. & Hope, D. (2006) From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution*, 21, 186–191.
- Shustack, D.P. & Rodewald, A.D. (2011) Nest predation reduces benefits to early clutch initiation in northern cardinals *Cardinalis cardinalis. Journal of Avian Biology*, 42, 204–209.
- Solonen, T. (2001) Breeding of the great tit and blue tit in urban and rural habitats in southern Finland. *Ornis Fennica*, 78, 49–60.
- Solonen, T. (2014) Timing of breeding in rural and urban tawny owls Strix aluco in southern Finland: effects of vole abundance and winter weather. Journal of Ornithology, 155, 27–36.
- Solonen, T. & Ursin, K.A. (2008) Breeding of tawny owls *Strix aluco* in rural and urban habitats in southern Finland. *Bird Study*, 55, 216–221.
- Stout, W.E., Anderson, R.K. & Papp, J.M. (1998) Urban, suburban and rural red-tailed hawk nesting habitat and populations in southeast Wisconsin. *Journal of Raptor Research*, 32, 221–228.
- Stracey, C.M. & Robinson, S.K. (2012) Are urban habitats ecological traps for a native songbird? Season-long productivity, apparent survival, and site fidelity in urban and rural habitats. *Journal of Avian Biology*, 43, 50–60.
- Strubbe, D., Salleh Hudin, N., Teyssier, A., Vantieghem, P., Aerts, J. & Lens, L. (2020) Phenotypic signatures of urbanization are scale-dependent: a multi-trait study on a classic urban exploiter. *Landscape and Urban Planning*, 197, 103767.
- Sumasgutner, P., Nemeth, E., Tebb, G., Krenn, H.W. & Gamauf, A. (2014) Hard times in the city-attractive nest sites but insufficient food supply lead to low reproduction rates in a bird of prey. *Frontiers in Zoology*, 34, 17–31.
- Thompson, M.J., Capilla-Lasheras, P., Dominoni, D.M., Réale, D. & Charmantier, A. (2022) Phenotypic variation in urban environments: mechanisms and implications. *Trends in Ecology & Evolution*, 37, 171–182.
- Thornton, M., Todd, I. & Roos, S. (2017) Breeding success and productivity of urban and rural Eurasian Sparrowhawks Accipiter nisus in Scotland. Ecoscience, 24, 115–116.
- Trikalinos, T. & Ioannidis, J.P.A. (2005) Assessing the evolution of effect sizes over time. In: Rothstein, H., Sutton, A. & Borenstein, M. (Eds.) *Publication bias in meta-analysis*. Chichester: John Wiley, pp. 241–259.
- Uchida, K., Blakey, R.V., Burger, J.R., Cooper, D.S., Niesner, C.A. & Blumstein, D.T. (2021) Urban biodiversity and the importance of scale. *Trends in Ecology and Evolution*, 36, 123–131.
- Via, S. & Lande, R. (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39, 505–522.
- Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, 36, 1–48.
- Wawyrzyniak, J., Kaliński, A., Glądalski, M., Bańbura, M., Markowski, M., Skwarska, J. et al. (2015) Long-term variation in laying date and clutch size of the great tit *Parus major* in Central Poland: a comparison between Urban Parkland and Deciduous Forest. *Ardeola*, 62, 311–322.

- Welch-Acosta, B.C., Skipper, B.R. & Boal, C.W. (2019) Comparative breeding ecology of Mississippi Kites in urban and exurban areas of West Texas. *Journal of Field Ornithology*, 90, 248–257.
- Westgate, M.J. (2019) revtools: an R package to support article screening for evidence synthesis. *Research Synthesis Methods*, 10, 606–614.
- Yeh, P.J. & Price, T.D. (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. *The American Naturalist*, 164, 531–542.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article. How to cite this article: Capilla-Lasheras, P., Thompson, M.J., Sánchez-Tójar, A., Haddou, Y., Branston, C.J. & Réale, D. et al. (2022) A global meta-analysis reveals higher variation in breeding phenology in urban birds than in their non-urban neighbours. *Ecology Letters*, 25, 2552–2570. Available from: https://doi.org/10.1111/ele.14099

APPENDICE F

Gervais et al. 2024 Under revision

Behavioral Ecology



Behavioral Ecology

Behavioral variation changes across an urbanization gradient in a population of great tits

Journal:	Behavioral Ecology
Manuscript ID	BEHECO-2024-0082
Manuscript Type:	Original article
Keywords:	among-individual variance, city, coefficient of variation, multiple-spatial scale, repeatability, trait variation

SCHOLARONE[™] Manuscripts

Lay summary : 72 words

Urbanization has been shown to alter trait means and more recently to affect the variance of life history and morphological traits, for example by increasing diversity in laying dates. However, the influence of urbanization on behavioral variation is not well understood. Using a long-term study of great tits, we show that individuals in the city may have more diverse behavioral stress responses, yet display stronger similarity in their behavioral responses to novelty.

io Review Only

Behavioral variation changes across an urbanization gradient in a population of great tits

Abstract : 247 words

Urbanization is occurring globally at an unprecedented rate and, despite the ecoevolutionary importance of individual variation in adaptive traits, we still have very limited insight on how phenotypic variation is modified by anthropogenic environmental change. Urbanization can increase individual differences in some contexts, but whether this is generalizable to behavioral traits, which directly affect how organisms interact with and respond to environmental variation, is not known. Here we examine variation across three behavioral traits (breath rate, handling aggression and exploration behaviour) in great tits Parus major along an urbanization gradient (n > 1000 phenotyped individuals accross nine years) to determine whether among-individual variance in behavior increases with the degree of urbanization and spatial heterogeneity. Urban birds were more aggressive and faster explorers than forest birds. They also displayed higher among-individual variation for breath rate and aggression (1.5 and 1.8 times increase, respectively), but lower among-individual variation for exploration (3.3 times decrease). Only individual variation in exploration clearly changed along the continuous urbanization gradient; individual differences in exploration declined with increasing impervious surface area. Collectively our results suggest that individuals in the city may have more diverse behavioral stress responses, yet display stronger similarity in their behavioral responses to novelty. Our results suggest that generalizations about urbanization's impacts on behavioral variation are not appropriate. Instead our results suggest that urbanization can shape individual variation differently across behavioral functions and we may expect decreased individual diversity in urban birds for traits related to behavioral response to novelty.

Key words: among-individual variance, city, coefficient of variation, multiple-spatial scale, repeatability, trait variation

Introduction

Environmental change is a widespread process that occurs naturally across space and time, but humans have caused changes at an unprecedented scale and speed, that pose new challenges to many organisms (Merilä, 2012; Pelletier & Coltman, 2018; Vitousek et al., 1997). One of the main challenges is urbanization, *i.e.* the ultimate replacement of natural landscapes by man-made infrastructures (Dansereau 1957), resulting in a variety of environmental alterations, such as increased noise pollution, impervious surfaces or disturbance by human presence (Niemelä et al., 2011). While some organisms struggle in the face of new selective pressures induced by these changes, others survive or even thrive in urban environments, based on features they acquired in the past or by adjusting their phenotype via individual plasticity or genetic evolution (Hendry et al., 2008; Merilä & Hendry, 2014).

An increasing number of studies has documented urban-associated phenotypic change in a variety of taxa and traits (e.g. pigmentation in Lepidoptera moths, Kettlewell, 1956; beak morphology and vocal performance in house finches Haemorhous mexicanus, Giraudeau et al., 2014; toxin tolerance in killifish, Reid et al., 2016). To date, studies of urban-associated phenotypic shifts have mostly reported changes in mean phenotypes. Phenotypic change can occur not only through a shift in mean, but also through a shift in variation, with important implications for ecoevolutionary processes in the context of urban adaptation. Indeed, phenotypic variation is central to any evolutionary response to environmental change as it is the prerequisite for selection to act and thus influences the direction and magnitude of the response to selection. Furthermore, phenotypic variance can itself be shaped by eco-evolutionary processes (e.g. plasticity, dispersal, (epi)genetic variation, Des Roches et al., 2018; Draghi, 2019; Reed et al., 2011). In the framework of urban evolutionary ecology, however, surprisingly little is known about the relationship between urbanization and phenotypic variation. Recent reviews hypothesized that phenotypic variation could increase in urban environments due to multiple nonexclusive mechanisms (e.g., limited dispersal, relaxed or heterogeneous selection,

increased exposure to mutagens, developmental plasticity; Capilla-Lasheras et al., 2022; Thompson et al., 2022). To date, however, fewer than ten studies have investigated and supported this hypothesis (*e.g.* morphology in great tit *Parus major* and blue tits Cyanistes caeruleus; Thompson et al., 2022, life-history traits in bird species globally, n=35 species; Capilla-Lasheras et al., 2022).

Animal behaviour variation has been extensively studied within the personality framework (Réale et al., 2007; Sih et al., 2004; Wolf & Weissing, 2012), a growing field at the intersection between wild quantitative genetics (Charmantier et al., 2014) and behavioral ecology (Dingemanse & Dochtermann, 2014). Specifically, this framework aims at exploring among-versus within-individual (co)variation in behavioral traits, their origin, and their adaptive nature. Despite the abundant studies exploring the within-species diversity in behaviour, few studies have examined how urbanization affects behavioral variation (n=24, published between 2010 and 2022, see Burkhard, Dochtermann & Charmantier (2023) metanalysis on behaviour responses to urban environments for more details). Recent attempts to tackle this question have compared repeatability, i.e. the proportion of total phenotypic variation due to among-individual variance, between urban and rural populations (n=24 studies, e.g. Dammhahn et al., 2020; Fossett & Hyman, 2021, see Burkhard, Dochtermann & Charmantier, 2023 for the complete list) For example, speckled wood butterflies (Pararge aegeria) from urban landscapes were found to be more repeatable in boldness (i.e. latency to approach feeder) than rural ones (0.50[0.39-0.56] vs. 0.15[0.09-0.22]; Kaiser et al., 2019), which was partly explained by higher amongindividual variance paired with lower within-individual variance in butterflies from urban landscapes. In contrast, boldness in song sparrows (Melospiza melodia) did not differ between urban and rural habitats (repeatability of 0.24; Fossett & Hyman, 2021), but among and within-individual variances were not reported. However, the absence of differences in these repeatability ratios does not imply the absence of differences in among- and within-individual variance (Dochtermann & Royauté, 2019) between urban and non-urban habitats.

Page 5 of 44

Behavioral Ecology

Differences in among-individual variance across habitats may occur if they are underpinned by different genetic variances (e.g. due to different heterogeneous selection across habitats, Barrett & Schluter, 2008; Hedrick, 1986). It may also occur due to plasticity in response to the environment experienced during development leading to permanent differences between individuals (*e.g.* due to different exposure to stress during early life, Kristensen et al., 2018; Lazić et al., 2015; Lindström, 1999; see Thompson et al., 2022 for an exhaustive review of underpinned mechanisms). In the literature, urban dwellers have been shown to have higher among-individual variance (e.g. in woodchucks (Marmota monax); Lehrer et al., 2012 or shrews (Crocidura russula & Sorex araneus), von Merten et al., 2022). Such diversity might buffer urban populations from new or fluctuating selective pressures if urban individuals exploit a greater diversity of habitats and resources (Møller, 2010). Differences in repeatability may also result from differences in within-individual variation, partly as a result of individual behavioral plasticity. In the literature, most urban dwellers have greater behavioral plasticity (Dammhahn et al., 2020; Hendry et al., 2008; Sol & Lefebvre, 2000), which should help them adjust quickly to novel challenges in the city and, in some cases, could also hinder or facilitate adaptive evolution (Caspi et al., 2022). Both among and within-individual components are likely to play an important role in urban adaptation (Lowry et al., 2013). In short, 1) amongindividual variance is frequently established as the upper limit for genetic variance, contributing to population persistence and facilitating adaptive evolution (Hughes et al., 2008) and 2) within-individual variance has been demonstrated to either constrain (Diamond & Martin, 2016; Huey et al., 2003) or, in certain cases, facilitate evolution (Caspi et al., 2022; Levis & Pfennig, 2016), and in some instances, even undergo evolutionary changes itself (Diamond et al., 2018). Hence, examining how urbanization impacts behavioral variation would allow a more comprehensive view on the processes that impact urban populations and their evolutionary potential.

Quantifying the degree of urbanization and its impacts is both challenging and crucial because sampling locations classified as urban and rural are not necessarily homogeneous. Within cities for example, the amount of urbanization at sampling

Behavioral Ecology

locations can vary (*e.g.*, city center versus urban park) and cities can be highly heterogeneous depending on the spatial or temporal scale considered (Mohring et al., 2021; Moll et al., 2020; Strubbe et al., 2020). Therefore, the ability to detect landscape effects linked to urbanization may depend on which urban locations are considered (Evans et al., 2009) or the choice of appropriate spatial scale (Levin, 1992; Martin, 2018). For example, urban grasshoppers were shyer than rural grasshoppers only when urbanization was measured at a scale of 0.5 km, but not 3 and 5 km (Waterschoot et al., 2023). Despite growing availability of remote sensing data, the spatial scale at which environmental urbanization impacts organisms is rarely known (Moll et al., 2020; Perrier et al., 2018; Uchida et al., 2021) and even less is known about how it can influence conclusions of eco-evolutionary investigations.

Here we used a long-term monitoring study of great tits living in an urban mosaic, ranging from a natural forest habitat to highly urbanized areas, to explore differences in among- and within-individual variance in behaviors along an urbanization gradient assessed at multiple spatial scales. In this study system, urban great tits show slightly reduced gene flow compared to forest areas, with some genomic evidence of local adaptation (Perrier et al., 2018) which can promote differences in phenotypic variation between habitats. Previous research has shown that urban great tits are more aggressive, faster explorers and have faster breath rates than those from the forest (Caizergues et al., 2022), but less is known about differences in variance.

We tested if phenotypic variance was higher in the city in these three behaviors compared to the forest (P1). Throughout, we determined whether differences in phenotypic variance were due to differences in among-individual variance, differences in within-individual variance, or both. For most species, cities offer more spatially heterogeneous territories than wild habitats (Alberti et al., 2020; Cadenasso et al., 2007), which could be associated with urban selection for more diverse behavioral types. Consequently, we expect that due to higher environmental heterogeneity in the city for breeding great tits, we will find more among-individual variance in the city (P2a) than in the forest and this will be consistent across different sampling locations within the city (P2b). Finally, within the city, we expect to find greater among-

individual variance within sampling locations that have the highest spatial heterogeneity in urbanization (P3) or that are the most urbanized (P4). We did not make directional predictions regarding differences in within-individual variation according to urbanization, since there are mixed findings in the literature (*e.g.* higher within-individual variation in city, Dammhahn et al., 2020; in forest Prange et al., 2004; no difference between city and forest, Sprau & Dingemanse, 2017).

Mat & Met

Study system

Great tits (*Parus major*) were monitored in southern France in La Rouvière (ROU), an oak forest 20 km northwest of Montpellier that has been monitored since 1992 with 230 nest boxes for blue tits (*Cyanistes caeruleus*) and great tits (Blondel et al., 2006). We also monitored tits at eight locations across an urbanization gradient in the city of Montpellier, which includes around 247 nest boxes monitored since 2011 and hosting mostly great tits (Charmantier et al., 2017; Demeyrier et al., 2016)(Figure 1).

During the breeding season, nest boxes were visited at least weekly to follow reproduction. Adults were captured in nest boxes when feedingtheir 10-15 days old nestlings. All nestlings and adults were individually ringed with a unique metal ring provided by the French CRBPO (Centre de Recherche par le Baguage des Populations d'Oiseaux) and parents underwent behavioral assays (see below for more details). Behavioral assays were performed on both forest and urban parents captured between 2014 and 2022. See Table S1 for summary statistics on the traits studied. All protocols were approved by the local ethics committee for animal experimentation of Languedoc Roussillon (CEEA-LR. 05/06/2018) and regional institutions (Prefecture decree no. 2012167-003). The captures were carried out under personal ringing permits issued by the CRBPO for the research ringing program number 369.

Behavioral assays and description

Once a bird was captured in its nest box, we assessed two reactions to the stress of being handled. First, we immediately recorded its handling aggression (HA) score as soon as we removed the bird from the nest box. The bird was handled while facing

away from the observer and provoked with a finger of the free hand. The observer assigned a score ranging from 0 (unresponsive bird) to 3 (aggressive bird spreading wings and tail) in increments of 0.5 following a standardized protocol (see FigS2A in Caizergues et al., 2022 and table S1 in Dubuc-Messier et al., 2017). Immediately after the HA test, the bird was isolated in a cloth bag for 5 minutes for a standardized period of rest. Following these 5 minutes, the bird was removed from the bag and held on its back by the handler, who measured its breath rate index (BRI). From 2013 to 2016, BRI was estimated as the number of chest movements during 30 seconds, whereas since 2017, the protocol was updated to measure the time to complete 30 chest movements (Caizergues et al., 2022, Figure S2B). Measurements from 2013 to 2016 were therefore converted to approximate the time required to complete 30 chest movements to obtain the measurements on the same scale. BRI was taken consecutively twice in a row and averaged across these two measures. We consider breath rate index a behavioral trait because breath rate reflects both the physiological function of respiration (*i.e.*, O2 and CO2 exchange) and respiratory behaviour (*i.e.*, breath rate can be altered by classical and operant conditioning, Ley, 1994). BRI correlates with heart rate under restraint (Dubuc Messier et al., 2017) and is often used as a proxy for acute stress response (Carere & van Oers, 2004; Krams et al., 2013), with a lower BRI (and therefore faster breath rate) reflecting a greater stress response. Finally, the bird underwent an open field test using an open field cage with similar dimensions as in Stuber et al., (2013), to evaluate its exploration behaviour in a novel environment (Caizergues et al., 2022; Stuber et al., 2013). The bird was placed in an acclimation compartment adjacent to the main open-field cage for 2 min before being released into the exploration room. The videos were analyzed using the BORIS software (Friard & Gamba, 2016) to generate an exploration score (ES) by counting the number of flights and hops during the 4 min exploration trial. For a detailed protocol see Charmantier et al., (2017) and Caizergues et al., (2022), figure S3. Note that these three behaviors are not correlated among or within individual great tits in our populations (Caizergues et al., 2022).

Page 9 of 44

Quantification of urbanization

We quantified the degree of urbanization at each nest box where at least one parent was captured (N = 301) using the proportion of impervious surface area (ISA), defined as sealed non-natural surfaces (e.g., roads, railways, buildings), using the imperviousness density raster datasets from the Copernicus on-line database (resolution 10m. tiles: E38N22 & E38N23. Projection: LAEA EPSG 3035; EEA, 2020). ISA has previously been shown to correlate with other urban factors such as high temperature (Diamond & Martin, 2020), high noise and light pollution, low tree cover, and short distance from roads (Szulkin et al., 2020). The spatial scale at which environmental urbanization impacts organisms is rarely known and may vary across focal traits (Uchida et al., 2021; Waterschoot et al., 2023) and so we quantified the proportion of ISA around each nest box at three different spatial scales: 100, 250, and 1000 meters. We chose this range to explore different effects of urbanization at small, medium, and large spatial scales for great tits as they tend to have smaller home ranges during breeding (approx. 60-160m, van Overveld et al., 2015; Wilkin et al., 2006) but can have extensive natal dispersal (up to 900m on average in females, Dingemanse et al., 2003; Garant et al., 2005; Szulkin & Sheldon, 2008). Using circular radius buffers at these spatial scales in QGIS (v3.22.0; QGIS Development Team 2022), we counted the number of pixels associated with impervious surfaces and calculated an ISA proportion index (range = 0-1. Where 1 = all ISA) around each nest box by dividing by the total number of pixels within each buffer. When considering all nest boxes together, the amount of urbanization correlated moderately between the three spatial scales (rho > 0.75), with most discrepancy at nest boxes in the middle or at the edges of urban parks (Figure 1). We classified sampling locations as forest if the mean ISA measurements were below 5% (ROU) and urban if they were above 5% (CEF, BOT, MOS, MAS, FONT, GRAM, FAC, ZOO, Figure 1). The mean proportion of ISA around each forest nest box was zero at 100 and 250 meters and 0.0007 at 1000 meters, while the mean proportion of ISA around each urban nest box was 0.48, 0.51, and 0.53 at 100, 250, and 1000 meters, respectively, and ranged from 0 to 1 (see Table S2 for more details for each sampling location and Figure 1). To assess spatial heterogeneity within the city, we also calculated the within-site variance of ISA (Table S2).


Figure 1. Spatial map of the eight urban locations (A,B) and forest location (C,D) and their associated proportion of urbanization at 100 m (A,C) and 1000 m (B,D) around each nest-box in the Montpelier area, France. Each sampling location is delineated by a black polygon. Each circle corresponds to a buffer around a nest. Each buffer is characterized by the average proportion of ISA (*i.e.,* impervious surface area) where increasing urbanization moves towards red.

Statistical analysis

We investigated differences in phenotypic means and variances between urban and forest habitats across the three behavioral traits which are known to be repeatable, not correlated, and have habitat-specific means (*i.e.*, urban vs. forest mean, Caizergues et al., 2022). For each trait, we used a Bayesian generalized linear mixed effects model (GLMM) that allowed the mean, among-, and within-individual variances to differ between habitats (also known as heterogeneous variance model, Gianola, 1986). We chose the error distribution to fit each trait, *i.e.* Gaussian for BRI, threshold for HA, and Poisson for ES. We ensured that effective sample sizes for each model were higher than 1000. We assessed the convergence of all parameters graphically as well as using the Heidelberger and Walch test of the 'coda' package (Plummer et al., 2006). Finally, we graphically controlled the residual assumptions with diagnostic.mcmc from the MCMC.qpcr package (Matz et al., 2013) when residuals were not fixed in the model.

a) Comparison between city and forest

To assess whether phenotypic (P1) and among-individual variance (P2a) is higher in urban than forest habitats, we first ran a heterogeneous variance model with two habitat categories (*i.e.*, two separate random intercepts for urban and forest groups of individuals). We estimated the phenotypic mean, among-individual (Vi), annual (Vy) and residual variances (Vr) for each habitat and their corresponding 95% credible intervals. We included individual identity and year as random effects with heterogeneous variance across random effect variances and error variance (model a). For all traits we included an interaction between habitat (urban/forest) and fixed effects known to influence traits: sex and age (adult *vs.* juveniles) (Caizergues et al., 2021, 2022; Charmantier et al., 2017). The interaction between habitat and decimal hour of measure was also fitted as a continuous quadratic fixed effect since behaviour and metabolism can change throughout the day (Caizergues et al., 2020,2022). To additionally account for possible habituation to multiple captures or tests, we included assay (*i.e.*, number of previous assays) as a continuous fixed effect. As the protocol for BRI changed during the study (see Caizergues et al., 2022), we included protocol type as a fixed effect for this trait. Finally, for BRI and HA, we accounted for among-observer variance by fitting observer identity as a random effect and included heterogeneous variance for each habitat like the other random effects. As amongobserver variance is not biologically relevant we did not include it in the total phenotypic variance estimate. Thus, we estimated the total phenotypic variance for each habitat type as **Vp=Vi+Vy+Vf+Vr**, where Vf is the variance in biologically relevant fixed effects only (*i.e.* sex and decimal hour of the day linked to circadian rhythm, in our specific case, de Villemereuil et al., 2018).

To compare behavioral means between urban and forest birds we calculated the natural logarithm of the ratio between urban and forest means as **InRR=log(mean_urb/mean_rur)** for each sex and age category, and its 95 % credible interval. As means and variances are related in many cases, we estimated the meanstandardized coefficient of variation of each variance component such that CV = sd(variance)/phenotypic trait mean and its 95 % credible interval. This approach allows a direct comparison of the magnitudes of variation across traits measured on different scales between groups. The posterior distributions of predictions were generated using the phenotypic mean across sex and age categories (table S2). To compare variance between urban and forest birds, we estimated the natural logarithm of the ratio between the coefficients of variations from urban and forest (i.e. coefficient of variation ratio, InCVR, Nakagawa et al., 2014) and its 95 % credible interval such that InCVR= log(CV_urb/CV_rur) for each variance component (InCVR P, InCVR I, InCV R,InCVR F, InCVR Y for total phenotypic, among-individual, residual, fixed-effect, year components respectively). Traits with a higher mean or variance in urban habitats will have positive InRR and InCVR. We also estimated adjusted repeatability **rpt=Vi/Vi+Vy+Vf+Vr** and tested differences in repeatability by calculating the log repeatability ratio **InRPT= rpt urb/rpt rur** to allow comparisons to similar estimates in the literature . We interpret InRR, InCVR and InRPT (i.e. effect sizes) as evidence for a difference between urban and forest when 95% CI does not overlap with zero.

b) Comparison across sampling locations

To compare whether among-individual variance at each urban sampling location was higher than the forest location (P2b), we ran a heterogeneous variance model for each trait that estimated variance components separately for each of the 9 locations (model b, *i.e.*, nine separate random intercepts grouping individuals by sampling location). The number of observations between sampling locations was fairly balanced except for the urban CEF and BOT locations, for which we have less than 30 individuals (see Supplementary Table S4). The heterogeneous variance model had the same structure as described for model a; we kept the same fixed effects but we removed the interaction term between habitat and sex, age, and the quadratic effect of decimal hour to avoid over-fitting the model. In addition, we fitted homogeneous instead of heterogeneous variance structure across the year and observer random effects as there was no evidence for differences in variance explained by year between urban and forest habitats (HA: LNCVR Y=-0.14[-2.06;1.89]; BRI: 1[-0.66;3.83]; ES: -0.64[-3.14;2.03]) or observer (HA: LNCVR_O=-0.48 [-1.34, 0.41]; BRI: -0.53 [-1.48, 0.50]). All variance components and derived values were estimated and tested in the same way as outlined above.

c) Phenotypic variance across the urban gradient

Finally, to investigate whether among-individual variance within the city increased with the level of spatial heterogeneity (P3) and urbanization (P4), we estimated the strength and direction of the association between the mean-standardized among-individual variances (CVI, from model b mentioned above) with the variance (*i.e.,* spatial heterogeneity) and mean of ISA measures of each sampling location. As the means and variances of ISA were on very different scales, we centered and scaled them (x - mean(x)) / sd(x), where x is mean or variance ISA. We focused only on urban locations because preliminary analyses suggested that the forest location alone drove the correlation for some traits. In addition, two locations within the city had less than 30 observations and high uncertainty around the variances of model b mentioned above, so we decided to exclude these locations (CEF and BOT, table S4, note that conclusions were not sensitive to their inclusion) and used the remaining six urban

locations for this analysis. To estimate the correlation between behavioral variance and ISA metrics, we ran a Bayesian regression model on the mean-standardized posterior variance estimated within each iteration of model b, thus generating the uncertainty around the phenotypic mean and variance components. We included both mean ISA and variance ISA as fixed effects, both measured on the same spatial scale. Mean and variance ISA were not colinear as the absolute values of the correlations between both variables were well below 0.8 (Young, 2018) ($\rho_{spearman} = -0.12$, p-value = 0.793; -0.57, p-value = 0.15; and -0.26, p-value = 0.53 for 100, 250, and 1000m scales, respectively). We used each iteration from model b to run these new models (one model per iteration of model b) with the three different spatial scales of ISA independently. To determine which spatial scale was most relevant, we calculated the model fit by estimating Bayesian-R² (*i.e.*, the variance of the predicted values divided by the variance of the predicted values plus the expected variance of the errors, Gelman et al., 2019) and averaged the estimates across the different models generated at each spatial scale. We define here the spatial scale at which the Bayesian-R² is the strongest as the "scale of effect" (Martin & Fahrig, 2012).

The analyses for models a and b were conducted using the MCMCglmm package (Hadfield, 2010) with uninformative priors. For our last analysis, the model was run on the posterior distributions generated from the MCMCglmm (model b), independently utilizing the rstanarm package (Goodrich et al., 2018), which allows for linear-regression models. The analyses were performed on R version 4.3.0 (released on 2023-04-21).

Results

1. Birds from the city are faster explorers and more aggressive.

Urban birds were faster explorers (i.e., had higher exploration scores) than forest birds regardless of sex and age (InRR_male=0.91[0.5; 1.72]; InRR_female=0.63[0.32; 1.11]; InRR_adult=0.77[0.44; 1.29]; InRR_juvenile=0.76[0.41; 1.27]). By contrast, urban and forest birds did not significantly differ in either mean breath rate (InRR=-0.04[-0.14, 0.05] across sex and age, Figure 2) or mean handling aggression (InRR_female=-0.1[-13]).

Behavioral Ecology

0.36; 0.19]). However, we found that handling aggression response varied by sex, with urban males tending to be more aggressive than forest males (i.e. credible interval slightly overlapping zero); lnRR_male=0.22[-0.01; 0.43]).

Across urban locations, we observed consistent differences in mean behaviour between urban and forest locations (i.e., effect size of the same sign), but the magnitude and precision of the effect varied between sampling locations (Figure S1, S2). Yet we observed a significant faster breath rate and increased handling aggression at certain city locations, despite the overall trait means being similar between the city and the forest (see Figure S1 and S3).

2. City birds had higher among-individual variance in breath rate and handling aggression but less in exploration than rural ones

a) Breath rate index

We found no evidence that urban birds were phenotypically more variable in breath rate (InCVR_P=0.04[-0.06;0.16]) than forest birds (Figure 2). This was explained by urban birds having increased among-individual variance (InCVR_I=0.25[0.03;0.53]) but decreased within-individual variance [InCVR_R=-0.13[-0.3;0.04]], thus balancing the effect size near zero at the overall phenotypic level. As a result of this difference in among-individual variance and within-individual variance, urban birds tended to be more repeatable in breath rate (credible interval slightly overlapping zero; InRPT=0.31[-0.05;0.77]). All other variance components were unaffected by habitat (*i.e.* observer, fixed-effect and year variance, Table S3).

Our results showed varying among-individual variance among urban locations, with greater (positive InCVR_I, in blue, Figure 3B) and lower (negative InCVR_I, in red, Figure 3A) variance depending on which urban locations were compared to the forest. While the overall trend across city and forest (model a) indicated higher among-individual variance for the city, two of the eight urban locations exhibited lower among-individual variance, although the credible interval overlapped zero (InCVR_I=-0.19 [-2.05; 0.5] and -0.38 [-2.56; 0.6], for MOS and CEF, respectively). All others urban 14

locations had higher among-individual variance (though the credible interval overlapped zero for ZOO, MAS and BOT, figure S1). Despite the expectation of greater among-individual variance for more urbanized locations (P4), no consistent pattern was observed in this direction for the breath rate index. For instance, MAS and MOS, which are more urbanized than FONT and GRAM, respectively (all spatial scales confounded - Table S2), displayed lower among-individual variance. Additionally, CEF and FONT, with similar levels of average urbanization (all spatial scales confounded, Table S2), exhibited different among-individual variance. Finally, we observed both greater and lower within-individual variation (positive or negative InCVR_R) depending on which urban locations were compared to the forest, with no apparent pattern (Figure 3A, upper triangle). See figure S4A,B,C for detailed estimates related to Figure 3.

b) Handling aggression

Urban birds were 1.28 times more phenotypically variable in their handling aggression (InCVR P=0.25[0.15;0.35]) than forest birds. This was in part attributed to urban birds having 1.5 times more among-individual variance (InCVR_I=0.41[0.1;0.71]) and 2.53 attributed times more variance to sex, age, and decimal hour (InCVR F=0.93[0.2;1.81]). It was not possible to assess whether differences in phenotypic variance could also be due to differences in within-individual variation as this component cannot be estimated in a threshold model. Consistent with the higher among-individual variance, there was evidence that urban birds were more repeatable in handling aggression (InRPT=0.48[0.2;0.83]). All other variance components remained unchanged (Table S3).

Accounting for between-sampling location variation (model b), all urban sites except CEF (InCVR_I=-0.17[-0.97; 0.07]) exhibited higher among-individual variance than the forest (*i.e.*, higher InCVR_I, blue tiles in Figure 3B). The comparison within the city did not reveal a clear pattern due to a lot of heterogeneity in the sign of InCVR_I (red, white, and blue tiles in Figure 3B, lower triangle) and a considerable amount of uncertainty around the effect size.

c) Exploration score 15

Page 17 of 44

Behavioral Ecology

Results for the exploration score were opposite to our predictions for phenotypic variance (P1) and to the patterns found for the first two behaviors. Phenotypic variance for exploration was 2.8 time lower in city than in forest birds (InCVR_P=-1.03[-1.19;-0.88]). The reduced phenotypic variance variability in exploration among urban birds was explained by urban birds having 3.3 times less among-individual variance (InCVR_I=-1.2[-1.63;-0.8]) and 1.15 times less variance due to sex, age, and decimal hour (InCVR_f=-0.14[-0.36;0.02]). There was no evidence of a difference in within-individual variance (InCVR_R=0.21[-0.16;0.66]) across habitats. Finally, consistent with the lower among-individual variance, there was evidence that urban birds were less repeatable in exploration (InRPT=-0.24[-0.45;-0.06]). The variance due to the year effect remained unchanged (Table S3).

When we compared the urban locations one by one, we found that all eight urban locations exhibited less among-individual variance than the forest location. Across urban locations, the patterns of effect size for among-individual variance and within-individual variation differences were quite homogeneous (all of the same sign within each triangle, aka same colour) (Figure 3C, upper and lower triangle, respectively).

3. Among-individual variance correlates with urbanization gradient for exploration but not for stress-related behaviors.

When using different spatial scales to estimate the proportion of ISA around each nest-box, the locations classification changed depending on the spatial scale considered (see Figure 1, Table S2). For instance, the botanical garden (BOT) was one of the most urbanized locations (*i.e.*, highest level of mean ISA) at the 1000-m scale (ISA=0.93), but had intermediate levels of urbanization at the 100-m scale (ISA=0.56) because it is a small green haven in the center of town. An examination of which spatial scale of urbanization explained the most variation in among-individual variance (*i.e.*, scale of effect) showed heterogeneous results across traits leading to interesting insight into the relevant scale for each trait. The scale of effect was 250 m for breath rate index (R-squared=0.31), 100 m for handling aggression (R-squared= 0.48), and 1000 meters for exploration score (Rsq=0.63) (Figure S5). Note that although 250 m

for the breathing rate index had the highest Bayesian R-squared, 100 and 1000 m were very close (R-squared=0.28 and 0.29 respectively). We will only present the following results for the corresponding scale effect (see Figure S5 for the other scales).

Contrary to our prediction (P3), we found no clear evidence for greater amongindividual variance within urban environments that are more spatially heterogeneous in terms of urbanization (*i.e.*, variance in ISA). There was no evidence for greater among-individual variance in BRI and HA in environments with higher levels of urbanization (P4, mean ISA, $\beta_{meanISA}=0.01[-0.03;0.06]$ and 0.02[-0.05;0.11], respectively) or greater spatial heterogeneity ($\beta_{varianceISA}=0.01[-0.006;0.03]$ and 0.07[-0.02;0.20], respectively, Figure 4A,B and S4A, S4B). In contrast, we found that amongindividual variance in exploration score decreased linearly with more urbanized areas within the city (with higher ISA) ($\beta_{meanISA}=-0.12[-0.23;-0.004]$, Figure 4C and figure S4C), but there was no evidence of a correlation with spatial heterogeneity ($\beta_{varianceISA}=-0.07[-0.17;0.07]$). Note that the direction of the relationship between among-individual variance and spatial heterogeneity varied depending on the spatial scale analyzed. It changed from positive at small and medium scales to negative at large scales for both the breath rate index and exploration (see Figure S4)



Figure 2. Log-response ratio (InRR), log-coefficient variance ratio (InCVR) and their 95% credible interval for behavioral traits (BRI: Breath Rate Index, HA: handling aggression, ES : Exploration score) in great tits in forest *vs.*. urban environment Montpelier area. Traits have a higher mean (InRR) or are more variable (higher InCVR) in urban habitats when estimates are positive (*i.e.*, right of the zero dashed line). Rpt corresponds to the repeatability and the 95% credible interval for the urban (on the right) and forest (on the left) habitat. Dots and triangles represent the mean of the posteriors distributions for InRR and InCVR respectively.



Figure 3. Pattern of pairwise log-coefficient variance ratio (InCVR) for breath rate index BRI (A), handling aggression HA (B) and exploration score ES (C) between the 9 locations of the study system. The 9 locations are ordered by ascending mean ISA (buffer 1000m), which is also represented in an orange gradient, with dark orange

indicating higher levels of ISA. InCVR for within individual variance (InCVR_R) is above the diagonal while InCVR for among-individual variance (InCVR_I) is below the diagonal. Note that for HA, we represented InCVR for total phenotypic variance (InCVR_P) as the threshold model does not estimate residual variance. Positive, zero and negative InCVR are shown in blue, white and red respectively. A positive (blue) value means that the location on the x-axis has a greater coefficient of variation (and so associated variance) than the location on the y-axis. See the supplementary materials to have access to all the values.



Figure 4. Relationship between predicted mean-standardized among-individual variance (CVI) and mean ISA (on the left), variance ISA (on the right) for Breath Rate Index (BRI, A), Handling aggression (HA, B) and exploration score (ES, C) and their corresponding «scale effect». CVI used here are estimated from the posteriors distributions of model b. Each line represents the predictions of one Bayesian model (one model per iteration of model b). Note that CVI are expressed on the latent-scale for HA and ES. β is the coefficient of relation between mean/variance ISA among-individual variance (CVI).

Discussion

Recent work has demonstrated that phenotypic variance across individuals, the raw material for selection, may be higher in urban populations than non-urban ones in some contexts (Thompson et al., 2022, Capilla-Lasheras et al., 2022 but see Burkhard, Dochtermann & Charmantier, 2023 Sanderson et al., 2022). We examined if urban populations contained more behavioral variation (P1) and whether this was due to higher among-individual variance (*i.e.*, between individual differences, P2a, P2b). We find that urban birds are faster explorer and more aggressive than forest birds. In addition urban birds are more variable than forest birds for breath rate and handling aggression, but less variable in their exploration of a novel environment. These results are fairly robust when examining behavioral variation at each urban sampling location compared to the forest, but also highlighted differences among urban locations in the individual diversity they contained, which is especially evident for breath rate. Finally, we find decreasing individual heterogeneity in exploration with increasing urbanization in the city, but no support for linear relationships between increasing urbanization and individual differences for the other behavioral traits. Collectively our results suggest that individuals in the city may have more diverse behavioral stress responses, yet display stronger similarity in their behavioral responses to novelty.

Behavioral differences between city and forest

In agreement with previous findings (Charmantier et al., 2017, Caizergues et al., 2022), we confirm that birds in urban areas are faster explorers than conspecifics living in the forest, and we also find a trend that males in urban areas are more aggressive.

Although we find faster urban breath rates as in Caizergues et al., (2022), the uncertainty around the estimates are larger and do not allow us to conclude on a significant difference. Such disparity in the magnitude and uncertainty of effect sizes between the two studies could be explained by 1) the use of different fixed effects and 2) the use of heterogeneous variance structures (one intercept and variance per location). Interestingly, there is clear evidence of faster breath rate at three urban sites (GRAM, ZOO and FONT, Fig S1) and so our results suggest that different environmental conditions among sampling locations within a city can drive stronger differences in breath rate means. In the literature, this trait has conflicting patterns with urbanization, with some studies showing that birds breathe faster in the city (Caizergues et al., 2022, Torné-Noguera et al., 2014), while others show no difference (Abolins-Abols et al., 2016; Senar et al., 2017). Given our findings, these discrepancies could result from non-accounted for habitat heterogeneity within urban and forest habitats (e.g. human disturbance, food and predator abundance). Further studies that account for environmental heterogeneity at multiple spatial scales are needed to understand whether it might alter our interpretation of the urbanization effect on average traits.

Higher among-individual variation in two urban behaviors

While earlier studies found higher phenotypic variance in life-history and morphological traits among urban versus non-urban birds (Thompson et al., 2022; Capilla-Lasheras et al., 2022), our present study reveals increased phenotypic variance (P1) exclusively in one behavioral trait—handling aggression. Inconsistent support for P1 across traits aligns with Sanderson et al.'s (2023) recent findings that human-related disturbances can both increase and decrease phenotypic variation across different trait types. Consistent with our second prediction, we find higher among-individual variance (P2a) in urban habitats for breath rate and handing aggression, which translates into higher urban repeatability. Previous studies on great tits showed contrasting patterns with both higher (Charmantier et al., 2017) and lower (Hardman & Dalesman, 2018) repeatability of handling aggression for urban compared to forest

Page 23 of 44

Behavioral Ecology

populations. However, these studies did not report variance estimates and thus did not conclude on whether changes in repeatability were due to higher or lower amongindividual variance. Burkhard, Dochtermann & Charmantier (2023) only found seven studies testing explicitly for differences in behavioral variance at the among- and within-individual levels between urban and non-urban environments in other birds and mammals species for behavioral traits (Bokony et al., 2012; Kaiser et al., 2019; Lehrer et al., 2012; Thompson et al., 2018; von Merten et al., 2022; Williams et al., 2020). For aggressive behaviour, only one study had reported differences in amongindividual variance in an urbanization context (Von Merten et al., 2022, in shrews), where they showed higher repeatability due to higher among-individual variance in urban shrews, consistent with our results. Our results illustrate that examining amongand within-individual variance component, that have different eco-evolutionary implications, can be critical to reveal differences that might otherwise remain cryptic. For example, higher among- and lower within-individual variation in urban breath rates (Fig 2B) contribute to similar amounts of total phenotypic variation between the city and forest. This could suggest that variance is shaped by different processes in the city, e.g., less plasticity in breath rates in the city than the forest, an inference that would be missed if not evaluating among and within- individual variance separately. While high among-individual variance in behaviour is often associated with increased population stability and resilience (Wolf & Weissing, 2012), it is important to note that for this to hold true, among-individual variance should partly include genetic variance, which is vital for adaptive evolution and long-term population persistence (Barrett & Schluter, 2008). The greater among-individual variance we find in the city can be underpinned by genetic variance in response to relaxed or fluctuating selection or by developmental plasticity in response to spatial environmental heterogeneity (Wolf & Weissing 2010). Studies comparing urban and forest great tit genomes found evidence of differences in genes (Perrier et al., 2018; Riyahi et al., 2017), in DNA methylation (Caizergues, Le Luyer, et al., 2022; Riyahi et al., 2015), or expression of genes (Watson et al., 2017) that are inextricably linked to behaviour (e.g., genes linked to the nervous system and stress responses). Specifically, urbanization has been repeatedly associated with shifts in allele frequencies in the SERT gene (Mueller et al., 2013; van Dongen et al., 2015) which affects a wide diversity of behavioral traits such as stress sensitivity and handling aggression in great tits (Craig & Halton, 2009; Savitz & Ramesar, 2004), but also in other bird and mammals species (Grunst et al., 2021, Mueller et al., 2013, 2020 ,Savitz & Ramesar, 2004). In our study system, reduced genomic diversity was found in the most urbanized locations and genetic differentiation increased with urbanization differences (maximum FST=0.009, Perrier et al., 2018) suggesting that the high among-individual variance we uncovered for aggression and breath rate in the city may not necessarily comprise high among-individual genetic variation (Yates et al., 2019). While they are difficult to implement in vertebrates, common garden and quantitative genetic (genomic) approaches may be the most useful opportunities to uncover the mechanisms underpinning trait variation and further understand how urbanization impacts the ability of species to persist and evolve (Schell, 2018).

Higher homogeneity in exploration among urban birds

Contrary to our predictions P1, P2a and P4 we find that urban birds exhibit both lower phenotypic and among-individual variance in exploration compared to forest birds (Fig 2D). Exploration is closely linked to resource acquisition and risk avoidance (Toscano et al., 2016) and faster explorers have been shown to be better at detecting environmental cues associated with resources (in blue tits, Delaitre et al., *unpublished*). It suggests that urban birds might employ less diverse strategies for resource acquisition and predator avoidance in the city. While there is evidence in the literature that urban individuals tend to be more homogeneous in predator avoidance behaviors (Geffroy et al., 2020) and that the repeatability of exploration is lower in urban great tits (Charmantier et al., 2017) and house finches (Weaver et al., 2019), it is unknown whether there are consequences for intra-specific competition, predation, or even demography (Araújo et al., 2011; Bolnick et al., 2003). A reduction in among-individual variance is hypothesized to have consequences for population viability, as it may render the population vulnerable to new or fluctuating selective pressures due to individuals exploiting a smaller diversity of habitats and resources (Møller, 2010).

Behavioral Ecology

However, fast exploring great tits (such as urban birds in our study system) have been shown to adjust their behaviour more rapidly in response to changes in food resources and find new food resources more quickly than their slower counterparts (Coomes et al., 2022; van Overveld & Matthysen, 2009). In the literature there are conflicting hypotheses linking plasticity to the fast-slow exploration continuum. While the behavioral flexibility hypothesis predicts that fast explorers are less behaviorally flexible (forming routine-like behaviour and taking more time to adjust; Coppens et al., 2010; Logan, 2016; Mazza et al., 2018), the information gathering hypothesis predicts the opposite where higher sampling behaviors allow more rapid responses; Arvidsson & Matthysen, 2016; Herborn et al., 2014; Rojas-Ferrer et al., 2020). Further studies would be needed to investigate whether urban individuals, in our study system, could adjust to environmental conditions as a result of individual differences in plasticity despite low among-individual variance in average behaviour.

Lower total phenotypic variation and among-individual variance in exploration of urban birds could partly be a consequence of genetic diversity loss. While we did not investigate differences in genetic variation for this trait among habitats, exploration has been shown to harbour genetic variance for some species (e.g. Careau et al., 2009 in deer mice, Peromyscus maniculatus, Dingemanse et al., 2012 in stickleback Gasterosteus aculeatus) including great tits (Drent et al., 2003, Dingemanse et al., 2002), so we could hypothesize that some of the differences we found arise from differences in genetic variance across habitats. However, given we do not see consistent patterns of variation along the urbanization gradient across all the behavioral traits, it is likely that other mechanisms such as matching habitat choice (Edelaar et al., 2017) or plasticity explain reduced individual diversity of exploration in more urbanized contexts. For example, urban individuals could plastically adjust to novel stimuli in the same direction through habituation (as shown in blue-tailed skinks, Williams et al., 2021) and, in our novel environment assay, urban tits may similarly reduce responses to novelty and explore more quickly while forests tits may have shown more varied exploration responses. Indeed, a recent meta-analysis suggests that decreases in phenotypic variation in human-disturbed habitats may be more

common for behavioral traits (Sanderson et al., 2023), which would support this alternative explanation.

Mean urbanization, not spatial heterogeneity, influences among-individual variation in exploration

Our measure of impervious surface assessed at three different spatial scales, illustrated how locations within the same city are characterized by different levels of impervious surface (*i.e.* proxy for urbanization) (Table S2). The ordering of locations by urbanization intensity depends on the spatial scales considered (e.g., FAC is the most urbanized at 100 m, but BOT is the most urbanized at 1000 m). Such heterogeneity in urbanization metrics within a single city may explain why our ability to detect urbanization effects depends on the specific urban locations considered (Evans et al., 2009). Differences in behavioral variation for the same trait among our urban sampling locations highlights a need to examine variation at finer spatial scales within cities and investigate other unmeasured environmental or ecological features (e.g., native and non-native tree composition; Jensen et al., 2023) that may drive heterogeneity in phenotypic variation across the urban matrix. Note that the historical nest box set-up in our focal locations was not optimal to explore such heterogeneity since we only had one fairly homogeneous forest location versus multiple urban locations. Hence, going beyond analyzing overall variation in the city and estimating variation at finer scales was a crucial step. While differences in trait variation between city and forest can not be solely attributed to the effect of urbanization, the urban gradient approach allows for a more comprehensive exploration of the effects of urbanization proxies. For instance, our findings illustrate that the average impervious surface partially explains the reduction in among-individual variance in great tit exploration within the urban environment (Fig 4C). By combining multiple approaches (city vs. forest, location heterogeneity and urbanization gradient), we show inconsistencies among these approaches for breath rate and handling aggression but robust conclusions across the approaches for exploration. For example, there a are higher urban individual differences for breath rate and handling aggression when comparing urban and forest habitat categories, but there are no strong relationships between individual variation in these behaviors across the urbanization gradient. This

Behavioral Ecology

highlights the value of incorporating at least two different approaches to comprehensively understand effects of urbanization on wild populations.

Recent studies suggest that higher among-individual variance in behavior should be favored in environments with greater spatial heterogeneity as it would allow the exploitation of more diverse resources or niches (Montiglio et al., 2013; Pamela Delarue et al., 2015; von Merten et al., 2022). Partly in line with these studies and our prediction P2a, we find greater among-individual variance for breath rate and handling aggression in the city than in the forest, although we could not demonstrate that this was related to spatial heterogeneity in impervious surface (P3). This is surprising given that previous research has demonstrated a positive association between trait variance and land cover spatial heterogeneity within cities, particularly for life-history traits in great tits (Capilla-Lasheras et al., 2022). One limitation of our study is that we do not account for non-linear relationships between mean and spatial heterogeneity in impervious surface. Locations with extreme proportions of impervious surface (0 or 1) inherently exhibit less variability in their impervious surface cover. In contrast, two locations with an intermediate level of imperviousness has more variation in the spatial distribution of impervious surfaces. For example in our study system, CEF and FONT have the same average percentage of impervious surface (*i.e.* 70%), but the variability in impervious surface is 50 times higher in FONT. Under these conditions, if we hypothesize that among-individual variance in behavior is influenced by spatial heterogeneity, we might expect lower among-individual variance at the extremes of the urban gradient and higher among-individual variance in the middle. While a more suitable approach would involve using an independent, more integrative, and continuous measure of spatial heterogeneity (e.g., Simpson diversity of land cover), it is not feasible to assess this at smaller spatial resolutions in our study system. Another limitation of our study is that in our study system, birds occupy urban locations with high spatial variability in human disturbances, such as pedestrian frequency, car and tramway density, or air and light pollution. This high variability in urban stressors could influence the diversity of behavioral stress responses among urban tits, but these stressors may not correlate strongly to

impervious surface. Exploring these other dimensions of the urban matrix would be particularly useful to identify which urban environmental features shape variation in different avian behaviors (Alberti et al., 2020; Rivkin et al., 2019; Szulkin et al., 2020).

Behaviors are influenced by the environment at different spatial scales

The spatial scale that urbanization affects organisms is an important yet still overlooked issue (Moll et al., 2020), while the increasing availability of remote sensing data provides a great opportunity to extract environmental heterogeneity at multiple scales (Kuenzer et al., 2014). The urbanization gradient approach applied at multiple scales highlights that the most relevant spatial scale for the effect of urbanization (*i.e.* scale of effect) is dependent on the trait studied (Capilla-Lasheras et al., 2022; Martin, 2018; Waterschoot et al., 2023). The «scale of effect» for exploration was a buffer radius of 1000 meters, which aligns with great tits exploring and foraging at large spatial scales around their nest (approximately 3,500-4,000 m2, 95% KDE density, according to Naef-Daenzer, 2000). In contrast, the «scale of effect» for stress-related behaviors reflected more local impacts of the environment on breath rate (250 m) and handling aggression (100 m). Similar results have been shown in blue tits, where average exploration and handling aggression were influenced by large and small scale ecological conditions respectively (Dubuc-Messier et al., 2017). The use of remote sensing data opens an exciting avenue for investigating the different temporal and spatial scale effects of urban-driven evolutionary processes, while providing standardized environmental metrics that allow comparison of effect sizes across studies (Szulkin et al., 2020).

Conclusion and perspectives

This study aimed at exploring the impacts of urbanization on both the total phenotypic and among-individual variance for three behavioral traits. Higher among-individual variance may enhance a population's ecological success, as demonstrated by studies indicating that populations with greater diversity are less susceptible to environmental changes, more demographically stable, more successful in colonizing

new environments, and less prone to extinction compared to less variable populations (Forsman & Wennersten, 2016). Our findings present a contrasting scenario wherein urban birds exhibited higher among-individual variance in stress-related behaviors but lower diversity in novelty-related exploration compared to forest birds. This suggests that urban great tit populations may possess the ability to quickly adapt to changes in stressors, such as human disturbance, albeit adaptation could be slowed down and countered by a low diversity of exploration strategies. While among-individual variance is frequently regarded as the upper limit for genetic variance, additional studies are necessary to examine whether cities contain populations with higher genetic variance or, alternatively, higher individual variation in plasticity. This will be essential to fully comprehend whether wild populations can continue to thrive in a context of increasing global urbanization.

References

Abolins-Abols, M., Hope, S. F., & Ketterson, E. D. (2016). Effect of acute stressor on reproductive behavior differs between urban and rural birds. Ecology and Evolution, 6(18), 6546–6555. https://doi.org/10.1002/ece3.2347

Alberti, M., Palkovacs, E. P., Roches, S. D., Meester, L. D., Brans, K. I., Govaert, L., Grimm, N. B., Harris, N. C., Hendry, A. P., Schell, C. J., Szulkin, M., Munshi-South, J., Urban, M. C., & Verrelli, B. C. (2020). The Complexity of Urban Eco-evolutionary Dynamics. BioScience, 70(9), 772–793. https://doi.org/10.1093/biosci/biaa079

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. Ecology Letters, 14(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Arvidsson, L. K., & Matthysen, E. (2016). Individual differences in foraging decisions: Information-gathering strategies or flexibility? Behavioral Ecology, 27(5), 1353–1361. https://doi.org/10.1093/beheco/arw054

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. Trends in Ecology & Evolution, 23(1), 38–44. https://doi.org/10.1016/j.tree.2007.09.008

Blondel, J., Thomas, D. W., Charmantier, A., Perret, P., Bourgault, P., & Lambrechts, M. M. (2006). A thirty-year study of phenotypic and genetic variation of blue tits in Mediterranean habitat mosaics. Bioscience, 56(8), 661–673.

Bokony, V., Kulcsar, A., Toth, Z., & Liker, A. (2012). Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (Passer domesticus). PloS One, 7(5), e36639.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The Ecology of Individuals: Incidence and Implications of Individual Specialization. The American Naturalist, 161(1), 1–28. https://doi.org/10.1086/343878

Burkhard, T., Dochtermann, N., & Charmantier, A. (2023). Global meta-analysis of behavioral response to urban environments. *Under review* https://doi.org/10.17605/OSF.IO/GSXBM

Cadenasso, M. L., Pickett, S. T. A., & Schwarz, K. (2007). Spatial heterogeneity in urban ecosystems: Reconceptualizing land cover and a framework for classification. Frontiers in Ecology and the Environment, 5(2), 80–88. https://doi.org/10.1890/1540-9295(2007)5[80:SHIUER]2.0.CO;2

Caizergues, A. E., Charmantier, A., Lambrechts, M. M., Perret, S., Demeyrier, V., Lucas, A., & Grégoire, A. (2021). An avian urban morphotype: How the city environment shapes great tit morphology at different life stages. Urban Ecosystems, 24(5), 929–941. https://doi.org/10.1007/s11252-020-01077-0

Caizergues, A. E., Grégoire, A., Choquet, R., Perret, S., & Charmantier, A. (2022). Are behaviour and stress-related phenotypes in urban birds adaptive? Journal of Animal Ecology, 91(8), 1627–1641. https://doi.org/10.1111/1365-2656.13740

Caizergues, A. E., Le Luyer, J., Grégoire, A., Szulkin, M., Senar, J.-C., Charmantier, A., & Perrier, C. (2022). Epigenetics and the city: Non-parallel DNA methylation modifications across pairs of urban-forest Great tit populations. Evolutionary Applications, 15(1), 149–165. https://doi.org/10.1111/eva.13334

Capilla-Lasheras, P., Thompson, M. J., Sánchez-Tójar, A., Haddou, Y., Branston, C. J., Réale, D., Charmantier, A., & Dominoni, D. M. (2022). A global meta-analysis reveals higher variation in breeding phenology in urban birds than in their non-urban neighbours. Ecology Letters, 25(11), 2552–2570. https://doi.org/10.1111/ele.14099

Careau, V., Bininda-Emonds, O. R. P., Thomas, D. W., Réale, D., & Humphries, M. M. (2009). Exploration strategies map along fast-slow metabolic and life-history continua in muroid rodents. Functional Ecology, 150–156.

Carere, C., & van Oers, K. (2004). Shy and bold great tits (Parus major): Body temperature and breath rate in response to handling stress. Physiology & Behavior, 82(5), 905–912. https://doi.org/10.1016/j.physbeh.2004.07.009

Caspi, T., Johnson, J. R., Lambert, M. R., Schell, C. J., & Sih, A. (2022). Behavioral plasticity can facilitate evolution in urban environments. Trends in Ecology & Evolution, 37(12), 1092–1103. https://doi.org/10.1016/j.tree.2022.08.002

Wild. OUP Oxford.

Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S., & Grégoire, A. (2017). Urbanization Is Associated with Divergence in Pace-of-Life in Great Tits. Frontiers in

Charmantier, A., Garant, D., & Kruuk, L. E. B. (2014). Quantitative Genetics in the

Coomes, J. R., Davidson, G. L., Reichert, M. S., Kulahci, I. G., Troisi, C. A., & Quinn, J. L. (2022). Inhibitory control, exploration behaviour and manipulated ecological context are associated with foraging flexibility in the great tit. Journal of Animal Ecology,

Coppens, C. M., Boer, S. F. de, & Koolhaas, J. M. (2010). Coping styles and behavioral flexibility: Towards underlying mechanisms. Philosophical Transactions of the Royal

Craig, I. W., & Halton, K. E. (2009). Genetics of human aggressive behaviour. Human

Dammhahn, M., Mazza, V., Schirmer, A., Göttsche, C., & Eccard, J. A. (2020). Of city

environments. Scientific Reports, 10(1), Article 1. https://doi.org/10.1038/s41598-

Dansereau, P. (1957). Biogeography. An ecological perspective. Biogeography. An

de Villemereuil, P., Morrissey, M. B., Nakagawa, S., & Schielzeth, H. (2018). Fixedeffect variance and the estimation of repeatabilities and heritabilities: Issues and

Demeyrier, V., Lambrechts, M. M., Perret, P., & Grégoire, A. (2016). Experimental

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., & Martin, R. A. (2018). Evolution

of thermal tolerance and its fitness consequences: Parallel and non-parallel

demonstration of an ecological trap for a wild bird in a human-transformed

Delaitre, S., Graham, J., Buatois, B., De Franceschi, C., Giovannini, P., Lucas, A., Bonadonna, F., Caro, SP. (*under review*). Olfactory communication from trees to birds: Effects of herbivore-induced plant defences on a songbird behaviours and

and village mice: behavioral adjustments of striped field mice to urban

Ecological Perspective. The Ronald Press Company, New York.

solutions. Journal of Evolutionary Biology, 31(4), 621–632.

environment. Animal Behaviour, 118, 181–190. https://doi.org/10.1016/j.anbehav.2016.06.007

variation. Nature Ecology & Evolution, 2(1), Article 1.

https://doi.org/10.1038/s41559-017-0402-5

Ecology and Evolution, 5. https://doi.org/10.3389/fevo.2017.00053

91(2), 320-333. https://doi.org/10.1111/1365-2656.13600

Society B: Biological Sciences, 365(1560), 4021–4028.

https://doi.org/10.1098/rstb.2010.0217

Genetics, 126, 101–113.

reproductive decisions.

https://doi.org/10.1111/jeb.13232

020-69998-6

1	
2	
3	
4	
5	
7	
8	
9	
10	
11	
12	
13	
14	
15	
17	
18	
19	
20	
21	
22	
23	
25	
26	
27	
28	
29	
30 31	
32	
33	
34	
35	
36	
37	
39	
40	
41	
42	
43	
44 45	
45	
47	
48	
49	
50	
51 52	
52 53	
54	
55	
56	
57	
58	
59 60	
00	

responses to urban heat islands across three cities. Proceedings of the Royal Society B: Biological Sciences, 285(1882), 20180036. https://doi.org/10.1098/rspb.2018.0036

Diamond, S. E., & Martin, R. A. (2016). The interplay between plasticity and evolution in response to human-induced environmental change (5:2835). F1000Research. https://doi.org/10.12688/f1000research.9731.1

Diamond, S. E., & Martin, R. A. (2020). Evolutionary Consequences of the Urban Heat Island. In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), Urban Evolutionary Biology (p. 0). Oxford University Press. https://doi.org/10.1093/oso/9780198836841.003.0006

Dingemanse, N. J., Barber, I., Wright, J., & Brommer, J. E. (2012). Quantitative genetics of behavioral reaction norms: Genetic correlations between personality and behavioral plasticity vary across stickleback populations: behavioral reaction norm quantitative genetics. Journal of Evolutionary Biology, 25(3), 485–496. https://doi.org/10.1111/j.1420-9101.2011.02439.x

Dingemanse, N. J., Both, C., Drent, P. J., van Oers, K., & van Noordwijk, A. J. (2002). Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour, 64(6), 929–938. https://doi.org/10.1006/anbe.2002.2006

Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal dispersal and personalities in great tits (Parus major). Proceedings of the Royal Society B: Biological Sciences, 270(1516), 741–747. https://doi.org/10.1098/rspb.2002.2300

Dingemanse, N. J., & Dochtermann, N. A. (2014). Individual behaviour: behavioral ecology meets quantitative genetics. Quantitative Genetics in the Wild, 54–67.

Dochtermann, N. A., & Royauté, R. (2019). The mean matters: Going beyond repeatability to interpret behavioral variation. Animal Behaviour, 153, 147–150. https://doi.org/10.1016/j.anbehav.2019.05.012

Draghi, J. (2019). Phenotypic variability can promote the evolution of adaptive plasticity by reducing the stringency of natural selection. Journal of Evolutionary Biology, 32(11), 1274–1289. https://doi.org/10.1111/jeb.13527

Drent, P. J., Oers, K. V., & Noordwijk, A. J. V. (2003). Realized heritability of personalities in the great tit (Parus major). Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1510), 45–51. https://doi.org/10.1098/rspb.2002.2168

Dubuc-Messier, G., Réale, D., Perret, P., & Charmantier, A. (2017). Environmental heterogeneity and population differences in blue tits personality traits. Behavioral Ecology, 28(2), 448–459. https://doi.org/10.1093/beheco/arw148

Edelaar, P., Jovani, R., & Gomez-Mestre, I. (2017). Should I Change or Should I Go? Phenotypic Plasticity and Matching Habitat Choice in the Adaptation to

1	
2	
3	
4	
5	
6	
7	
8	
q	
10	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
37	
22	
24	
34 25	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
52	
52	
22	
54 55	
55	
56	
57	
58	
59	
60	

Environmental Heterogeneity. The American Naturalist, 190(4), 506–520. https://doi.org/10.1086/693345

Eggenberger, H., Frey, D., Pellissier, L., Ghazoul, J., Fontana, S., & Moretti, M. (2019). Urban bumblebees are smaller and more phenotypically diverse than their rural counterparts. Journal of Animal Ecology, 88(10), 1522–1533. <u>https://doi.org/10.1111/1365-2656.13051</u>

European Environment Agency & European Environment Agency. (2020). Imperviousness Density 2018 (raster 10 m), Europe, 3-yearly, Aug. 2020 (01.00) [GeoTIFF]. European Environment Agency. https://doi.org/10.2909/3BF542BD-EEBD-4D73-B53C-A0243F2ED862

Evans, K. L., Gaston, K. J., Sharp, S. P., McGowan, A., & Hatchwell, B. J. (2009). The effect of urbanization on avian morphology and latitudinal gradients in body size. Oikos, 118(2), 251–259. https://doi.org/10.1111/j.1600-0706.2008.17092.x

Falvey, C. H., Aviles-Rodriguez, K. J., Hagey, T. J., & Winchell, K. M. (2020). The finer points of urban adaptation: Intraspecific variation in lizard claw morphology. Biological Journal of the Linnean Society, 131(2), 304–318. https://doi.org/10.1093/biolinnean/blaa123

Forsman, A., & Wennersten, L. (2016). Inter-individual variation promotes ecological success of populations and species: Evidence from experimental and comparative studies. Ecography, 39(7), 630–648. https://doi.org/10.1111/ecog.01357

Fossett, T. E., & Hyman, J. (2021). The effects of habituation on boldness of urban and rural song sparrows (Melospiza melodia). Behaviour, 159(3–4), 243–257.

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution, 7(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Garant, D., Kruuk, L. E. B., Wilkin, T. A., McCleery, R. H., & Sheldon, B. C. (2005). Evolution driven by differential dispersal within a wild bird population. Nature, 433(7021), Article 7021. https://doi.org/10.1038/nature03051

Geffroy, B., Sadoul, B., Putman, B. J., Berger-Tal, O., Garamszegi, L. Z., Møller, A. P., & Blumstein, D. T. (2020). Evolutionary dynamics in the Anthropocene: Life history and intensity of human contact shape antipredator responses. PLOS Biology, 18(9), e3000818. https://doi.org/10.1371/journal.pbio.3000818

Gelman, A., Goodrich, B., Gabry, J., & Vehtari, A. (2019). R-squared for Bayesian Regression Models. The American Statistician, 73(3), 307–309. https://doi.org/10.1080/00031305.2018.1549100

Gianola, D. (1986). On selection criteria and estimation of parameters when the variance is heterogeneous. TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik, 72(5), 671–677. https://doi.org/10.1007/BF00289007

Giraudeau, M., Nolan, P. M., Black, C. E., Earl, S. R., Hasegawa, M., & McGraw, K. J. (2014). Song characteristics track bill morphology along a gradient of urbanization in house finches (Haemorhous mexicanus). Frontiers in Zoology, 11(1), 83. https://doi.org/10.1186/s12983-014-0083-8

Goodrich, B., Gabry, J., Ali, I., & Brilleman, S. (2018). rstanarm: Bayesian applied regression modeling via Stan. R package version 2.17. 4. Online< Http://Mc-Stan. Org.

Grunst, A. S., Grunst, M. L., Staes, N., Thys, B., Pinxten, R., & Eens, M. (2021). Serotonin transporter (SERT) polymorphisms, personality and problem-solving in urban great tits. Scientific Reports, 11, 24270. <u>https://doi.org/10.1038/s41598-021-03466-7</u>

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. Journal of Statistical Software, 33(2), 1–22.

Hardman, S. I., & Dalesman, S. (2018). Repeatability and degree of territorial aggression differs among urban and rural great tits (Parus major). Scientific Reports, 8(1), Article 1. https://doi.org/10.1038/s41598-018-23463-7

Hedrick, P. W. (1986). Genetic polymorphism in heterogeneous environments: A decade later. Annual Review of Ecology and Systematics, 17(1), 535–566.

Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology, 17(1), 20–29. https://doi.org/10.1111/j.1365-294X.2007.03428.x

Herborn, K. A., Heidinger, B. J., Alexander, L., & Arnold, K. E. (2014). Personality predicts behavioral flexibility in a fluctuating, natural environment. Behavioral Ecology, 25(6), 1374–1379. https://doi.org/10.1093/beheco/aru131

Huey, R. B., Hertz, P. E., & Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: A null model approach. The American Naturalist, 161(3), 357–366. https://doi.org/10.1086/346135

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. Ecology Letters, 11(6), 609–623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Jensen, J. K., Ekroos, J., Watson, H., Salmón, P., Olsson, P., & Isaksson, C. (2023). Urban tree composition is associated with breeding success of a passerine bird, but effects vary within and between years. Oecologia, 201(3), 585–597. https://doi.org/10.1007/s00442-023-05319-8

Kaiser, A., Merckx, T., & Van Dyck, H. (2019). behavioral repeatability is affected by early developmental conditions in a butterfly. Animal Behaviour, 157, 219–226.

Krams, I., Vrublevska, J., Cirule, D., Kivleniece, I., Krama, T., Rantala, M. J., Kaasik, A., Hõrak, P., & Sepp, T. (2013). Stress, Behaviour and Immunity in Wild-Caught

Wintering Great Tits (Parus major). Ethology, 119(5), 397–406. https://doi.org/10.1111/eth.12075
Kristensen, N. P., Johansson, J., Chisholm, R. A., Smith, H. G., & Kokko, H. (2018). Carryover effects from natal habitat type upon competitive ability lead to trait divergence or source-sink dynamics. Ecology Letters, 21(9), 1341–1352. https://doi.org/10.1111/ele.13100
Kuenzer, C., Ottinger, M., Wegmann, M., Guo, H., Wang, C., Zhang, J., Dech, S., & Wikelski, M. (2014). Earth observation satellite sensors for biodiversity monitoring: Potentials and bottlenecks. International Journal of Remote Sensing, 35(18), 6599– 6647. https://doi.org/10.1080/01431161.2014.964349
Lazić, M. M., Carretero, M. A., Crnobrnja-Isailović, J., & Kaliontzopoulou, A. (2015). Effects of Environmental Disturbance on Phenotypic Variation: An Integrated Assessment of Canalization, Developmental Stability, Modularity, and Allometry in Lizard Head Shape. The American Naturalist, 185(1), 44–58. https://doi.org/10.1086/679011
Lehrer, E. W., Schooley, R. L., & Whittington, J. K. (2012). Survival and antipredator behavior of woodchucks (Marmota monax) along an urban–agricultural gradient. Canadian Journal of Zoology, 90(1), 12–21. https://doi.org/10.1139/z11-107
Levin, S. A. (1992). The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. Ecology, 73(6), 1943–1967. https://doi.org/10.2307/1941447
Levis, N. A., & Pfennig, D. W. (2016). Evaluating 'Plasticity-First' Evolution in Nature: Key Criteria and Empirical Approaches. Trends in Ecology & Evolution, 31(7), 563– 574. https://doi.org/10.1016/j.tree.2016.03.012
Ley, R. (1994). An introduction to the psychophysiology of breathing. Biofeedback and Self-Regulation, 19(2), 95–96. https://doi.org/10.1007/BF01776482
Lindström, J. (1999). Early development and fitness in birds and mammals. Trends in Ecology & Evolution, 14(9), 343–348.
Logan, C. J. (2016). Behavioral flexibility in an invasive bird is independent of other behaviors. PeerJ, 4, e2215. https://doi.org/10.7717/peerj.2215
Lowry, H., Lill, A., & Wong, B. B. M. (2013). behavioral responses of wildlife to urban environments. Biological Reviews, 88(3), 537–549. https://doi.org/10.1111/brv.12012
Martin, A. E. (2018). The Spatial Scale of a Species' Response to the Landscape Context Depends on which Biological Response You Measure. Current Landscape Ecology Reports, 3(1), 23–33. https://doi.org/10.1007/s40823-018-0030-z
Martin, A. E., & Fahrig, L. (2012). Measuring and selecting scales of effect for landscape predictors in species–habitat models. Ecological Applications, 22(8), 2277– 2292. https://doi.org/10.1890/11-2224.1 34

Matz, M. V., Wright, R. M., & Scott, J. G. (2013). No Control Genes Required: Bayesian Analysis of gRT-PCR Data. PLOS ONE, 8(8), e71448. https://doi.org/10.1371/journal.pone.0071448 Mazza, V., Eccard, J. A., Zaccaroni, M., Jacob, J., & Dammhahn, M. (2018). The fast and the flexible: Cognitive style drives individual variation in cognition in a small mammal. Animal Behaviour, 137, 119–132. https://doi.org/10.1016/j.anbehav.2018.01.011 Merilä, J. (2012). Evolution in response to climate change: In pursuit of the missing evidence. BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology, 34(9), 811–818. https://doi.org/10.1002/bies.201200054 Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. Evolutionary Applications, 7(1), 1–14. https://doi.org/10.1111/eva.12137 Mohring, B., Henry, P.-Y., Jiguet, F., Malher, F., & Angelier, F. (2021). Investigating temporal and spatial correlates of the sharp decline of an urban exploiter bird in a large European city. Urban Ecosystems, 24(3), 501–513. https://doi.org/10.1007/s11252-020-01052-9 Moll, R. J., Cepek, J. D., Lorch, P. D., Dennis, P. M., Robison, T., & Montgomery, R. A. (2020). At what spatial scale(s) do mammals respond to urbanization? Ecography, 43(2), 171–183. https://doi.org/10.1111/ecog.04762 Møller, A. P. (2010). Interspecific variation in fear responses predicts urbanization in birds. Behavioral Ecology, 21(2), 365–371. https://doi.org/10.1093/beheco/arp199 Montiglio, P.-O., Ferrari, C., & Réale, D. (2013). Social niche specialization under constraints: Personality, social interactions and environmental heterogeneity. Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1618), 20120343. https://doi.org/10.1098/rstb.2012.0343 Mueller, J. C., Partecke, J., Hatchwell, B. J., Gaston, K. J., & Evans, K. L. (2013). Candidate gene polymorphisms for behavioral adaptations during urbanization in blackbirds. Molecular Ecology, 22(13), 3629–3637. https://doi.org/10.1111/mec.12288 Mueller, J. C., Carrete, M., Boerno, S., Kuhl, H., Tella, J. L., & Kempenaers, B. (2020). Genes acting in synapses and neuron projections are early targets of selection during urban colonization. Molecular Ecology, 29(18), 3403–3412. https://doi.org/10.1111/mec.15451 Naef-Daenzer, B. (2000). Patch time allocation and patch sampling by foraging Great and Blue Tits. Animal Behaviour, 59, 989–999. https://doi.org/10.1006/anbe.1999.1380 Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M., & Senior, A. M. (2015). Meta-analysis of variation: Ecological and evolutionary

applications and beyond. Methods in Ecology and Evolution, 6(2), 143–152. https://doi.org/10.1111/2041-210X.12309
Niemelä, J., Breuste, J.H., Guntenspergen, G., McIntyre, N.E., Elmqvist, T., and James, P. (eds) (2011). Urban Ecology: Patterns, Processes, and Applications. OxfordUniversity Press, Oxford.
Delarue E.M.P., E. M., Kerr, S. E., & Lee Rymer, T. (2015). Habitat complexity, environmental change and personality: A tropical perspective. behavioral Processes, 120, 101–110. https://doi.org/10.1016/j.beproc.2015.09.006
Pelletier, F., & Coltman, D. W. (2018). Will human influences on evolutionary dynamics in the wild pervade the Anthropocene? BMC Biology, 16(1), 7. https://doi.org/10.1186/s12915-017-0476-1
Perrier, C., Lozano del Campo, A., Szulkin, M., Demeyrier, V., Gregoire, A., & Charmantier, A. (2018). Great tits and the city: Distribution of genomic diversity and gene–environment associations along an urbanization gradient. Evolutionary Applications, 11(5), 593–613. https://doi.org/10.1111/eva.12580
Pickett, S. T. A., Cadenasso, M. L., Rosi-Marshall, E. J., Belt, K. T., Groffman, P. M., Grove, J. M., Irwin, E. G., Kaushal, S. S., LaDeau, S. L., Nilon, C. H., Swan, C. M., & Warren, P. S. (2017). Dynamic heterogeneity: A framework to promote ecological integration and hypothesis generation in urban systems. Urban Ecosystems, 20(1), 1–14. https://doi.org/10.1007/s11252-016-0574-9
Plummer, M., Best, N., Cowles, K., & Vines, K. (2006). CODA: Convergence diagnosis and output analysis for MCMC. R News, 6(1), Article 1.
Prange, S., Gehrt, S. D., & Wiggers, E. P. (2004). Influences of anthropogenic resources on raccoon (Procyon lotor) movements and spatial distribution. Journal of Mammalogy, 85(3), 483–490.
QGIS.org, 2022. QGIS 3.28. Geographic Information System Developers Manual. QGIS Association. Electronic document: https://docs.qgis.org/3.28/en/docs/developers_guide/index.html
R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R- project.org/.
Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. Biological Reviews, 82(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x
Reed, T. E., Schindler, D. E., Hague, M. J., Patterson, D. A., Meir, E., Waples, R. S., & Hinch, S. G. (2011). Time to Evolve? Potential Evolutionary Responses of Fraser River Sockeye Salmon to Climate Change and Effects on Persistence. PLOS ONE, 6(6), e20380. https://doi.org/10.1371/journal.pone.0020380

Reid, N. M., Proestou, D. A., Clark, B. W., Warren, W. C., Colbourne, J. K., Shaw, J. R., Karchner, S. I., Hahn, M. E., Nacci, D., Oleksiak, M. F., Crawford, D. L., & Whitehead, A. (2016). The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. Science, 354(6317), 1305–1308. https://doi.org/10.1126/science.aah4993

Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., de Keyzer, C. W., Diamond, S. E., Fortin, M.-J., Frazee, L. J., Gorton, A. J., Hendry, A. P., Liu, Y., Losos, J. B., MacIvor, J. S., Martin, R. A., McDonnell, M. J., Miles, L. S., Munshi-South, J., Ness, R. W., Newman, A. E. M., ... Johnson, M. T. J. (2019). A roadmap for urban evolutionary ecology. Evolutionary Applications, 12(3), 384–398. https://doi.org/10.1111/eva.12734

Riyahi, S., Björklund, M., Mateos-Gonzalez, F., & Senar, J. C. (2017). Personality and urbanization: behavioral traits and DRD4 SNP830 polymorphisms in great tits in Barcelona city. Journal of Ethology, 35(1), 101–108. https://doi.org/10.1007/s10164-016-0496-2

Riyahi, S., Sánchez-Delgado, M., Calafell, F., Monk, D., & Senar, J. C. (2015). Combined epigenetic and intraspecific variation of the DRD4 and SERT genes influence novelty seeking behavior in great tit Parus major. Epigenetics, 10(6), 516– 525. https://doi.org/10.1080/15592294.2015.1046027

Rojas-Ferrer, I., Thompson, M. J., & Morand-Ferron, J. (2020). Is exploration a metric for information gathering? Attraction to novelty and plasticity in black-capped chickadees. Ethology, 126(4), 383–392. https://doi.org/10.1111/eth.12982

Sanderson, S., Beausoleil, M.-O., O'Dea, R. E., Wood, Z. T., Correa, C., Frankel, V., Gorné, L. D., Haines, G. E., Kinnison, M. T., Oke, K. B., Pelletier, F., Pérez-Jvostov, F., Reyes-Corral, W. D., Ritchot, Y., Sorbara, F., Gotanda, K. M., & Hendry, A. P. (2022). The pace of modern life, revisited. Molecular Ecology, 31(4), 1028–1043. https://doi.org/10.1111/mec.16299

Savitz, J. B., & Ramesar, R. S. (2004). Genetic variants implicated in personality: A review of the more promising candidates. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 131B(1), 20–32. https://doi.org/10.1002/ajmg.b.20155

Schell, C. J. (2018). Urban Evolutionary Ecology and the Potential Benefits of Implementing Genomics. Journal of Heredity, 109(2), 138–151. https://doi.org/10.1093/jhered/esy001

Senar, J. C., Garamszegi, L. Z., Tilgar, V., Biard, C., Moreno-Rueda, G., Salmón, P., Rivas, J. M., Sprau, P., Dingemanse, N. J., Charmantier, A., Demeyrier, V., Navalpotro, H., & Isaksson, C. (2017). Urban Great Tits (Parus major) Show Higher Distress Calling and Pecking Rates than Rural Birds across Europe. Frontiers in Ecology and Evolution, 5. https://www.frontiersin.org/articles/10.3389/fevo.2017.00163

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral Syndromes: An Integrative Overview. The Quarterly Review of Biology, 79(3), 241–277. https://doi.org/10.1086/422893

1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
20 21	
21 22	
22 22	
23	
24	
25	
20	
2/	
20	
29	
30	
31	
32	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	

Sol, D., & Lefebvre, L. (2000). behavioral flexibility predicts invasion success in birds introduced to New Zealand. Oikos, 90(3), 599–605. https://doi.org/10.1034/j.1600-0706.2000.900317.x

Sprau, P., & Dingemanse, N. J. (2017). An Approach to Distinguish between Plasticity and Non-random Distributions of Behavioral Types Along Urban Gradients in a Wild Passerine Bird. Frontiers in Ecology and Evolution, 5. https://doi.org/10.3389/fevo.2017.00092

Strubbe, D., Salleh Hudin, N., Teyssier, A., Vantieghem, P., Aerts, J., & Lens, L. (2020). Phenotypic signatures of urbanization are scale-dependent: A multi-trait study on a classic urban exploiter. Landscape and Urban Planning, 197, 103767. https://doi.org/10.1016/j.landurbplan.2020.103767

Stuber, E. F., Araya-Ajoy, Y. G., Mathot, K. J., Mutzel, A., Nicolaus, M., Wijmenga, J. J., Mueller, J. C., & Dingemanse, N. J. (2013). Slow explorers take less risk: A problem of sampling bias in ecological studies. Behavioral Ecology, 24(5), 1092–1098. https://doi.org/10.1093/beheco/art035

Szulkin, M., Garroway, C. J., Corsini, M., Kotarba, A. Z., & Dominoni, D. (2020). How to Quantify Urbanization When Testing for Urban Evolution? In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), Urban Evolutionary Biology (p. 0). Oxford University Press. https://doi.org/10.1093/oso/9780198836841.003.0002

Szulkin, M., & Sheldon, B. C. (2008). Dispersal as a means of inbreeding avoidance in a wild bird population. Proceedings of the Royal Society B: Biological Sciences, 275(1635), 703–711. https://doi.org/10.1098/rspb.2007.0989

Taylor, R. W., Boon, A. K., Dantzer, B., Réale, D., Humphries, M. M., Boutin, S., Gorrell, J. C., Coltman, D. W., & McAdam, A. G. (2012). Low heritabilities, but genetic and maternal correlations between red squirrel behaviours. Journal of Evolutionary Biology, 25(4), 614–624. https://doi.org/10.1111/j.1420-9101.2012.02456.x

Theodorou, P., Baltz, L. M., Paxton, R. J., & Soro, A. (2021). Urbanization is associated with shifts in bumblebee body size, with cascading effects on pollination. Evolutionary Applications, 14(1), 53–68. https://doi.org/10.1111/eva.13087

Thompson, M. J., Capilla-Lasheras, P., Dominoni, D. M., Réale, D., & Charmantier, A. (2022). Phenotypic variation in urban environments: Mechanisms and implications. Trends in Ecology & Evolution, 37(2), 171–182. https://doi.org/10.1016/j.tree.2021.09.009

Thompson, M. J., Evans, J. C., Parsons, S., & Morand-Ferron, J. (2018). Urbanization and individual differences in exploration and plasticity. Behavioral Ecology, 29(6), 1415–1425. https://doi.org/10.1093/beheco/ary103

Torné-Noguera, A., Pagani-Núñez, E., & Senar, J. C. (2014). Great Tit (Parus major) breath rate in response to handling stress: Urban and forest birds differ. Journal of Ornithology, 155(1), 315–318. https://doi.org/10.1007/s10336-013-1025-5

Toscano, B. J., Gownaris, N. J., Heerhartz, S. M., & Monaco, C. J. (2016). Personality, foraging behavior and specialization: Integrating behavioral and food web ecology at the individual level. Oecologia, 182(1), 55–69. https://doi.org/10.1007/s00442-016-3648-8

Uchida, K., Blakey, R. V., Burger, J. R., Cooper, D. S., Niesner, C. A., & Blumstein, D. T. (2021). Urban Biodiversity and the Importance of Scale. Trends in Ecology & Evolution, 36(2), 123–131. https://doi.org/10.1016/j.tree.2020.10.011

van Dongen, W. F. D., Robinson, R. W., Weston, M. A., Mulder, R. A., & Guay, P.-J. (2015). Variation at the DRD4 locus is associated with wariness and local site selection in urban black swans. BMC Evolutionary Biology, 15(1), 253. https://doi.org/10.1186/s12862-015-0533-8

van Overveld, T., Adriaensen, F., & Matthysen, E. (2015). No evidence for correlational selection on exploratory behaviour and natal dispersal in the great tit. Evolutionary Ecology, 29(1), 137–156. https://doi.org/10.1007/s10682-014-9737-4

van Overveld, T., & Matthysen, E. (2009). Personality predicts spatial responses to food manipulations in free-ranging great tits (Parus major). Biology Letters, 6(2), 187–190. https://doi.org/10.1098/rsbl.2009.0764

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human Domination of Earth's Ecosystems. Science, 277(5325), 494–499. https://doi.org/10.1126/science.277.5325.494

von Merten, S., Oliveira, F. G., Tapisso, J. T., Pustelnik, A., Mathias, M. da L., & Rychlik, L. (2022). Urban populations of shrews show larger behavioral differences among individuals than rural populations. Animal Behaviour, 187, 35–46. https://doi.org/10.1016/j.anbehav.2022.02.012

Waterschoot, B. O. G., Bataille, G., & Van Dyck, H. (2023). Spatial scale-dependent effects of urbanization on phenotypic traits in a thermophilous grasshopper. Behavioral Ecology and Sociobiology, 77(5), 54. https://doi.org/10.1007/s00265-023-03325-7

Watson, H., Videvall, E., Andersson, M. N., & Isaksson, C. (2017). Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. Scientific Reports, 7(1), Article 1. https://doi.org/10.1038/srep44180

Wilkin, T. A., Garant, D., Gosler, A. G., & Sheldon, B. C. (2006). Density effects on lifehistory traits in a wild population of the great tit Parus major: Analyses of long-term data with GIS techniques. Journal of Animal Ecology, 75(2), 604–615. https://doi.org/10.1111/j.1365-2656.2006.01078.x

Williams, D. M., Nguyen, P.-T., Chan, K., Krohn, M., & Blumstein, D. T. (2020). High human disturbance decreases individual variability in skink escape behavior. Current Zoology, 66(1), 63–70.

Wolf, M., & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1560), 3959–3968.

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. Trends in Ecology & Evolution, 27(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Yates, M. C., Bowles, E., & Fraser, D. J. (2019). Small population size and low genomic diversity have no effect on fitness in experimental translocations of a wild fish. Proceedings of the Royal Society B: Biological Sciences, 286(1916), 20191989. <u>https://doi.org/10.1098/rspb.2019.1989</u>

Figure legends :

Figure 1. Spatial map of the eight urban locations (A,B) and forest location (C,D) and their associated proportion of urbanization at 100 m (A,C) and 1000 m (B,D) around each nest-boxin the Montpelier area, France. Each sampling location is delineated by a black polygon. Each circle corresponds to a buffer around a nest. Each buffer is characterized by the average proportion of ISA (*i.e.,* impervious surface area) where increasing urbanization moves towards red.

Figure 2. Log-response ratio (InRR), log-coefficient variance ratio (InCVR) and their 95% credible interval for behavioral traits (BRI: Breath Rate Index, HA: handling aggression, ES : Exploration score) in great tits in forest *vs.*. urban environment Montpelier area. Traits have a higher mean (InRR) or are more variable (higher InCVR) in urban habitats when estimates are positive (*i.e.*, right of the zero dashed line). Rpt corresponds to the repeatability and the 95% credible interval for the urban (on the right) and forest (on the left) habitat. Dots and triangles represent the mean of the posteriors distributions for InRR and InCVR respectively.

Figure 3. Pattern of pairwise log-coefficient variance ratio (InCVR) for breath rate index BRI (A), handling aggression HA (B) and exploration score ES (C) between the 9 locations of the study system. The 9 locations are ordered by ascending mean ISA (buffer 1000m), which is also represented in an orange gradient, with dark orange indicating higher levels of ISA. InCVR for within individual variance (InCVR_R) is above the diagonal while InCVR for among-individual variance (InCVR_I) is below the diagonal. Note that for HA, we represented InCVR for total phenotypic variance (InCVR_P) as the threshold model does not estimate residual variance. Positive, zero and negative InCVR are shown in blue, white and red respectively. A positive (blue) value means that the location on the x-axis has a greater coefficient of variation (and so associated variance) than the location on the y-axis. See the supplementary materials to have access to all the values.

Figure 4. Relationship between predicted mean-standardized among-individual variance (CVI) and mean ISA (on the left), variance ISA (on the right) for Breath Rate Index (BRI, A), Handling aggression (HA, B) and exploration score (ES, C) and their corresponding «scale effect». CVI used here are estimated from the posteriors

distributions of model b. Each line represents the predictions of one Bayesian model (one model per iteration of model b). Note that CVI are expressed on the latent-scale for HA and ES. β is the coefficient of relation between mean/variance ISA among-individual variance (CVI).

Figures :



Figure 1



















Figure 4

APPENDICE G Charmantier et al. 2024 Under revision

How does urbanisation affect natural selection?

Authors: Anne Charmantier₁, Tracy Burkhard_{1,2}, Laura Gervais_{1,3}, Charles Perrier₄, Albrecht I. Schulte-Hostedde₅, Megan J. Thompson_{1,6}

Centre d'Ecologie Fonctionnelle et Evolutive, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France
Institut des Sciences de l'Evolution de Montpellier ISEM, Univ Montpellier, CNRS, IRD, Montpellier, France

3 Station d'Ecologie Théorique et Expérimentale du CNRS, UAR 2029, Moulis, France

4 UMR CBGP, INRAE, CIRAD, IRD, Institut Agro, Univ Montpellier, Montpellier, France

5 School of Natural Sciences, Laurentian University, Sudbury, Ontario, Canada, P3E 6H8

6 Département des sciences biologiques, Université du Québec à Montréal, 141 Avenue du Président-Kennedy, Montréal, QC H2X 1Y4, Canada

Acknowledgements: We thank all WAMBAM 2023 attendees for useful discussions on this review, Luis Miguel Chevin & Michael Morrissey for advice that substantially improved Table 2, Tom Reed, Juan Carlos Senar, Aude Caizergues, Claire Doutrelant, Lisa Sandmeyer and Marta Szulkin, for various discussions on the challenges of measuring selection and identifying agents of selection. AC thanks the Functional Ecology Review Editor Dr Liza Holeski for the invitation to write this Calow-Grace review and the ANR for funding (URBANTIT project ANR-19-CE34-0008-05 and ACACIA project AAPG-2022-252886).

Author contributions: From an original idea conceived by AC, all authors designed the research in a teamwork coordinated by AC; MJT performed the phenotypic reference search; TB & LG performed the genomic reference search; sections 1 and 4 were written by AC, section 2 by MJT & AC, section 3 by LG, TB & CP, and all authors contributed to editing the paper.
How does urbanisation affect natural selection?

Abstract

1. Urbanisation is one of the most significant contributors to the Anthropocene, and urban evolutionary ecology has become an important field of research. While it is commonly assumed that cities impose new and/or stronger selection, the contradictory assertion that selection may be relaxed in cities is also frequently mentioned, and overall our understanding of the effects of urbanisation on natural selection is incomplete.

2. In this review, we first conduct a literature search to find evidence for patterns of natural selection on phenotypic traits including morphology, physiology, behaviour and life history, in urban populations of animals and plants. This search reveals that coefficients of natural selection in the context of urbanisation are scarce (n = 8 studies providing selection gradients/differentials that include a total of n = 200 coefficients) and a lack of standardized methods hinders quantitative comparisons across studies (e.g., with meta-analysis). These studies, however, provide interesting insight on the agents shaping natural selection in cities and improve our mechanistic understanding of selection processes at different spatial scales.

3. We then perform a second literature search to review genomic studies assessing selection intensity in cities on the genome of non-human natural populations. While this search returned >1k articles, only 51 of these truly investigated footprints of selection associated with urbanisation, and only one study provided genomic selection coefficients. Here again, we found highly heterogeneous approaches, yet studies provide strong evidence of genomic footprints of urban adaptation.

4. In neither the phenotypic nor genomic literature review were we able to quantitatively assess natural selection across urban versus non-urban habitats. Thus, we propose a roadmap of how future studies should provide standardised metrics to facilitate mega- or meta-analyses and explore generalised effects of urbanisation on selection.

Keywords: Urban adaptation, evolution, natural populations, literature review, selection coefficient, phenotypic, genomic, city

1. Introduction

The Anthropocene is defined as the period during which human activity has demonstrably impacted the planet's geology, climate and ecosystems (Waters et al. 2016). The consequences of human impacts on the planet have included rapid shifts in the phenotypes of wild organisms in response to anthropogenic disturbances (Hendry, Farrugia & Kinnison 2008), such as a reduction in the size of harvested (i.e. fished or hunted) animals (Darimont et al. 2009) or the repeated evolution of extreme pollution tolerance in populations of Atlantic killifish (Fundulus heteroclitus) of urban estuaries (Whitehead et al. 2012). These human-induced phenotypic shifts are driven by both rapid plasticity and genetic responses (Palumbi 2001). Human impacts include pervasive alterations of ecological processes, e.g. via substantial habitat degradation, climate change, pollution, exotic species introduction, or over-exploitation of resources. These ecological alterations may in turn influence evolutionary processes such as gene flow, mutation rate, genetic drift and natural selection. The related eco-evolutionary feedback loops impede analysis of these individual processes, and predicting the future eco-evolutionary consequences of the human footprint on biodiversity is challenging (Pelletier et al. 2007; Hendry, Gotanda & Svensson 2017; Otto 2018; Wood et al. 2021).

Despite the difficulty in studying these processes independently, there is a general consensus that "human activities have reshaped selection pressures" (Otto 2018). Perhaps there is already a general agreement on this statement because of long-standing evidence that humans can intentionally initiate and control artificial selection such as during plant or animal domestication (Driscoll, Macdonald & O'Brien 2009). In fact, from Darwin to modern quantitative genetics, agricultural domestication has resulted in huge steps in our understanding of natural selection and adaptive evolution (e.g. Thompson 2008; Gregory 2009). Humans can also unintentionally influence the direction, shape and strength of natural selection. Contrary to the common expectation that human activities will result in novel and strong selection (Pelletier & Coltman 2018), a review of phenotypic selection coefficients across 37 different species found no evidence for stronger selection on average in human-disturbed compared to natural habitats, with notable exceptions such as strong size-related selection in fisheries (Fugere & Hendry 2018). Despite these findings, there is still a pervasive assumption in the literature that human altered environments, and in particular urban habitats, are "a powerful selective force" (Grimm et al. 2008) that can "increase the total strength of selection" (Alberti, Marzluff & Hunt 2017).

Cities are increasingly being recognized as agents of evolutionary change that can provide unique insight on patterns of evolution, specifically rapid adaptation (Donihue & Lambert 2015; Johnson & Munshi-South 2017; Thompson, Rieseberg & Schluter 2018; Lambert et al. 2021). Cities occupy only 3% of Earth's landmass, while hosting more than half of the human population. The UN projects that cities will continue to grow, with 68% of the population living in cities by 2050 (United Nations 2019). The rapid pace of urbanisation creates many challenges for an ever-growing urban human population as well as for preserving urban biodiversity. The nascent field of urban evolutionary biology studies adaptation of species in urban environment compared to less anthropogenic ones in order to predict how wildlife can cope with growing urbanisation. In parallel, this field uses cities as laboratories to explore eco-evolutionary processes involved in species rapid adaptations to new/modified environments (Szulkin, Munshi-South & Charmantier 2020). A recent horizon scan for urban evolutionary ecology points to the challenges faced by this field, and in particular the difficulty in reaching general conclusions on how urbanisation affects natural selection (Verrelli et al. 2022). The answer to this question can depend on the target of selection, the spatial and temporal scales considered, the age and history of cities, and the numerous agents of selection in cities such as air, light and sound pollution (Verrelli et al. 2022; Winchell et al. 2022).

Theoretically, the many constraints associated with urban life, such as reduced access to high quality food resources or shelter and strong disturbances by humans, cars and domestic pets, could shift the fitness landscape, decreasing mean absolute fitness, and increasing maladaptation and the opportunity for selection (Fig. 1 in Fugere & Hendry 2018). In contrast, urban conditions could also lead to relaxed selection. First, this can happen if human habitat alteration makes a costly trait no longer adaptative. For example, eutrophication and algae invasion in the Baltic Sea have rendered the male red nuptial coloration of three-spined sticklebacks (Gasterosteus aculeatus) ineffective in sexual courtship due to poor visibility, which has weakened natural selection (Candolin, Salesto & Evers 2007). Second, urban environments can be more homogeneous than natural habitats for some species, buffering organisms from environmental variation. For example the urban heat island (UHI) effect keeps cities warmer during extreme cold (Yang & Bou-Zeid 2018). Such buffering could decrease among-individual variation in fitness and relax selection pressures (see e.g. Rodewald & Arcese 2017). Note that a given city characteristic such as the UHI can have both positive and negative influences on fitness depending on the species or the season (see e.g. in humans, Macintyre et al. 2021).

Cronin et al. (2022) reviewed evidence for divergent sexual selection in urban habitats and drivers, such as pollution or resource availability, that shape selection pressures acting on sexual traits. They identified 104 studies published between 1980 and 2021, providing insight on urban influences on sexual signalling (Table S1 in Cronin et al., 2022). For instance, trade-offs between signal transmission and attractiveness lead to altered male songs in urban areas, with higher minimum frequencies for several bird species (Slabbekoorn & den Boer-Visser 2006; Wood & Yezerinac 2006; Dowling, Luther & Marra 2012). Although interpreted

in the light of new sexual selection pressures (e.g. selection for increased signal transmission or alternative signals in the above example of male bird song), the majority of these case studies provide no estimates of the strength of selection.

Natural selection can be defined in "purely phenotypic terms" (Arnold & Wade 1984) because it involves differences in fitness resulting from differences in phenotypic traits. As such, selection has been historically approached by estimating the covariance between a phenotypic trait and relative fitness (Price 1970). Another approach examining natural selection in urban habitats determines how genomes have been shaped by urban adaptation or urban-specific demography, gene flow or drift (Johnson et al. 2018). While these attempts rarely produce robust conclusions on natural selection without associated fitness measures, studies that provide genetic selection coefficients can compare the magnitude of natural selection acting on genetic variants in urban versus non-urban habitats. Thurman and Barrett (2016) gathered 3416 directional selection shapes genomes, for instance revealing stronger selection over shorter timescales. They highlighted the limited data available compared to the huge potential to estimate similar genetic selection coefficients across taxa and contexts. This study inspired us to perform a similar literature search, restricted to urban versus non-urban contexts, with the hope that Thurman and Barrett's call would lead us to numerous new estimates.

The aim of our study is to assess how urbanisation alters natural selection, via 1. evaluating whether the strength of phenotypic selection is generally stronger or weaker in cities when compared to natural habitats and 2. measuring the force of urban-related selection on adaptive genomic variants. We reviewed the literature comparing coefficients of natural selection in urban and non-urban habitats using both phenotypic and genomic approaches with the initial aim of conducting meta-analyses to quantitatively assess trends for given traits or taxa. The scarcity of studies prevented such an analysis, and thus we provide a qualitative assessment of how natural selection can differ in urban versus non-urban environments, and outline a roadmap for how future studies should provide standardised metrics to facilitate mega- or meta-analyses and explore generalised effects of urbanisation on selection.

2. Urban natural selection on wild phenotypes

Understanding how natural selection varies across wild populations, but also in time, is considered a central question in evolutionary ecology (Hoekstra et al. 2001; Siepielski, DiBattista & Carlson 2009; Bell 2010; Morrissey & Hadfield 2012). It has led to an abundant literature, in particular following the publication of

Lande and Arnold's (1983) accessible introduction on how to measure linear and non-linear selection gradients using multivariate regression on individual phenotypic and fitness data (Svensson 2023).

To determine whether urbanisation affects the strength of selection, we first synthesized studies on phenotypic traits that i) estimated selection coefficients i.e. selection gradients or differentials in urban and non-urban environments and ii) provided compelling evidence for altered urban selection. Studies reviewed in section 2.1. were found by searching Google Scholar for studies that cited Lande & Arnold (1983; following approach in Fugere & Hendry 2018) and mentioned either urban*, city*, town*, or metro* (conducted 15 January, n = 6 relevant studies out of 439 hits). We also conducted a Web of Science Core collection search (15 January 2024, see Table S1 for full search terms) that included articles containing urban-related search terms (Topic = "urban* OR city* or town* OR metro*") and either "selection gradient*" or "selection differential*" across all fields, which did not return any new articles (n = 2previously identified studies of 7 hits). As we were aware of two other relevant studies that did not appear in either of these searches, we also included these studies (n = 2; Badyaev et al. 2008; Branston et al. 2021). From the Google Scholar search, we identified an additional n = 8 studies that estimated coefficients of selection in either urban, suburban, or agricultural environments only (Sol, Jovani & Torres 2003; Gregoire et al. 2004; Yeh & Price 2004; Price, Yeh & Harr 2008; Ryder et al. 2012; Lambrecht, Mahieu & Cheptou 2016; Houle et al. 2020; Spear et al. 2023) and so we highlight their importance towards future metaanalyses but do not elaborate on their findings here.

Overall, studies estimating wild selection coefficients were all conducted in avian or plant taxa (five studies on three avian species and three studies on three plant species; Figure F.1; Table F.1 & Supporting information Table S2), with a particular focus on morphological and phenological traits. In section 2.2., we include a non-exhaustive list of studies that illustrate how urbanisation can strengthen or weaken selection, which broadens examples to more diverse traits across mammals and insects. Our review highlights a crucial gap of knowledge on the specific urban drivers that modify the strength of selection, which are often speculated upon but very rarely demonstrated. Most of the reviewed studies mentioned plausible mechanisms that alter the strength of selection in urban areas, and some attempted to measure these possible mechanisms (e.g. Irwin, Warren & Adler 2018; Branston et al. 2021; Palacio & Ordano 2023), but we found no unequivocal evidence linking altered selection to a specific urban agent of selection.

Table F.1. Studies estimating selection gradients or differentials in urban and non-urban environments. Order follows discussion in the main text. ISA= Impervious surface area, CMR= capture-mark-recapture.

Taxa	Species	Phenotypes	Fitness proxy	Coefficient	Results	Details	Reference
Birds	House finch (Carpodacus mexicanus)	Morphology: Bill length, width, depth	Survival: Survival to following year	Differentials (linear & quadratic)	Stronger urban directional selection	Coefficients computed separately for urban vs desert from phenotypic distributions before and after survival	(Badyaev et al. 2008)
	Great tit (Parus major) Blue tit (Cyanistes caeruleus)	Morphology: Body mass	Survival: Survival to fledging	Differentials (linear)	Stronger urban selection in both species, but urban selection significantly higher only in great tits	Coefficients computed separately for high and low ISA categories	(Corsini <i>et al.</i> 2021)
	Great tit (Parus major)	Morphology: Body mass, wing length, tail length Life-history: Lay date, clutch size	Reproduction: Number of fledglings	Differentials & gradients (linear, quadratic, correlational)	Selection on traits overall weak in both habitats, but significantly weaker urban selection for lay date and body mass (males)	Coefficients computed separately by habitat and sex Morphological traits corrected by tarsus length	(Caizergues, Gregoire & Charmantier 2018)
	Great tit (Parus major)	Behaviour: Exploration, aggression, stress response	Reproduction: Number of fledglings	Differentials & gradients (linear, quadratic, correlational)	Selection on traits overall weak in both habitats, but significantly weaker urban selection for exploration (males)	Also consider selection using CMR survival models	(Caizergues et al. 2022a)
	Great tit (Parus major) Blue tit (Cyanistes caeruleus)	Life-history: Lay date, clutch size	Reproduction: Number of fledglings	Gradients (linear & quadratic)	Selection was significantly stronger in the forest for both traits only in blue tits, no significant difference in great tits.	Also measure caterpillar availability	(Branston <i>et al.</i> 2021)
Plants	Yellow jessamine (Gelsemium sempervirens)	Morphology: Floral size, display size & shape Physiology: Chemical defence	Reproduction: Seeds per plant	Gradients (linear, quadratic, correlational)	Significantly stronger urban selection for larger floral display size	Coefficients separated by each urban and non- urban study site Also measure pollen receipt	(Irwin, Warren & Adler 2018)
	Blue passionflower (Passiflora caerulea)	Morphology: Fruit diameter, mean seed number, crop size, peel carbohydrate content	Reproduction: Fruit removal	Gradients (linear, quadratic, correlational)	Weaker urban and semiurban selection for fruit crop size, but not statistically compared	Coefficients reported separately for urban, semiurban, and rural populations Fruit removal by birds observed at each location	(Palacio & Ordano 2023)
	Common ragweed (Ambrosia artemisiifolia)	Morphology: Plant height Phenology: Transition to reproduction, first open male flower, male to female flower	Reproduction: Number of flowers & fruits	Differentials & gradients (linear, quadratic)	Net selection favoured larger plants and earlier phenology overall, but stronger selection on foreign genotypes	Common garden / reciprocal transplant design	(Gorton, Moeller & Tiffin 2018)

2.1. Phenotypic selection differentials and gradients

2.1.1. Stronger urban natural selection

Stronger estimates of urban selection have so far been reported in bird and plant species (Table 1). The limited number of studies in Table 1 suggests that in birds, selection in urban habitats may more strongly act on morphological traits than behaviour or life-history traits, with the underlying drivers often linked to changes in urban diet. Urban bird feeding activities have shifted urban diets towards larger seed resources which has presumably contributed to phenotypic divergence in bill morphology of urban house finches (Carpodacus mexicanus); urban finches tend to have larger bills than finches occupying more natural desert environments (Badyaev et al. 2008). In line with this divergence, there was strong selection favouring longer bills in urban finches whereas selection was weaker and favoured smaller bills in desert finches. Another example comes from a study of great and blue tits (Parus major, Cyanistes caeruleus) along an urbanisation gradient in Warsaw (Corsini et al. 2021). Caterpillars are key resources fed to nestlings during reproduction and a lower abundance of these resources in densely built urban areas (Seress et al. 2020; Branston et al. 2021) limits growth and reduces the probability of nestling survival in both species (Corsini et al. 2021). This study revealed stronger selection in more urbanised areas favouring higher body mass at hatching for great tits, most likely related to the reduction in food resources (Corsini et al. 2021).

As in birds, evidence for stronger urban selection in plants has been documented in morphology and, more specifically related to increases in floral size. In the Yellow jessamine (Gelsemium sempervirens), selection for larger floral displays is significantly stronger in urban areas compared to non-urban ones (Irwin, Warren & Adler 2018). The authors suggest selection for larger urban floral displays could be driven by reductions in urban pollinators as urban yellow jessamines tend to have lower pollen receipt and they show that, in urban areas, pollen receipt can directly act to increase floral display size in this system. Although the study supports stronger urban selection for this trait, the authors highlight that they find only modest selection estimates for the other traits considered, suggesting that urbanisation may not contribute to sweeping changes in phenotypic selection as commonly expected (Irwin, Warren & Adler 2018).

2.1.2. Weaker urban natural selection

Evidence in birds for weaker urban selection come from two tit species, but range across morphological, life-history, and behavioural traits. Urban great tits in the city of Montpellier tend to be smaller, faster explorers, more aggressive, and more stressed during handling, and tend to lay earlier and smaller clutches

than tits living in a nearby forest habitat (Charmantier et al. 2017; Caizergues et al. 2022a). Selection gradients across these traits were overall weak in both habitats and, in some cases, patterns of selection were in the opposite direction to the documented phenotypic divergence. For example, there were significantly different patterns of selection for both lay date and male body mass between habitat types but, in both cases, selection favoured earlier lay dates and lighter males in the forest, whereas selection in the city was significantly weaker (Caizergues, Gregoire & Charmantier 2018). Further, selection for higher exploration tendencies in male great tits was significantly stronger in forest rather than urban habitats (Caizergues et al. 2022a). In higher latitude populations of blue tits around Glasgow, there is selection on these traits was significantly weaker in urban and forest habitats but, again, the strength of selection, however, did not significantly differ between urban and forest populations of great tits in these study areas (Branston et al. 2021). The authors show that their urban study sites possess fewer caterpillars and so hypothesize that weaker environmental cues in urban areas could contribute to relaxed urban selection, especially if blue tits are more sensitive to absences of these resources (Branston et al. 2021).

We describe above that urban selection can be stronger on floral display size, but we find opposite support for weaker urban selection on other plant morphological traits. Bird visitation was found to exert selection on fruit crop size across populations of the blue passionflower (Passiflora caerulea) promoting fruit removal, but this selection was weaker in the urban and semi-urban populations, and strongest at the rural population (Palacio & Ordano 2023). The authors suggest that relaxed urban selection in this case could be a result of either i) urban populations being closer to the phenotypic optimum as they tended to have larger average fruit crop sizes or ii) generalist bird species in urban areas being less selective of which fruits (and associated traits) they forage. This calls for an integration of species interactions to unravel the agents driving novel selection pressures in cities. In another example, selection favoured taller height and earlier phenology across common ragweeds (Ambrosia artemisiifolia) from urban and rural origins planted in both urban and rural study sites (Gorton, Moeller & Tiffin 2018). Urban and rural common ragweeds were found to have genetically differentiated across multiple phenological traits (e.g., plants from urban origins tended to flower earlier regardless of habitat type) and the strength of selection tended to be stronger on foreign plants (i.e., stronger selection acting on plants from rural origins when planted at urban study sites and vice versa). Genetic differentiation and stronger selection on foreign genotypes provide consistent support for local adaptation in ragweed, although plants from rural origins tended to have higher overall lifetime fitness when reared in both urban and rural study sites providing conflicting support (Gorton, Moeller & Tiffin 2018). Although selection was not directly compared between urban and rural plants reared in their native environments in this study, the authors suggest these mixed findings could be a result of weaker selection in the urban environment, potentially because urban habitats are more spatially heterogeneous (Gorton, Moeller & Tiffin 2018).

2.1.3. Insight from urban phenotypic selection gradients

As summarized above, there are several examples pointing to urbanisation both increasing and decreasing the strength of selection, at least in bird and plant taxa. Across these examples, no obvious generalizations have emerged that enable directional predictions on how urbanisation impacts selection across different traits and species. Our original aim was to conduct a formal quantitative analysis to determine whether urbanisation increases or decreases the strength of selection. Given the few studies that have quantified selection coefficients (n = 8), we cannot determine how urbanisation affects phenotypic selection. We list in the Supporting Information (Table S2) 200 effect sizes (n = 101 urban and 99 non-urban) of linear selection gradients and differentials reported in these studies. We consider this a first step to inspire research interest in reporting these selection coefficients and their associated uncertainty, so that meta-analyses can be conducted in the future. Descriptively, the median (absolute) urban and non-urban selection gradients are similar (n urban = 70 with median: 0.091, range: 0.0001-1.06; n non-urban = 68 with median: 0.10, range: 0.004-1.37), and within the range of previously reported summaries of selection in natural populations (see e.g. Kingsolver et al. 2012; Morrissey & Hadfield 2012). Similar median strength of selection between urban and non-urban habitats is somewhat unsurprising given there is evidence of both stronger and weaker urban phenotypic selection (as summarized above) and selection estimation is classically associated with large measurement error (e.g. Dingemanse, Araya-Ajoy & Westneat 2021). Overall, our summary on phenotypic coefficients of selection implies that we so far are unable to generalize on how urbanisation impacts the strength of selection. Further comparative research of selection in and outside cities across diverse systems is needed before this conclusion can be re-addressed and any generalities can emerge.

When collecting effect sizes of selection across studies, we became aware of some methodological differences between studies. We do not properly account for these methodological differences in our summary above, but these constraints will need to be addressed in future meta-analyses and we provide suggestions and considerations that researchers can use when measuring urban selection (see section 4). Studies examining selection in only urban environments will also be useful to consider in future work. For instance, larger size was also under strong selection in holy hawksbeard (Crepis sancta) in urban environments (Lambrecht, Mahieu & Cheptou 2016), but it is unknown whether selection for plant size is stronger or weaker for this species outside the urban setting.

2.2. Compelling evidence of modified selection

Since the literature search conducted above provided reduced power to conclude on how selection differentials or gradients changed between non-urban and urban areas, we move beyond this attempt to quantify results on selection coefficients and discuss here studies that have provided complementary crucial knowledge on how urban natural selection can vary compared to non-urban natural selection. While these studies do not allow a quantitative comparison across species and contexts, they provide unique insight into the mechanisms that may drive novel, stronger or weaker selection in cities. Demonstrating the causal agents of selection has always been a great challenge in evolutionary ecology, and it is usually not possible to firmly identify the drivers via a correlational approach (Mitchell-Olds & Shaw 1987; Svensson 2023). This challenge is particularly salient in the context of cities which are characterized by multifarious urban stressors such as multiple pollutions, heat, and altered and fragmented habitats (Diamond & Martin 2021). Only experimental manipulations such as alteration of the biotic (e.g. predation pressure) or abiotic (e.g. temperature) agents can clearly identify agents driving differences in selection in urban environments (Wade & Kalisz 1990; MacColl 2011).

2.2.1. Relaxed selection under altered resource and predator regimes

In their extensive review on the effects of urbanisation on sexual selection, Cronin et al (Cronin et al. 2022) cited resource availability as one of the main biotic drivers influencing the strength and form of sexual selection in an urban context. While food is necessary for somatic growth and maintenance, it is also a key determinant of colourful ornaments. In the Northern Cardinal Cardinalis cardinalis, the stunningly red plumage colour of males is produced from carotenoid pigments that they need to find in their diet. A study of northern cardinals by Rodewald and colleagues 2011 (Rodewald, Shustack & Jones 2011) across a rural-urban gradient in Ohio, USA, revealed that in more urban landscapes, brightness of male plumage no longer correlated with breeding phenology or reproductive success. The authors suggest that this relaxed selection for colour arises because of the over-abundance of carotenoid-rich exotic fruits in cities, such as honeysuckles. We find this example particularly striking because in other bird species with carotenoid-related colours but more insectivorous than frugivorous diet, urban birds are generally paler, although with strong variation across cities, and no insight yet on the force of natural / sexual selection on these carotenoid colours (Salmón et al. 2023).

One of our favourite demonstrations of relaxed selection in cities was very recently published and echoes the emblematic and precursor study of industrial melanism in the peppered moth Biston betularia in industrial England (see e.g. Cook & Saccheri 2013). Following observations of parallel high prevalence of melanic eastern gray squirrels (Sciurus carolinensis) across 43 cities in North America, Cosentino and colleagues (Cosentino, Vanek & Gibbs 2023) translocated 76 gray squirrels from urban areas in Syracuse to both urban and rural novel areas. The subsequent survival monitoring revealed that while gray squirrels had much higher survival than melanic squirrels in rural habitats, there was no such evidence for survival selection in the city. The authors favour the hypothesis that weaker selection against the conspicuous melanic morph in the city results from lower predation and hunting pressure.

Finally, the role of altered predator communities is also cited as a possible agent of relaxed selection for fast growth rates in urban damselflies Coenagrion puella (Tüzün et al. 2017). A common garden experiment reveals weaker selection for growth rates in urban ponds, most probably because of warmer water allowing for longer growing seasons, and possibly also temperature-related alteration in predator pressure.

2.2.2. Reversed selection and identifying multiple agents of selection

Evidence for reversed selection in urban compared to non-urban habitats is very scarce but it was shown in the common urban-dweller and model species great tit. Using 17 years of capture-mark-recapture data and measures of the size of the black breast stripe of male great tits in and around Barcelona, Senar and colleagues (Senar et al. 2014) found that survival was positively associated with the size of this sexual ornament in forest males, but negatively correlated in urban males. While the size of the black tie in male great tits has been positively associated with dominance status (e.g. Jarvi & Bakken 1984), tie size is also negatively correlated with exploration speed (Nicolaus et al. 2016). Senar thus hypothesizes that smaller ties in city birds is likely a by-product of selection on personality (Senar, pers.com.), which aligns with findings that urban great tits are bolder and faster explorers (Charmantier et al. 2017; Riyahi et al. 2017).

A global study of the white clover (Trifolium repens) illustrates the difficult task of identifying agents of selection in a complex urban system. A large-scale study of 20 Canadian cities revealed parallel clines with decreased plant production of hydrogen cyanide (HCN) in response to urbanisation, indicating parallel evolution resulting from parallel selection favouring lower chemical defence in cities, since the authors found no evidence for genetic drift (Johnson et al. 2018). Despite the large number of cities in this study, agents of selection causing these evolved differences remained unclear, HCN being both an anti-herbivore chemical defence but also reducing freezing and drought tolerance. In an even larger scale study across a monumental 160 cities, Santangelo and colleagues analysed environmental predictors of HCN clines,

concluding that herbivory selected for higher HCN in rural areas while lower drought selected for lower HCN in urban areas (Santangelo et al. 2022).

It is sobering to note that for most studies discussed in this section, even with extensive efforts across decades, agents of selection inducing new selective forces in urban contexts remain hypothetical interpretations from field experts. We also note that, and again despite tremendous effort, most of these studies would be unable to provide standardized selection differentials or gradients that would have contributed to Table 1 and allowed a quantitative meta-analysis, in the absence of individual fitness measurements. In comparison to the demanding challenge of collecting data that allow estimating phenotypic selection coefficients, selection coefficients from genomic data may be more tractable for many taxa.



Figure F.1: Illustrating some studies that have demonstrated divergent natural selection in urban habitats, with potential explanations regarding the agents that can lead to weaker, stronger, or reversed natural selection in cities. Studies either compared selection differentials / gradients between urban and non-urban habitats (solid lines, discussed in section 2.1), or used other approaches (dotted lines, section 2.2). List of species and references: 1: Great tit Parus major in (Caizergues, Gregoire & Charmantier 2018) ; Blue tit Cyanistes caeruleus in (Branston et al. 2021); Northern Cardinal Cardinalis cardinalis in (Rodewald, Shustack & Jones 2011); 2: Common ragweed Ambrosia artemisiifolia in (Gorton, Moeller & Tiffin 2018); 3: Blue passionflower Passiflora caerulea in (Palacio & Ordano 2023); Eastern gray squirrel Sciurus carolinensis in (Cosentino, Vanek & Gibbs 2023); 4: Damselfly Coenagrion puella in (Tüzün et al. 2017); 5: House finches Carpodacus mexicanus in (Badyaev et al. 2008); 6: Great tit in (Corsini et al. 2021); 7: Yellow jessamine Gelsemium sempervirens in (Irwin, Warren & Adler 2018); 8: Great tit in (Senar et al. 2014); 9: White clover Trifolium repens (Santangelo et al. 2022). All drawings from MJT.

3. Genomic insight on measuring urban selection coefficients

Estimates of genomic selection coefficients can provide important knowledge on the nature of selection acting on adaptive traits and their underlying genetic architecture. These estimates can be an addition to or substitute for selection coefficient estimates based on phenotypic and fitness data, depending on whether such phenotypic-based approaches have already been performed or are even possible. While linking phenotypes and fitness measured in the wild can provide insights into the functional significance of specific

traits in a given environment, genomic selection coefficients quantify the overall past strength of natural selection on specific genetic variants, based on genomic data only. Genetic selection coefficients can be measured by genotyping one of a few candidate loci previously identified as being under selection and/or associated to phenotypic variations. Advances in genotyping methods, principally through democratization of high-throughput sequencing, have increased the possibility, especially for natural populations of non-model species, to scan genomes in order to detect potentially many loci under selection and/or associated to phenotypic variations and estimate genomic selection coefficients at these loci (Nielsen 2005; Barrett & Hoekstra 2011; Bank et al. 2014; Manel et al. 2016). In particular, in this section, we describe general methods for calculating genomic selection coefficients, then review their application in an urban context. Finally, we highlight important gaps in the current literature and we propose in the next section future steps that can be taken to help advance our knowledge of selection in urban environments.

3.1. Overview of genetic and genomic approaches to measure selection coefficients

As the scope of our article is to review the state of the literature, and not to describe in detail the methodology for calculating selection coefficients, we refer interested readers to comprehensive reviews of popular and useful methods for calculating genomic selection coefficients by Linnen & Hoekstra (2009) and Bank et al (2014).

In brief, the most straightforward strategies for measuring selection based on genomic data depend on the availability of measures of individual fitness (or fitness-related traits) and individual genotypes for causal loci or genome-wide variants (e.g., SNPs). When individual fitness measures and genotypes are available, it is possible to directly link fitness to underlying genetic architecture. In the simplest cases, such as at a single Mendelian locus causing discrete polymorphic phenotypes, selection coefficients can be calculated from estimates of the relative fitness (w) for a given genotype (Eanes 1999). Advances in genomic techniques now allow such estimates for quantitative trait loci and further, can aid in discovery of candidate loci, circumventing the need for prior knowledge of causal loci. One popular contemporary strategy is to use genome scans or Genome-Wide Association Study (GWAS) to identify loci underlying variation in fitness or fitness-related traits, and then measure selection at these candidate loci by associating allelic variation and individual fitness. For example, Bérénos et al. (Berenos et al. 2015) combined genomic SNP data with fitness and phenotypic measures to investigate the genetic architecture of body size traits. The authors used a GWAS to identify SNPs associated with body size (i.e. hind length), tested the association between fitness and genotypes for outlier SNPs, then estimated selection coefficients at these SNPs. Their study illustrates how contemporary population genomics techniques paired with traditional phenotypic and

fitness measures can both facilitate the discovery of candidate loci underlying quantitative traits and improve our understanding of how selection affects these loci in natural populations.

Many studies may lack fitness and phenotypic data. In these cases, selection coefficients can be calculated from changes in allelic frequencies over time (i.e., multiple generations) or over geographic space (i.e., clines). If the candidate gene is unknown, it can be analysed by applying first genome scans (methods reviewed in Hohenlohe et al. 2010) and then by estimating selection coefficient on identified outlier loci. Otherwise the selection coefficient for known candidate genes can be directly estimated. Selection coefficients are estimated by computing the probability of the underlying changes in allele frequencies over multiple generations or geographic locations, often using a likelihood-based approach (e.g. software package SelEstim, Vitalis et al. 2014) or approximate Bayesian computation (ABC) approaches (e.g. Bank et al. 2014; Stern, Wilton & Nielsen 2019). Importantly, these methods are in general very sensitive to sample size (Pinsky et al. 2021) as well as the spatial and/or temporal distribution of sampling. As an example of this strategy relying on genomic data only, Walden and colleagues (2020) estimated selection coefficients at genes implicated in evolutionary response to spatially heterogeneous climatic conditions in Arabidopsis lyrata. They first used GWAS, and Environmental Association Analysis (EAA) on POOLseq data to identify outlier SNPS and genes associated with local climatic variations. Then, they estimated mean genomic selection coefficients for these genes using SelEstim (Vitalis et al. 2014). They found increased selection coefficients for outlier genes compared to non-outlier ones, suggesting their importance for climate adaptation. The implementation of such methodologies finds seamless applicability in urban contexts, where temperatures persistently rise, and our comprehension of the selection pressures induced by these escalating temperatures remains constrained. Consequently, in the absence of accessible phenotype and fitness measures, this approach affords the capability to discern genes intricately linked to climate adaptation and it facilitates the estimation and comparison of selection intensities between urban and forest environments.

3.2. A review of genomic selection coefficients in the wild urban context

While genomic selection coefficients can provide valuable insight to understanding basic questions in evolutionary biology, there remains limited knowledge about the distribution of selection coefficients (s) in natural populations. A meta-analysis by Thurman and Barrett (2016) aimed to quantify natural selection at the genetic level, reviewing over 2000 papers and ultimately extracting ~3000 estimates from 79 studies. While their analysis provided valuable insight into the magnitude and tempo of selection in natural environments, for example, suggesting that selection is stronger over shorter timescales, their study also underscored that a critical lack of published selection coefficients constrained their ability to conclusively

address these topics. We anticipated that in the years since Thurman and Barrett's review, an increased number of studies would report genomic selection coefficients, including studies set in an urban context. Thus, our objective was to replicate their meta-analysis and literature search to retrieve and analyse estimates of selection, with a focus on estimates of selection coefficients in urban contexts.

3.2.1. Systematic review and data extraction

We identified relevant papers with literature searches using the Web of Science Core Collection (conducted 8 November 2023, see Table S1 for full search terms), filtering results to include only primary articles in evolutionary biology containing urban-related search terms ("Document Types: Article; Research Areas: Evolutionary Biology; Topic = "urban* OR city* or town* OR metro*"). We conducted three independent searches using different sets of search terms. In Search 1, we specified key terms used by Thurman and Barrett (2016) to find published estimates of natural selection acting at the genetic level: Topic = ("selection coefficient* OR genotyp* selection OR adapt* gene"). In Search 2, we broadened our criteria to include selection gradients: Topic = ("selection coefficient OR selection gradient"). In Search 3, we targeted potentially relevant papers by specifying popular approaches for quantifying the strength of selection from genomic data, as identified from Linnen & Hoekstra's (2009) review: Topic = ("MK test OR McDonald Kreitman test OR dn/ds test OR nonsynonymous substitutions synonymous substitutions OR allele frequenc* ecotone OR allele frequenc* cline width OR CLR test OR composite-likelihood-ratio test"). These searches returned 264, 15, and 133 articles respectively, for a total of 412 papers.

De-duplication resulted in 355 articles that we screened for inclusion. Our initial criteria for inclusion in our review were that each study: 1) provided a selection coefficient or selection gradient for a genetic unit (allele, SNP, QTL, chromosome, etc); 2) provided the selection statistic for both an urban and a non-urban population; and 3) focused on natural populations (e.g., not laboratory or domesticated plants and animals). No paper met all criteria, however, so we relaxed our criteria to encompass studies that 1) provided genomic evidence for selection or selection coefficients/gradients estimated from genomic data; 2) provided this genomic evidence for at least one urban population; and 3) focused on natural population; and 3) focused on natural population.

3.2.2. Insights from the literature review and discussion

Of the 355 articles that we reviewed, no papers met all initial criteria for inclusion, and only 34 (9.6%) met our relaxed criteria for inclusion. Many of the remaining 321 articles were irrelevant to our focus despite our specified criteria (e.g., they concerned agricultural populations, lacked urban context, or did not report genomic data). Our difficulty in identifying relevant studies mirrors challenges reported by Thurman & Barrett in their meta-analysis (Thurman & Barrett 2016): of the 2200 studies reviewed for inclusion, they were able to accept only 79 (3.5%). Anticipating this challenge, we sought to improve the relevancy of our results and the efficacy of our search by complementing Thurman & Barrett's key terms (Search 1) with independent searches for more broad key terms (Search 2) and for key terms explicitly targeting methods used to calculate genomic selection coefficients (Search 3). Still, we retrieved few relevant papers. In fact, Search 2 and Search 3 combined were less successful than Search 1: while Search 1 returned 29 relevant papers out of 264 total papers (11.0%), Search 2 returned only 1 relevant paper out of 15 total papers (6.7%) while Search 3 returned only 2 relevant papers out of 133 total studies (1.5%). Together, however, these three searches incorporated a range of both broad and targeted keywords. We therefore suggest that our detection of few relevant papers reflects an apparent lack of genomic selection coefficients reported in the urban eco-evolutionary literature.

Of the 34 papers we retained for our study (Table S3), 29 included urban-associated genomic "signatures of selection", e.g., using genomic scans to identify candidate SNPs for divergent selection between urban and non-urban environments (Nielsen 2005; Hohenlohe et al. 2010). Six papers presented genomic data that could be used to estimate the strength of selection. Of these, five studies reported urban-non-urban genetic clines; none of these papers used the documented genetic clines to calculate selection coefficients (Linnen & Hoekstra 2009). Only 1 study reported selection coefficients; however, this was for a single urban population without a non-urban statistical complement (see box F.1).

Box F.1. A single study to date reporting urban genomic selection coefficient

Baltzegar et al (Baltzegar et al. 2021) studied the evolution of a knockdown resistance (kdr haplotype) in the mosquito Aedes aegypti in response to insecticide use in the city of Iquitos (Peru). The frequency of resistant alleles was expected to increase over time, with positive selection coefficients induced by insecticide exposure. The authors genotyped the kdr mutations associated with pyrethroid resistance, in 9882 mosquitoes sampled in several locations in Iquitos City before/during/after the use of insecticides (pyrethroids). Then, they estimated selection coefficients of the resistance alleles at each locus by using the Wright-Fisher approximate Bayesian computation method (WFABC) for temporally sampled data (Foll, Shim & Jensen 2015). The frequency of kdr resistance mutations increased rapidly after insecticides exposure, with very large selection coefficients of 0.313 (0.007, 0.821) and 0.485 (0.145, 0.969) for the two resistance mutations while they were increasing in frequency. The authors discuss that these selection coefficients may be underestimated because of several violations of the model's assumptions, including non-independence of the mutations and spatio-temporal heterogeneity of the selection pressure. The

authors also note that, although frequencies of resistant alleles shifted quickly during the studied period, genetic heterogeneity existed not only at the citywide scale but also on a very fine scale within the city. In the scope of our review, we note that this example does not address the question of whether urbanisation leads to stronger/weaker/reversed selection but that a comparison with similar estimates outside the urban context would allow stronger conclusions on urban-specific selection.

4. Roadmap for future phenotypic and genomic studies

This review has revealed how difficult it is to presently conduct a quantitative comparative analysis of studies exploring urban versus non-urban natural selection, both at the phenotypic and the genomic levels, using a systematic literature review and meta-analytical approach. What would it take for future studies to allow such a quantitative approach and derive more general interpretations on which traits in which taxa can be expected to be under stronger, weaker or reversed selection in the urban context?

4.1. Facing the challenge of standardised estimations of natural selection

For meta-analysts to combine summary statistics on natural selection, the primary studies estimating selection should ideally integrate all insight from the vast literature that delivers guidance for adequate statistical approaches, and provide coefficients with their error estimation informing on accuracy. Our objective here is not to provide an exhaustive insight on how to measure natural selection in the wild as others have covered this extensively (Brodie, Moore & Janzen 1995; Linnen & Hoekstra 2009; Stinchcombe, Kelley & Conner 2017). We provide in Table 2 a synthesised list of questions and challenges that must be addressed to estimate natural selection based on wild phenotypic and fitness data. We have split the challenges in three categories: 1. Questions that need addressing at the start of any project measuring coefficients of natural selection, 2. Outstanding biological questions regarding what aspect of the trait-fitness relationship we want to measure, 3. Statistical challenges that make biological questions more difficult to answer. Note that we recognise that there is strong subjectivity in whether a challenge is placed in the second or third category as many challenges in the third section can be considered outstanding questions of focal interest (e.g. spatio-temporal patterns of selection).

In fine, is it possible to gather comparable estimates of natural selection in the urban context despite "the tremendous quantitative and statistical sophistication that is being brought to measuring selection on phenotypes and genomes" (Stinchcombe, Kelley & Conner 2017)? The long list and associated (non-exhaustive) references in Table 2 are sobering and many of the issues raised here could be broken-down

into a number of sub-considerations. For instance, regarding the first question on estimating selection over a whole life cycle, the original theoretical work (Arnold & Wade 1984) was developed on non-overlapping generations, and models of selection with age structure are even more complex (see applications in e.g. Childs et al. 2011; Engen et al. 2012).

The challenge	References addressing it		
1. Initial fundamental considerations:			
How to estimate total selection over a life cycle?	(Lande 1982; Arnold & Wade 1984; Charlesworth		
What is the best estimate of fitness?	(Arnold 1983: Franklin & Morrissey 2017)		
Do we have sufficient power to detect selection?	(Amold 1965, Hankin & Wolfissey 2017) (Mitchell Olde & Show 1087; Hersch & Dhilling 2004)		
Do we have sufficient power to detect selection:	(Mitchell-Olds & Shaw 1987; Hersch & Phillips 2004)		
Which statistical methodology to implement	(Janzen & Stern 1998; Morrissey & Sakrejda 2013;		
measure?	Momssey 2014a; Momssey & Goudie 2022)		
How to standardize fitness and traits?	(Lande & Arnold 1983: Hereford Hansen & Houle		
	(2004)		
2. How to best estimate:			
- Direct and indirect selection, and causal pathways	(Lande & Arnold 1983; Arnold & Wade 1984; Scheiner,		
	Mitchell & Callahan 2000; Morrissey 2014b; Henshaw,		
	Morrissey & Jones 2020)		
- Linear and non-linear selection	(Lande & Arnold 1983; Stinchcombe <i>et al.</i> 2008; Langhau, & Zamal 2017)		
- Environmental sensitivity of selection	(Wade & Kalisz 1990: MacColl 2011: Chevin Visser &		
- Environmental sensitivity of selection	Tufto 2015)		
3. How to best account for:			
- Multicollinearity	(Lande & Arnold 1983; Mitchell-Olds & Shaw 1987;		
	Morrissey 2014a; Chong, Fung & Stinchcombe 2018;		
	Morrissey & Ruxton 2018)		
- Non-Gaussian trait distributions	(de Villemereuil et al. 2016; Bonamour et al. 2017; de		
	Villemereuil 2018; Pick <i>et al.</i> 2022)		
- Non-Gaussian fitness distribution	(Shaw & Geyer 2010)		
- Uncertainty and sampling error (in particular for	(Morrissey & Hadfield 2012; Stinchcombe, Simonsen &		
The problem of environmental covariance	Blows 2014; Momssey 2010) (Paushar 1002: Kruuk Marila & Shaldon 2003:		
- The problem of environmental covariance between trait and fitness	Morrissey Kruuk & Wilson 2010: Morrissey &		
between trait and miness	Henshaw 2022)		
- Individual interactions	(Hadfield & Thomson 2017)		
- Spatial autocorrelation in fitness	(Marrot, Garant & Charmantier 2015)		
- Temporal autocorrelation and fluctuating	(Chevin, Visser & Tufto 2015; de Villemereuil et al.		
selection	2020)		
- The missing traits and the invisible fraction	(Grafen 1988; Hadfield 2008; Mittell & Morrissey		
Trait variation across lifetime (i.e. labile traite)	2023)		
- 11 att variation across metime (<i>i.e.</i> fablie traits)	Aiov et al 2023)		
	Ајбу ст ин. 2023)		

TABLE 2. The challenges of estimating natural selection in the wild.

4.2. Towards more selection coefficient estimations in genomic studies of urban adaptation

Many challenges outlined in Table 2 are related to measuring and analysing fitness data, and hence will not apply when adopting a genomic perspective on selection. While population genomic studies performed in urban contexts did not yet report selection coefficients at loci underlying fitness variation, many studies did identify putative genomic footprints of selection. These studies mostly followed a similar strategy comparing populations in urban versus non-urban environments to 1) identify candidate SNPs under divergent selection through multiple approaches, and 2) identify biological pathways and functions involved in adaptation to urban environments (Harris & Munshi-South 2017; Theodorou et al. 2018; Caizergues et al. 2022b). In order to provide more selection coefficients a first simple step would be, when population genomics or quantitative genomics methods already identified potential genomic footprints of selection and/or loci associated to phenotypic variations, to use statistical methods estimating selection coefficients, for example the SelEstim software (Vitalis et al. 2014) and other aforementioned methods (section 3.1). Second, as statistical methods estimating selection coefficients usually require large sample sizes, and spatial and temporal coverage, implementing large populations' sampling and sequencing of multiple spatial and/or temporal data point, ideally combined with phenotypic and fitness measures, would allow precisely estimating genomic selection coefficients.

While genomic studies usually lack fitness estimation thus limiting the scope for adaptive interpretations of signatures of selection, they have the great advantage of providing insight on historical patterns of selection that have shaped current phenotypic divergence and clines across urban gradients. In contrast, phenotypic approaches reviewed in section II provide insight on current patterns of natural selection with often strong variation across years and study areas for a given trait and taxa. Phenotypic and genomic approaches should therefore be used as complementary approaches to study urban-related natural selection at micro and macro-temporal scales.

4.3. Final considerations: fitness landscapes, opportunity for selection and mega-analyses

As a final look toward the future, we outline three different approaches that provide a different perspective on natural selection in urban areas.

First, rather than aiming to model a fitness-trait relationship using constraining parametric approaches, a more flexible approach could be to model the fitness landscape using nonparametric approaches such as cubic splines (Schluter 1988; Morrissey & Sakrejda 2013). Alternatively, one can use parametric approaches that relate more explicitly to theoretical predictions than do classic selection gradients from Lande & Arnold (1983). In particular, models of moving optimum are popular in theory on adaptation to changing

environments (e.g. Kopp & Matuszewski 2014), and can be inferred empirically (Chevin, Visser & Tufto 2015). Using such models, one could compare the location, width and height of the fitness peak between urban and non-urban populations in a comparable way to studies that have used this approach to investigate temporal fluctuations of selection (Chevin, Visser & Tufto 2015; de Villemereuil et al. 2020). Note that this fitness landscape approach also bears methodological limitations, e.g. it requires strong assumptions but also large sample sizes, to test how both the height and width of the fitness optimum may change, and as such it may not facilitate a quantitative comparison across urbanisation gradients. It could however provide a powerful tool to partition selection episodes and to identify environmental drivers of urban-specific selection (Gamelon et al. 2018), and relate to the abundant literature on local adaptation involving quantitative traits (e.g. Kawecki & Ebert 2004; Yeaman 2015; Yeaman 2022). Understanding how natural selection on a given character changes across different episodes of selection (e.g. viability selection and fertility selection, Walsh & Lynch 2018) and different landscapes will provide crucial insight to understand how the trait may evolve in the future.

Second, while this review has covered studies that compare the relative strength of selection for a particular trait between urban and non-urban areas, we can also compare overall selection intensity between habitats. A comparison of overall selection between the two habitats could be better obtained using measures of the opportunity for selection I, or the variance in relative fitness (Crow 1958; Arnold & Wade 1984). Note that while I reflects the upper limit of the intensity of natural selection, recent findings show that it can be highly influenced by demographic stochasticity (Reed, Visser & Waples 2023). Ultimately, we should be able to link I with population demography, and determine the overall link with selection as we classically measure it (i.e. on specific traits and at specific times in the life cycle), and total fitness.

Third and finally, a promising solution for future quantitative approaches aiming at comparing urban and non-urban natural selection is to conduct mega-analyses on individual based data for phenotypes, genomic data, and fitness measures (Eisenhauer 2021) rather than meta-analyses on heterogeneous non comparable estimations. Mega-analyses pool raw data from multiple populations in order to use the same statistical treatment rather than gather estimates from different studies in meta-analyses. The success of such mega-analytical approaches rely heavily on data platforms and repositories that allow large-scale sharing of standardized data (such as SPI-birds, see Culina et al. 2021) following FAIR principles (aka Findability, Accessibility, Interoperability and Reusability, Wilkinson et al. 2016), while also promoting interactions and collaborations between data holders. A mega-analytic approach will not only address the challenges of estimating natural selection (Table 2) in a standardized way across all data, but will also allow the measurement of urban gradients in a homogeneous way. We call here for use of mega-analyses in the near

future as we envisage it will be a powerful way to assess how different natural selection can be in cities in a wide array of taxa and traits.

5 References

- Alberti, M., Marzluff, J. & Hunt, V.M. (2017) Urban driven phenotypic changes: empirical observations and theoretical implications for eco-evolutionary feedback. Philosophical Transactions of the Royal Society B-Biological Sciences, 372, 9.
- Araya-Ajoy, Y.G., Dingemanse, N.J., Westneat, D.F. & Wright, J. (2023) The evolutionary ecology of variation in labile traits: selection on its among- and within-individual components. Evolution, 77, 2246-2256.
- Arnold, S.J. (1983) Morphology, performance and fitness. American Zoologist, 23, 347-361.
- Arnold, S.J. & Wade, M.J. (1984) On the measurement of natural and sexual selection: theory. Evolution, 38, 709-719.
- Badyaev, A.V., Young, R.L., Oh, K.P. & Addison, C. (2008) Evolution on a local scale: Developmental, functional, and genetic bases of divergence in bill form and associated changes in song structure between adjacent habitats. Evolution, 62, 1951-1964.
- Baltzegar, J., Vella, M., Gunning, C., Vasquez, G., Astete, H., Stell, F., Fisher, M., Scott, T.W., Lenhart, A., Lloyd, A.L., Morrison, A. & Gould, F. (2021) Rapid evolution of knockdown resistance haplotypes in response to pyrethroid selection in <i>Aedes aegypti</i>. Evolutionary Applications, 14, 2098-2113.
- Bank, C., Ewing, G.B., Ferrer-Admettla, A., Foll, M. & Jensen, J.D. (2014) Thinking too positive? Revisiting current methods of population genetic selection inference. Trends in Genetics, 30, 540-546.
- Barrett, R.D.H. & Hoekstra, H.E. (2011) Molecular spandrels: tests of adaptation at the genetic level. Nature Reviews Genetics, 12, 767-780.
- Bell, G. (2010) Fluctuating selection: the perpetual renewal of adaptation in variable environments. Philosophical Transactions of the Royal Society B-Biological Sciences, 365, 87-97.
- Berenos, C., Ellis, P.A., Pilkington, J.G., Lee, S.H., Gratten, J. & Pemberton, J.M. (2015) Heterogeneity of genetic architecture of body size traits in a free-living population. Molecular Ecology, 24, 1810-1830.
- Bonamour, S., Teplitsky, C., Charmantier, A., Crochet, P.-A. & Chevin, L.-M. (2017) Selection on skewed characters and the paradox of stasis. Evolution, 71, 2703-2713.
- Branston, C.J., Capilla-Lasheras, P., Pollock, C.J., Griffiths, K., White, S. & Dominoni, D.M. (2021) Urbanisation weakens selection on the timing of breeding and clutch size in blue tits but not in great tits. Behavioral Ecology and Sociobiology, 75, 12.

- Brodie, E.D., Moore, A.J. & Janzen, F.J. (1995) Visualizing and Quantifying Natural-Selection. Trends in Ecology & Evolution, 10, 313-318.
- Caizergues, A.E., Gregoire, A. & Charmantier, A. (2018) Urban versus forest ecotypes are not explained by divergent reproductive selection. Proceedings of the Royal Society B-Biological Sciences, 285, 9.
- Caizergues, A.E., Gregoire, A., Choquet, R., Perret, S. & Charmantier, A. (2022a) Are behaviour and stressrelated phenotypes in urban birds adaptive? Journal of Animal Ecology, 91, 1627-1641.
- Caizergues, A.E., Le Luyer, J., Gregoire, A., Szulkin, M., Senar, J.-C., Charmantier, A. & Perrier, C. (2022b) Epigenetics and the city: Non-parallel DNA methylation modifications across pairs of urban-forest Great tit populations. Evolutionary Applications, 15, 149-165.
- Candolin, U., Salesto, T. & Evers, M. (2007) Changed environmental conditions weaken sexual selection in sticklebacks. Journal of Evolutionary Biology, 20, 233-239.
- Charlesworth, B. (1993) Natural selection on multivariate traits in age-structured populations. Proceedings of the Royal Society B-Biological Sciences, 251, 47-52.
- Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S. & Grégoire, A. (2017) Urbanization Is Associated with Divergence in Pace-of-Life in Great Tits. Frontiers in Ecology and Evolution, 5, 53.
- Chevin, L.M., Visser, M.E. & Tufto, J. (2015) Estimating the variation, autocorrelation, and environmental sensitivity of phenotypic selection. Evolution, 69, 2319-2332.
- Childs, D.Z., Coulson, T.N., Pemberton, J.M., Clutton-Brock, T.H. & Rees, M. (2011) Predicting trait values and measuring selection in complex life histories: reproductive allocation decisions in Soay sheep. Ecology Letters, 14, 985-992.
- Chong, V.K., Fung, H.F. & Stinchcombe, J.R. (2018) A note on measuring natural selection on principal component scores. Evolution Letters, 2, 272-280.
- Cook, L.M. & Saccheri, I.J. (2013) The peppered moth and industrial melanism: evolution of a natural selection case study. Heredity, 110, 207-212.
- Corsini, M., Schöll, E.M., Di Lecce, I., Chatelain, M., Dubiec, A. & Szulkin, M. (2021) Growing in the city: Urban evolutionary ecology of avian growth rates. Evolutionary Applications, 14, 69-84.
- Cosentino, B.J., Vanek, J.P. & Gibbs, J.P. (2023) Rural selection drives the evolution of an urban-rural cline in coat color in gray squirrels. Ecology and Evolution, 13, 17.
- Cronin, A.D., Smit, J.A.H., Munoz, M.I., Poirier, A., Moran, P.A., Jerem, P. & Halfwerk, W. (2022) A comprehensive overview of the effects of urbanisation on sexual selection and sexual traits. Biological Reviews, 97, 1325-1345.
- Crow, J.F. (1958) Some possibilities for measuring selection intensities in man. American Anthropologist, 60, 1-13.
- Culina, A., Adriaensen, F., Bailey, L.D., Burgess, M.D., Charmantier, A., Cole, E.F., Eeva, T., Matthysen, E., Nater, C.R., Sheldon, B.C., Saether, B.-E., Vriend, S.J.G., Zajkova, Z., Adamik, P., Aplin, L.M., Angulo, E., Artemyev, A., Barba, E., Barisic, S., Belda, E., Bilgin, C.C., Bleu, J., Both, C.,

Bouwhuis, S., Branston, C.J., Broggi, J., Burke, T., Bushuev, A., Camacho, C., Campobello, D., Canal, D., Cantarero, A., Caro, S.P., Cauchoix, M., Chaine, A., Cichon, M., Cikovic, D., Cusimano, C.A., Deimel, C., Dhondt, A.A., Dingemanse, N.J., Doligez, B., Dominoni, D.M., Doutrelant, C., Drobniak, S.M., Dubiec, A., Eens, M., Erikstad, K.E., Espin, S., Farine, D.R., Figuerola, J., Gulbeyaz, P.K., Gregoire, A., Hartley, I.R., Hau, M., Hegyi, G., Hille, S., Hinde, C.A., Holtmann, B., Ilyina, T., Isaksson, C., Iserbyt, A., Ivankina, E., Kania, W., Kempenaers, B., Kerimov, A., Komdeur, J., Korsten, P., Kral, M., Krist, M., Lambrechts, M., Lara, C.E., Leivits, A., Liker, A., Lodjak, J., Magi, M., Mainwaring, M.C., Mand, R., Massa, B., Massemin, S., Martinez-Padilla, J., Mazgajski, T.D., Mennerat, A., Moreno, J., Mouchet, A., Nakagawa, S., Nilsson, J.-A., Nilsson, J.F., Norte, A.C., van Oers, K., Orell, M., Potti, J., Quinn, J.L., Reale, D., Reiertsen, T.K., Rosivall, B., Russell, A.F., Rytkonen, S., Sanchez-Virosta, P., Santos, E.S.A., Schroeder, J., Senar, J.C., Seress, G., Slagsvold, T., Szulkin, M., Teplitsky, C., Tilgar, V., Tolstoguzov, A., Torok, J., Valcu, M., Vatka, E., Verhulst, S., Watson, H., Yuta, T., Zamora-Marin, J.M. & Visser, M.E. (2021) Connecting the data landscape of long-term ecological studies: The SPI-Birds data hub. Journal of Animal Ecology, 90, 2147-2160.

- Darimont, C.T., Carlson, S.M., Kinnison, M.T., Paquet, P.C., Reimchen, T.E. & Wilmers, C.C. (2009) Human predators outpace other agents of trait change in the wild. Proceedings of the National Academy of Sciences of the United States of America, 106, 952-954.
- de Villemereuil, P. (2018) Quantitative genetic methods depending on the nature of the phenotypic trait. Annals of the New York Academy of Sciences, 1422, 29-47.
- de Villemereuil, P., Charmantier, A., Arlt, D., Bize, P., Brekke, P., Brouwer, L., Cockburn, A., Cote, S.D., Dobson, F.S., Evans, S.R., Festa-Bianchet, M., Gamelon, M., Hamel, S., Hegelbach, J., Jerstad, K., Kempenaers, B., Kruuk, L.E.B., Kumpula, J., Kvalnes, T., McAdam, A.G., McFarlane, S.E., Morrissey, M.B., Part, T., Pemberton, J.M., Qvarnstrom, A., Rostad, O.W., Schroeder, J., Senar, J.C., Sheldon, B.C., van de Pol, M., Visser, M.E., Wheelwright, N.T., Tufto, J. & Chevin, L.-M. (2020) Fluctuating optimum and temporally variable selection on breeding date in birds and mammals. Proceedings of the National Academy of Sciences of the United States of America, 117, 31969-31978.
- de Villemereuil, P., Schielzeth, H., Nakagawa, S. & Morrissey, M. (2016) General Methods for Evolutionary Quantitative Genetic Inference from Generalized Mixed Models. Genetics, 204, 1281-+.
- Diamond, S.E. & Martin, R.A. (2021) Evolution in Cities. Annual Review of Ecology, Evolution, and Systematics, Vol 52, 2021 (ed. D.J. Futuyma), pp. 519-540. Annual Reviews, Palo Alto.
- Dingemanse, N.J., Araya-Ajoy, Y.G. & Westneat, D.F. (2021) Most published selection gradients are underestimated: Why this is and how to fix it. Evolution, 75, 806-818.
- Donihue, C.M. & Lambert, M.R. (2015) Adaptive evolution in urban ecosystems. Ambio, 44, 194-203.
- Dowling, J.L., Luther, D.A. & Marra, P.P. (2012) Comparative effects of urban development and anthropogenic noise on bird songs. Behavioral Ecology, 23, 201-209.
- Driscoll, C.A., Macdonald, D.W. & O'Brien, S.J. (2009) From wild animals to domestic pets, an evolutionary view of domestication. Proceedings of the National Academy of Sciences of the United States of America, 106, 9971-9978.

- Eanes, W.F. (1999) Analysis of selection on enzyme polymorphisms. Annual Review of Ecology and Systematics, 30, 301-326.
- Eisenhauer, J.G. (2021) Meta-analysis and mega-analysis: A simple introduction. Teaching Statistics, 43, 21-27.
- Engen, S., Sæther, B.E., Kvalnes, T. & Jensen, H. (2012) Estimating fluctuating selection in age-structured populations. Journal of Evolutionary Biology, 25, 1487-1499.
- Foll, M., Shim, H. & Jensen, J.D. (2015) WFABC: a Wright–Fisher ABC-based approach for inferring effective population sizes and selection coefficients from time-sampled data. Molecular Ecology Resources, 15, 87-98.
- Franklin, O.D. & Morrissey, M.B. (2017) Inference of selection gradients using performance measures as fitness proxies. Methods in Ecology and Evolution, 8, 663-677.
- Fugere, V. & Hendry, A.P. (2018) Human influences on the strength of phenotypic selection. Proceedings of the National Academy of Sciences of the United States of America, 115, 10070-10075.
- Gamelon, M., Tufto, J., Nilsson, A.L.K., Jerstad, K., Rostad, O.W., Stenseth, N.C. & Sæther, B.E. (2018) Environmental drivers of varying selective optima in a small passerine: A multivariate, multiepisodic approach. Evolution, 72, 2325-2342.
- Gorton, A.J., Moeller, D.A. & Tiffin, P. (2018) Little plant, big city: a test of adaptation to urban environments in common ragweed (Ambrosia artemisiifolia). Proc Biol Sci, 285.
- Grafen, A. (1988) On the uses of data on lifetime reproductive success. Reproductive success (ed. T. Clutton-Brock), pp. 454-471. University of Chicago Press, Chicago.
- Gregoire, A., Preault, M., Cezilly, F., Wood, M.J., Pradel, R. & Faivre, B. (2004) Stabilizing natural selection on the early expression of a secondary sexual trait in a passerine bird. Journal of Evolutionary Biology, 17, 1152-1156.
- Gregory, T.R. (2009) Artificial Selection and Domestication: Modern Lessons from Darwin's Enduring Analogy. Evolution: Education and Outreach, 2, 5-27.
- Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J.G., Bai, X.M. & Briggs, J.M. (2008) Global change and the ecology of cities. Science, 319, 756-760.
- Hadfield, J.D. (2008) Estimating evolutionary parameters when viability selection is operating. Proceedings of the Royal Society B-Biological Sciences, 275, 723-734.
- Hadfield, J.D. & Thomson, C.E. (2017) Interpreting selection when individuals interact. Methods in Ecology and Evolution, 8, 688-699.
- Harris, S.E. & Munshi-South, J. (2017) Signatures of positive selection and local adaption to urbanization in white-footed mice (Peromyscus leucopus). Molecular Ecology, 26, 6336-6350.
- Hendry, A.P., Farrugia, T.J. & Kinnison, M.T. (2008) Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology, 17, 20-29.

- Hendry, A.P., Gotanda, K.M. & Svensson, E.I. (2017) Human influences on evolution, and the ecological and societal consequences. Philosophical Transactions of the Royal Society B-Biological Sciences, 372, 20160028.
- Henshaw, J.M., Morrissey, M.B. & Jones, A.G. (2020) Quantifying the causal pathways contributing to natural selection. Evolution, 74, 2560-2574.
- Henshaw, J.M. & Zemel, Y. (2017) A unified measure of linear and nonlinear selection on quantitative traits. Methods in Ecology and Evolution, 8, 604-614.
- Hereford, J., Hansen, T.F. & Houle, D. (2004) Comparing strengths of directional selection: How strong is strong? Evolution, 58, 2133-2143.
- Hersch, E.I. & Phillips, P.C. (2004) Power and potential bias in field studies of natural selection. Evolution, 58, 479-485.
- Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hoang, A., Hill, C.E., Beerli, P. & Kingsolver, J.G. (2001) Strength and tempo of directional selection in the wild. Proceedings of the National Academy of Sciences of the United States of America, 98, 9157-9160.
- Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A. & Cresko, W.A. (2010) Population Genomics of Parallel Adaptation in Threespine Stickleback using Sequenced RAD Tags. Plos Genetics, 6.
- Houle, C., Pelletier, F., Bélisle, M. & Garant, D. (2020) Impacts of environmental heterogeneity on natural selection in a wild bird population*. Evolution, 74, 1142-1154.
- Irwin, R.E., Warren, P.S. & Adler, L.S. (2018) Phenotypic selection on floral traits in an urban landscape. Proc Biol Sci, 285.
- Janzen, F.J. & Stern, H.S. (1998) Logistic regression for empirical studies of multivariate selection. Evolution, 52, 1564-1571.
- Jarvi, T. & Bakken, M. (1984) The function of the variation in the breast stripe of the Great Tit (Parus major). Animal Behaviour, 32, 590-596.
- Johnson, M.T.J. & Munshi-South, J. (2017) Evolution of life in urban environments. Science, 358, eaam8327.
- Johnson, M.T.J., Prashad, C.M., Lavoignat, M. & Saini, H.S. (2018) Contrasting the effects of natural selection, genetic drift and gene flow on urban evolution in white clover (Trifolium repens). Proceedings of the Royal Society B-Biological Sciences, 285.
- Kawecki, T.J. & Ebert, D. (2004) Conceptual issues in local adaptation. Ecology Letters, 7, 1225-1241.
- Kingsolver, J.G., Diamond, S.E., Siepielski, A.M. & Carlson, S.M. (2012) Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. Evolutionary Ecology, 26, 1101-1118.
- Kopp, M. & Matuszewski, S. (2014) Rapid evolution of quantitative traits: theoretical perspectives. Evolutionary Applications, 7, 169-191.

- Kruuk, L.E.B., Merila, J. & Sheldon, B.C. (2003) When environmental variation short-circuits natural selection. Trends in Ecology & Evolution, 18, 207-209.
- Lambert, M.R., Brans, K.I., Des Roches, S., Donihue, C.M. & Diamond, S.E. (2021) Adaptive Evolution in Citites: Progress and Misconceptions. Trends in Ecology & Evolution, 36, 239-257.
- Lambrecht, S.C., Mahieu, S. & Cheptou, P.O. (2016) Natural selection on plant physiological traits in an urban environment. Acta Oecologica-International Journal of Ecology, 77, 67-74.
- Lande, R. (1982) A quantitative genetic theory of life-history evolution. Ecology, 63, 607-615.
- Lande, R. & Arnold, S.J. (1983) The measurement of selection on correlated characters. Evolution, 37, 1210-1226.
- Linnen, C.R. & Hoekstra, H.E. (2009) Measuring natural selection on genotypes and phenotypes in the wild. Cold Spring Harbor Symposia on Quantitative Biology, 74, 155-168.
- MacColl, A.D.C. (2011) The ecological causes of evolution. Trends in Ecology & Evolution, 26, 514-522.
- Macintyre, H.L., Heaviside, C., Cai, X.M. & Phalkey, R. (2021) The winter urban heat island: Impacts on cold-related mortality in a highly urbanized European region for present and future climate. Environment International, 154, 9.
- Manel, S., Perrier, C., Pratlong, M., Abi-Rached, L., Paganini, J., Pontarotti, P. & Aurelle, D. (2016) Genomic resources and their influence on the detection of the signal of positive selection in genome scans. Molecular Ecology, 25, 170-184.
- Marrot, P., Garant, D. & Charmantier, A. (2015) Spatial autocorrelation in fitness affects the estimation of natural selection in the wild. Methods in Ecology and Evolution, 6, 1474-1483.
- Mitchell-Olds, T. & Shaw, R.G. (1987) Regression analysis of natural selection: statistical inference and biological interpretation. Evolution, 41, 1149-1161.
- Mittell, E.A. & Morrissey, M.B. (2023) The missing fraction problem as an episodes of selection problem. bioRxiv, 2023.2004.2027.538558.
- Morrissey, M.B. (2014a) In search of the best methods for multivariate selection analysis. Methods in Ecology and Evolution, 5, 1095-1109.
- Morrissey, M.B. (2014b) Selection and evolution of causally covarying traits. Evolution, 68, 1748-1761.
- Morrissey, M.B. (2016) Meta-analysis of magnitudes, differences and variation in evolutionary parameters. Journal of Evolutionary Biology, 29, 1882-1904.
- Morrissey, M.B. & Goudie, I.B.J. (2022) Analytical results for directional and quadratic selection gradients for log-linear models of fitness functions. Evolution, 76, 1378-1390.
- Morrissey, M.B. & Hadfield, J.D. (2012) Directional Selection in Temporally Replicated Studies Is Remarkably Consistent. Evolution, 66, 435-442.
- Morrissey, M.B. & Henshaw, J.M. (2022) Phenotypic selection analysis and confounding environmental variables. bioRxiv, 2022.2006.2015.496257.

- Morrissey, M.B., Kruuk, L.E.B. & Wilson, A.J. (2010) The danger of applying the breeder's equation in observational studies of natural populations. Journal of Evolutionary Biology, 23, 2277-2288.
- Morrissey, M.B. & Ruxton, G.D. (2018) Multiple Regression Is Not Multiple Regressions: The Meaning of Multiple Regression and the Non-Problem of Collinearity. Philosophy, Theory, and Practice in Biology, 10.
- Morrissey, M.B. & Sakrejda, K. (2013) Unification of regression-based methods for the analysis of natural selection. Evolution, 67, 2094-2100.
- Nicolaus, M., Piault, R., Ubels, R., Tinbergen, J.M. & Dingemanse, N.J. (2016) The correlation between coloration and exploration behaviour varies across hierarchical levels in a wild passerine bird. Journal of Evolutionary Biology, 29, 1780-1792.
- Nielsen, R. (2005) Molecular signatures of natural selection. Annual Review of Genetics, 39, 197-218.
- Otto, S.P. (2018) Adaptation, speciation and extinction in the Anthropocene. Proceedings of the Royal Society B-Biological Sciences, 285, 9.
- Palacio, F.X. & Ordano, M. (2023) Urbanization shapes phenotypic selection of fruit traits in a seeddispersal mutualism. Evolution, 77, 1769-1779.
- Palumbi, S.R. (2001) Evolution-Humans as the world's greatest evolutionary force. Science, 293, 1786-1790.
- Pelletier, F., Clutton-Brock, T., Pemberton, J., Tuljapurkar, S. & Coulson, T. (2007) The evolutionary demography of ecological change: Linking trait variation and population growth. Science, 315, 1571-1574.
- Pelletier, F. & Coltman, D.W. (2018) Will human influences on evolutionary dynamics in the wild pervade the Anthropocene? Bmc Biology, 16, 10.
- Pick, J.L., Lemon, H.E., Thomson, C.E. & Hadfield, J.D. (2022) Decomposing phenotypic skew and its effects on the predicted response to strong selection. Nature Ecology & Evolution, 6, 774-+.
- Pinsky, M.L., Eikeset, A.M., Helmerson, C., Bradbury, I.R., Bentzen, P., Morris, C., Gondek-Wyrozemska, A.T., Baalsrud, H.T., Brieuc, M.S.O., Kjesbu, O.S., Godiksen, J.A., Barth, J.M.I., Matschiner, M., Stenseth, N.C., Jakobsen, K.S., Jentoft, S. & Star, B. (2021) Genomic stability through time despite decades of exploitation in cod on both sides of the Atlantic. Proceedings of the National Academy of Sciences of the United States of America, 118, 6.
- Price, G.R. (1970) Selection and covariance. Nature, 227, 520-521.
- Price, T.D., Yeh, P.J. & Harr, B. (2008) Phenotypic plasticity and the evolution of a socially selected trait following colonization of a novel environment. American Naturalist, 172, S49-S62.
- Rausher, M.D. (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. Evolution, 46, 616-626.
- Reed, T.E., Visser, M.E. & Waples, R.S. (2023) The opportunity for selection: A slippery concept in ecology and evolution. Journal of Animal Ecology, 92, 7-15.

- Riyahi, S., Björklund, M., Mateos-Gonzalez, F. & Senar, J.C. (2017) Personality and urbanization: behavioural traits and <i>DRD4</i> SNP830 polymorphisms in great tits in Barcelona city. Journal of Ethology, 35, 101-108.
- Rodewald, A.D. & Arcese, P. (2017) Reproductive Contributions of Cardinals Are Consistent with a Hypothesis of Relaxed Selection in Urban Landscapes. Frontiers in Ecology and Evolution, 5.
- Rodewald, A.D., Shustack, D.P. & Jones, T.M. (2011) Dynamic selective environments and evolutionary traps in human-dominated landscapes. Ecology, 92, 1781-1788.
- Ryder, T.B., Fleischer, R.C., Shriver, W.G. & Marra, P.P. (2012) The ecological–evolutionary interplay: density-dependent sexual selection in a migratory songbird. Ecology and Evolution, 2, 976-987.
- Salmón, P., López-Idiáquez, D., Capilla-Lasheras, P., Pérez-Tris, J., Isaksson, C. & Watson, H. (2023) Urbanisation impacts plumage colouration in a songbird across Europe: Evidence from a correlational, experimental and meta-analytical approach. Journal of Animal Ecology, 92, 1924-1936.
- Santangelo, J.S., Ness, R.W., Cohan, B., Fitzpatrick, C.R., Innes, S.G., Koch, S., Miles, L.S., Munim, S., Peres-Neto, P.R., Prashad, C., Tong, A.T., Aguirre, W.E., Akinwole, P.O., Alberti, M., Alvarez, J., Anderson, J.T., Anderson, J.J., Ando, Y., Andrew, N.R., Angeoletto, F., Anstett, D.N., Anstett, J., Aoki-Gonçalves, F., Arietta, A.Z.A., Arroyo, M.T.K., Austen, E.J., Baena-Díaz, F., Barker, C.A., Baylis, H.A., Beliz, J.M., Benitez-Mora, A., Bickford, D., Biedebach, G., Blackburn, G.S., Boehm, M.M.A., Bonser, S.P., Bonte, D., Bragger, J.R., Branquinho, C., Brans, K., Bresciano, J.C., Brom, P.D., Bucharova, A., Burt, B., Cahill, J.F., Campbell, K.D., Carlen, E.J., Carmona, D., Castellanos, M.C., Centenaro, G., Chalen, I., Chaves, J.A., Chávez-Pesqueira, M., Chen, X.Y., Chilton, A.M., Chomiak, K.M., Cisneros-Heredia, D.F., Cisse, I.K., Classen, A.T., Comerford, M.S., Fradinger, C.C., Corney, H., Crawford, A.J., Crawford, K.M., Dahirel, M., David, S., De Haan, R., Deacon, N.J., Dean, C., Del-Val, E., Deligiannis, E.K., Denney, D., Dettlaff, M.A., DiLeo, M.F., Ding, Y.Y., Domínguez-López, M.E., Dominoni, D.M., Draud, S.L., Dyson, K., Ellers, J., Espinosa, C., Essi, L., Falahati-Anbaran, M., Falcao, J.C.F., Fargo, H.T., Fellowes, M.D.E., Fitzpatrick, R.M., Flaherty, L.E., Flood, P.J., Flores, M.F., Fornoni, J., Foster, A.G., Frost, C.J., Fuentes, T.L., Fulkerson, J.R., Gagnon, E., Garbsch, F., Garroway, C.J., Gerstein, A.C., Giasson, M.M., Girdler, E.B., Gkelis, S., Godsoe, W., Golemiec, A.M., Golemiec, M., González-Lagos, C., Gorton, A.J., Gotanda, K.M., Granath, G., Greiner, S., Griffiths, J.S., Grilo, F., Gundel, P.E., Hamilton, B., Hardin, J.M., He, T., Heard, S.B., Henriques, A.F., Hernández-Poveda, M., Hetherington-Rauth, M.C., Hill, S.J., Hochuli, D.F., Hodgins, K.A., Hood, G.R., Hopkins, G.R., Hovanes, K.A., Howard, A.R., Hubbard, S.C., Ibarra-Cerdeña, C.N., Iñiguez-Armijos, C., Jara-Arancio, P., Jarrett, B.J.M., Jeannot, M., Jiménez-Lobato, V., Johnson, M., Johnson, O., Johnson, P.P., Johnson, R., Josephson, M.P., Jung, M.C., Just, M.G., Kahilainen, A., Kailing, O.S., Kariñho-Betancourt, E., Karousou, R., Kirn, L.A., Kirschbaum, A., Laine, A.L., LaMontagne, J.M., Lampei, C., Lara, C., Larson, E.L., Lázaro-Lobo, A., Le, J.H., Leandro, D.S., Lee, C., Lei, Y.T., León, C.A., Tamara, M.E.L., Levesque, D.C., Liao, W.J., Ljubotina, M., Locke, H., Lockett, M.T., Longo, T.C., Lundholm, J.T., MacGillavry, T., Mackin, C.R., Mahmoud, A.R., Manju, I.A., Mariën, J., Martínez, D.N., Martínez-Bartolomé, M., Meineke, E.K., Mendoza-Arroyo, W., Merritt, T.J.S., Merritt, L.E.L., Migiani, G., Minor, E.S., Mitchell, N., Bazargani, M.M., Moles, A.T., Monk, J.D., Moore, C.M., Morales-Morales, P.A., Moyers, B.T., Muñoz-Rojas, M., Munshi-South, J., Murphy, S.M., Murúa, M.M., Neila, M., Nikolaidis, O., Njunji, I., Nosko, P., Núñez-Farfán, J., Ohgushi, T., Olsen, K.M., Opedal, O.H., Ornelas, C., Parachnowitsch, A.L., Paratore, A.S., Parody-Merino, A.M., Paule, J., Paulo, O.S., Pena, J.C., Pfeiffer, V.W., Pinho, P., Piot, A., Porth, I.M., Poulos, N., Puentes, A., Qu, J., Quintero-Vallejo, E., Raciti, S.M., Raeymaekers, J.A.M., Raveala, K.M., Rennison, D.J., Ribeiro,

M.C., Richardson, J.L., Rivas-Torres, G., Rivera, B.J., Roddy, A.B., Rodriguez-Muñoz, E., Román, J.R., Rossi, L.S., Rowntree, J.K., Ryan, T.J., Salinas, S., Sanders, N.J., Santiago-Rosario, L.Y., Savage, A.M., Scheepens, J.F., Schilthuizen, M., Schneider, A.C., Scholier, T., Scott, J.L., Shaheed, S.A., Shefferson, R.P., Shepard, C.A., Shykoff, J.A., Silveira, G., Smith, A.D., Solis-Gabriel, L., Soro, A., Spellman, K., Whitney, K.S., Starke-Ottich, I., Stephan, J.G., Stephens, J.D., Szulc, J., Szulkin, M., Tack, A.J.M., Tamburrino, I., Tate, T.D., Tergemina, E., Theodorou, P., Thompson, K.A., Threlfall, C.G., Tinghitella, R.M., Toledo-Chelala, L., Tong, X., Uroy, L., Utsumi, S., Vandegehuchte, M.L., VanWallendael, A., Vidal, P.M., Wadgymar, S.M., Wang, A.Y., Wang, N., Warbrick, M.L., Whitney, K.D., Wiesmeier, M., Wiles, J.T., Wu, J.Q., Xirocostas, Z.A., Yan, Z.G., Yao, J.H., Yoder, J.B., Yoshida, O., Zhang, J.X., Zhao, Z.G., Ziter, C.D., Zuellig, M.P., Zufall, R.A., Zurita, J.E., Zytynska, S.E. & Johnson, M.T.J. (2022) Global urban environmental change drives adaptation in white clover. Science, 375, 1275-1281.

- Scheiner, S.M., Mitchell, R.J. & Callahan, H.S. (2000) Using path analysis to measure natural selection. Journal of Evolutionary Biology, 13, 423-433.
- Schluter, D. (1988) Estimating the form of natural selection on a quantitative trait. Evolution, 42, 849-861.
- Senar, J.C., Conroy, M.J., Quesada, J. & Mateos-Gonzalez, F. (2014) Selection based on the size of the black tie of the great tit may be reversed in urban habitats. Ecology and Evolution, 4, 2625-2632.
- Seress, G., Sándor, K., Evans, K.L. & Liker, A. (2020) Food availability limits avian reproduction in the city: An experimental study on great tits <i>Parus major</i>. Journal of Animal Ecology, 89, 1570-1580.
- Shaw, R.G. & Geyer, C.J. (2010) Inferring fitness landscapes. Evolution, 64, 2510-2520.
- Siepielski, A.M., DiBattista, J.D. & Carlson, S.M. (2009) It's about time: the temporal dynamics of phenotypic selection in the wild. Ecology Letters, 12, 1261-1276.
- Slabbekoorn, H. & den Boer-Visser, A. (2006) Cities change the songs of birds. Current Biology, 16, 2326-2331.
- Sol, D., Jovani, R. & Torres, J. (2003) Parasite mediated mortality and host immune response explain agerelated differences in blood parasitism in birds. Oecologia, 135, 542-547.
- Spear, M.M., Levi, S.J., Etterson, J.R. & Gross, B.L. (2023) Resurrecting urban sunflowers: Phenotypic and molecular changes between antecedent and modern populations separated by 36 years. Molecular Ecology, 32, 5241-5259.
- Stern, A.J., Wilton, P.R. & Nielsen, R. (2019) An approximate full-likelihood method for inferring selection and allele frequency trajectories from DNA sequence data. Plos Genetics, 15, 32.
- Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. (2008) Estimating nonlinear selection gradients using quadratic regression coefficients: Double or nothing ? Evolution, 62, 2435-2440.
- Stinchcombe, J.R., Kelley, J.L. & Conner, J.K. (2017) How to measure natural selection. Methods in Ecology and Evolution, 8, 660-662.
- Stinchcombe, J.R., Simonsen, A.K. & Blows, M.W. (2014) Estimating uncertainty in multivariate responses to selection. Evolution, 68, 1188-1196.

- Svensson, E.I. (2023) Phenotypic selection in natural populations: what have we learned in 40 years? Evolution, 77, 1493-1504.
- Szulkin, M., Munshi-South, J. & Charmantier, A. (2020) Urban Evolutionary Biology. Oxford University Press, Oxford.
- Theodorou, P., Radzevičiūtė, R., Kahnt, B., Soro, A., Grosse, I. & Paxton, R.J. (2018) Genome-wide single nucleotide polymorphism scan suggests adaptation to urbanization in an important pollinator, the red-tailed bumblebee (<i>Bombus lapidarius</i> L.). Proceedings of the Royal Society B: Biological Sciences, 285, 20172806.
- Thompson, K.A., Rieseberg, L.H. & Schluter, D. (2018) Speciation and the City. Trends in Ecology & Evolution, 33, 815-826.
- Thompson, R. (2008) Estimation of quantitative genetic parameters. Proceedings of the Royal Society B-Biological Sciences, 275, 679-686.
- Thurman, T.J. & Barrett, R.D.H. (2016) The genetic consequences of selection in natural populations. Molecular Ecology, 25, 1429-1448.
- Tüzün, N., Op de Beeck, L., Brans, K.I., Janssens, L. & Stoks, R. (2017) Microgeographic differentiation in thermal performance curves between rural and urban populations of an aquatic insect. Evolutionary Applications, 10, 1067-1075.
- United Nations, D.o.E.a.S.A., Population Division (2019) World Urbanization Prospects: The 2018 Revision (ST/ESA/SER.A/420). New York.
- Verrelli, B.C., Alberti, M., Des Roches, S., Harris, N.C., Hendry, A.P., Johnson, M.T.J., Savage, A.M., Charmantier, A., Gotanda, K.M., Govaert, L., Miles, L.S., Rivkin, L.R., Winchell, K.M., Brans, K.I., Correa, C., Diamond, S.E., Fitzhugh, B., Grimm, N.B., Hughes, S., Marzluff, J.M., Munshi-South, J., Rojas, C., Santangelo, J.S., Schell, C.J., Schweitzer, J.A., Szulkin, M., Urban, M.C., Zhou, Y.Y. & Ziter, C. (2022) A global horizon scan for urban evolutionary ecology. Trends in Ecology & Evolution, 37, 1006-1019.
- Vitalis, R., Gautier, M., Dawson, K.J. & Beaumont, M.A. (2014) Detecting and Measuring Selection from Gene Frequency Data. Genetics, 196, 799-817.
- Wade, M.J. & Kalisz, S. (1990) The causes of natural selection. Evolution, 44, 1947-1955.
- Walden, N., Lucek, K. & Willi, Y. (2020) Lineage-specific adaptation to climate involves flowering time in North American <i>Arabidopsis lyrata</i>. Molecular Ecology, 29, 1436-1451.
- Walsh, B. & Lynch, M. (2018) Evolution and Selection of Quantitative Traits. Oxford University Press.
- Waters, C.N., Zalasiewicz, J., Summerhayes, C., Barnosky, A.D., Poirier, C., Galuszka, A., Cearreta, A., Edgeworth, M., Ellis, E.C., Ellis, M., Jeandel, C., Leinfelder, R., McNeill, J.R., Richter, D.D., Steffen, W., Syvitski, J., Vidas, D., Wagreich, M., Williams, M., An, Z.S., Grinevald, J., Odada, E., Oreskes, N. & Wolfe, A.P. (2016) The Anthropocene is functionally and stratigraphically distinct from the Holocene. Science, 351, 137-+.

- Whitehead, A., Pilcher, W., Champlin, D. & Nacci, D. (2012) Common mechanism underlies repeated evolution of extreme pollution tolerance. Proceedings of the Royal Society B-Biological Sciences, 279, 427-433.
- Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.-W., da Silva Santos, L.B., Bourne, P.E., Bouwman, J., Brookes, A.J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C.T., Finkers, R., Gonzalez-Beltran, A., Gray, A.J.G., Groth, P., Goble, C., Grethe, J.S., Heringa, J., 't Hoen, P.A.C., Hooft, R., Kuhn, T., Kok, R., Kok, J., Lusher, S.J., Martone, M.E., Mons, A., Packer, A.L., Persson, B., Rocca-Serra, P., Roos, M., van Schaik, R., Sansone, S.-A., Schultes, E., Sengstag, T., Slater, T., Strawn, G., Swertz, M.A., Thompson, M., van der Lei, J., van Mulligen, E., Velterop, J., Waagmeester, A., Wittenburg, P., Wolstencroft, K., Zhao, J. & Mons, B. (2016) The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data, 3, 160018.
- Winchell, K.M., Aviles-Rodriguez, K.J., Carlen, E.J., Miles, L.S., Charmantier, A., De Leon, L.F., Gotanda, K.M., Rivkin, L.R., Szulkin, M. & Verrelli, B.C. (2022) Moving past the challenges and misconceptions in urban adaptation research. Ecology and Evolution, 12, 13.
- Wood, W.E. & Yezerinac, S.M. (2006) Song sparrow (Melospiza melodia) song varies with urban noise. Auk, 123, 650-659.
- Wood, Z.T., Palkovacs, E.P., Olsen, B.J. & Kinnison, M.T. (2021) The Importance of Eco-evolutionary Potential in the Anthropocene. Bioscience, 71, 805-819.
- Yang, J.C. & Bou-Zeid, E. (2018) Should Cities Embrace Their Heat Islands as Shields from Extreme Cold? Journal of Applied Meteorology and Climatology, 57, 1309-1320.
- Yeaman, S. (2015) Local Adaptation by Alleles of Small Effect. American Naturalist, 186, S74-S89.
- Yeaman, S. (2022) Evolution of polygenic traits under global vs local adaptation. Genetics, 220.
- Yeh, P.J. & Price, T.D. (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. American Naturalist, 164, 531-542.

BIBLIOGRAPHY / BIBLIOGRAPHIE

- Ágh, N., Pipoly, I., Szabó, K., Vincze, E., Bókony, V., Seress, G., & Liker, A. (2020). Does offspring sex ratio differ between urban and forest populations of great tits (*Parus major*)? *Biologia Futura*, 71(1–2), 99–108. https://doi.org/10.1007/s42977-020-00024-6
- Alberti, M. (2008). Advances in urban ecology: Integrating humans and ecological processes in urban ecosystems. Springer New York, NY.
- Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences of the United States of America*, 114(34), 8951–8956. https://doi.org/10.1073/pnas.1606034114
- Alberti, M., Marzluff, J., & Hunt, V. M. (2017). Urban driven phenotypic changes: Empirical observations and theoretical implications for eco-evolutionary feedback. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1712), 20160029. https://doi.org/10.1098/rstb.2016.0029
- Alberti, M., Palkovacs, E. P., Roches, S. D., Meester, L. D., Brans, K. I., Govaert, L., Grimm, N. B., Harris, N. C., Hendry, A. P., Schell, C. J., Szulkin, M., Munshi-South, J., Urban, M. C., & Verrelli, B. C. (2020). The complexity of urban eco-evolutionary dynamics. *BioScience*, 70(9), 772–793. https://doi.org/10.1093/biosci/biaa079
- Altermatt, F., & Ebert, D. (2016). Reduced flight-to-light behaviour of moth populations exposed to longterm urban light pollution. *Biology Letters*, 12(4), 20160111. https://doi.org/10.1098/rsbl.2016.0111
- Ancel, L. W. (2000). Undermining the Baldwin expediting effect: Does phenotypic plasticity accelerate evolution? *Theoretical Population Biology*, 58(4), 307–319. https://doi.org/10.1006/tpbi.2000.1484
- Antonio-Nkondjio, C., Fossog, B. T., Ndo, C., Djantio, B. M., Togouet, S. Z., Awono-Ambene, P., Costantini, C., Wondji, C. S., & Ranson, H. (2011). *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): Influence of urban agriculture and pollution. *Malaria Journal*, 10(1), 1–13. https://doi.org/10.1186/1475-2875-10-154
- Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W., & Ketterson, E. D. (2012). Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, 23(5), 960–969. https://doi.org/10.1093/beheco/ars059
- Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Price, T. D., & Ketterson, E. D. (2014). Hormonal, behavioral, and life-history traits exhibit correlated shifts in relation to population establishment in a novel environment. *The American Naturalist*, 184(6), E147–E160. https://doi.org/10.1086/678398
- Bailey, L. D., van de Pol, M., Adriaensen, F., Arct, A., Barba, E., Bellamy, P. E., Bonamour, S., Bouvier, J.-C., Burgess, M. D., & Charmantier, A. (2022). Bird populations most exposed to climate

change are less sensitive to climatic variation. *Nature Communications*, *13*(1), 2112. https://doi.org/10.1038/s41467-022-29635-4

- Bang, C., Sabo, J. L., & Faeth, S. H. (2010). Reduced wind speed improves plant growth in a desert city. *PLoS ONE*, *5*(6), e11061. https://doi.org/10.1371/journal.pone.0011061
- Barabás, G., & D'Andrea, R. (2016). The effect of intraspecific variation and heritability on community pattern and robustness. *Ecology Letters*, 19(8), 977–986. https://doi.org/10.1111/ele.12636
- Baxter-Gilbert, J., Riley, J. L., & Whiting, M. J. (2019). Bold new world: Urbanization promotes an innate behavioral trait in a lizard. *Behavioral Ecology and Sociobiology*, 73(8), 1–10. https://doi.org/10.1007/s00265-019-2713-9
- Biard, C., Brischoux, F., Meillère, A., Michaud, B., Nivière, M., Ruault, S., Vaugoyeau, M., & Angelier, F. (2017). Growing in cities: An urban penalty for wild birds? A study of phenotypic differences between urban and rural great tit chicks (*Parus major*). *Frontiers in Ecology and Evolution*, 5, 1– 14. https://doi.org/10.3389/fevo.2017.00079
- Biard, C., Surai, P. F., & Møller, A. P. (2006). Carotenoid availability in diet and phenotype of blue and great tit nestlings. *Journal of Experimental Biology*, 209(6), 1004–1015. https://doi.org/10.1242/jeb.02089
- Björklund, M. (1997). Variation in growth in the blue tit (*Parus caeruleus*). Journal of Evolutionary Biology, 10(2), 139–155. https://doi.org/10.1046/j.1420-9101.1997.10020139.x
- Björklund, M., Ruiz, I., & Senar, J. C. (2010). Genetic differentiation in the urban habitat: The great tits (*Parus major*) of the parks of Barcelona city. *Biological Journal of the Linnean Society*, 99(1), 9– 19. https://doi.org/10.1111/j.1095-8312.2009.01335.x
- Blackburn, G., Broom, E., Ashton, B. J., Thornton, A., & Ridley, A. R. (2022). Heat stress inhibits cognitive performance in wild Western Australian magpies, *Cracticus tibicen dorsalis*. *Animal Behaviour*, 188, 1–11. https://doi.org/10.1016/j.anbehav.2022.03.016
- Blumstein, D. T. (2016). Habituation and sensitization: New thoughts about old ideas. *Animal Behaviour*, *120*(1), 255–262. https://doi.org/10.1016/j.anbehav.2016.05.012
- Bókony, V., Balogh, E., Ujszegi, J., Ujhegyi, N., Szederkényi, M., & Hettyey, A. (2024). Tadpoles develop elevated heat tolerance in urban heat islands regardless of sex. *Evolutionary Biology*, 51(1), 209–216. https://doi.org/10.1007/s11692-024-09626-7
- Bókony, V., Kulcsár, A., Tóth, Z., & Liker, A. (2012). Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (*Passer domesticus*). *PLoS ONE*, 7(5), e36639–e36639. https://doi.org/10.1371/journal.pone.0036639
- Bókony, V., Ujhegyi, N., Hamow, K. Á., Bosch, J., Thumsová, B., Vörös, J., Aspbury, A. S., & Gabor, C.
 R. (2021). Stressed tadpoles mount more efficient glucocorticoid negative feedback in anthropogenic habitats due to phenotypic plasticity. *Science of the Total Environment*, 753, 141896. https://doi.org/10.1016/j.scitotenv.2020.141896
- Bókony, V., Üveges, B., Verebélyi, V., Ujhegyi, N., & Móricz, Á. M. (2019). Toads phenotypically adjust their chemical defences to anthropogenic habitat change. *Scientific Reports*, 9(1), 1–8. https://doi.org/10.1038/s41598-019-39587-3

- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology and Evolution*, 24(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008
- Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., Rudolf, V. H. W., Schreiber, S. J., Urban, M. C., & Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution*, 26(4), 183–192. https://doi.org/10.1016/j.tree.2011.01.009
- Bonamour, S., Chevin, L.-M., Charmantier, A., & Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: The importance of cue variation. *Philosophical Transactions of the Royal Society B*, 374(1768), 20180178. https://doi.org/10.1098/rstb.2018.0178
- Bonamour, S., Chevin, L.-M., Réale, D., Teplitsky, C., & Charmantier, A. (2020). Age-dependent phenological plasticity in a wild bird. *Journal of Animal Ecology*, 89(11), 2733–2741. https://doi.org/10.1111/1365-2656.13337
- Bonier, F. (2012). Hormones in the city: Endocrine ecology of urban birds. *Hormones and Behavior*, 61(5), 763–772. https://doi.org/10.1016/j.yhbeh.2012.03.016
- Bonnet, T., Morrissey, M. B., De Villemereuil, P., Alberts, S. C., Arcese, P., Bailey, L. D., Boutin, S., Brekke, P., Brent, L. J. N., Camenisch, G., Charmantier, A., Clutton-Brock, T. H., Cockburn, A., Coltman, D. W., Courtiol, A., Davidian, E., Evans, S. R., Ewen, J. G., Festa-Bianchet, M., ... Kruuk, L. E. B. (2022). Genetic variance in fitness indicates rapid contemporary adaptive evolution in wild animals. *Science*, *376*(6596), 1012–1016. https://doi.org/10.1126/science.abk0853
- Both, C., Van Asch, M., Bijlsma, R. G., Van Den Burg, A. B., & Visser, M. E. (2009). Climate change and unequal phenological changes across four trophic levels: Constraints or adaptations? *Journal* of Animal Ecology, 78(1), 73–83. https://doi.org/10.1111/j.1365-2656.2008.01458.x
- Bourgault, P., Thomas, D., Perret, P., & Blondel, J. (2010). Spring vegetation phenology is a robust predictor of breeding date across broad landscapes: A multi-site approach using the Corsican blue tit (*Cyanistes caeruleus*). *Oecologia*, 162, 885–892. https://doi.org/10.1007/s00442-009-1545-0
- Branch, C. L., Semenov, G. A., Wagner, D. N., Sonnenberg, B. R., Pitera, A. M., Bridge, E. S., Taylor, S. A., & Pravosudov, V. V. (2022). The genetic basis of spatial cognitive variation in a food-caching bird. *Current Biology*, 32(1), 210–219. https://doi.org/10.1016/j.cub.2021.10.036
- Brans, K. I., Almeida, R. A., & Fajgenblat, M. (2021). Genetic differentiation in pesticide resistance between urban and rural populations of a nontarget freshwater keystone interactor, *Daphnia magna. Evolutionary Applications*, 14(10), 2541–2552. https://doi.org/10.1111/eva.13293
- Brans, K. I., & De Meester, L. (2018). City life on fast lanes: Urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. *Functional Ecology*, 32(9), 2225–2240. https://doi.org/10.1111/1365-2435.13184
- Brans, K. I., Govaert, L., Engelen, J. M. T., Gianuca, A. T., Souffreau, C., & De Meester, L. (2017). Ecoevolutionary dynamics in urbanized landscapes: Evolution, species sorting and the change in zooplankton body size along urbanization gradients. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1712), 20160030. https://doi.org/10.1098/rstb.2016.0030

- Brans, K. I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R., & De Meester, L. (2017). The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. *Global Change Biology*, 23(12), 5218–5227. https://doi.org/10.1111/gcb.13784
- Brans, K. I., Stoks, R., & De Meester, L. (2018). Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. *Proceedings of the Royal Society B: Biological Sciences*, 285(1883). https://doi.org/10.1098/rspb.2018.0169
- Brans, K. I., Tüzün, N., Sentis, A., De Meester, L., & Stoks, R. (2022). Cryptic eco-evolutionary feedback in the city: Urban evolution of prey dampens the effect of urban evolution of the predator. *Journal* of Animal Ecology, 91(3), 514–526. https://doi.org/10.1111/1365-2656.13601
- Branston, C. J., Capilla-Lasheras, P., Pollock, C. J., Griffiths, K., White, S., & Dominoni, D. M. (2021). Urbanisation weakens selection on the timing of breeding and clutch size in blue tits but not in great tits. *Behavioral Ecology and Sociobiology*, 75(11), 155. https://doi.org/10.1007/s00265-021-03096-z
- Breitbart, S. T., Agrawal, A. A., Wagner, H. H., & Johnson, M. T. (2023). Urbanization and a green corridor do not impact genetic divergence in common milkweed (*Asclepias syriaca L.*). Scientific Reports, 13(1), 20437. https://doi.org/10.1038/s41598-023-47524-8
- Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378–400. https://doi.org/10.3929/ethz-b-000240890
- Brown, L. R., Cuffney, T. F., Coles, J. F., Fitzpatrick, F., McMahon, G., Steuer, J., Bell, A. H., & May, J. T. (2009). Urban streams across the USA: Lessons learned from studies in 9 metropolitan areas. *Journal of the North American Benthological Society*, 28(4), 1051–1069. https://doi.org/10.1899/08-153.1
- Bukor, B., Seress, G., Pipoly, I., Sándor, K., Sinkovics, C., Vincze, E., & Liker, A. (2022). Doublebrooding and annual breeding success of great tits in urban and forest habitats. *Current Zoology*, 68(5), 517–525. https://doi.org/10.1093/cz/zoab096
- Bürger, R., & Lynch, M. (1995). Evolution and extinction in a changing environment: A quantitativegenetic analysis. *Evolution*, 49(1), 151–163. https://doi.org/10.1111/j.1558-5646.1995.tb05967.x
- Bürkner, P.-C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, 80, 1–28. https://doi.org/10.18637/jss.v080.i01
- Cadenasso, M. L., Pickett, S. T. A., & Schwarz, K. (2007). Spatial heterogeneity in urban ecosystems: Reconceptualizing land cover and a framework for classification. *Frontiers in Ecology and the Environment*, 5(2), 80–88. https://doi.org/10.1890/1540-9295(2007)5[80:SHIUER]2.0.CO;2
- Caizergues, A. E., Charmantier, A., Lambrechts, M. M., Perret, S., Demeyrier, V., Lucas, A., & Grégoire, A. (2021). An avian urban morphotype: How the city environment shapes great tit morphology at different life stages. Urban Ecosystems, 45(5), 929–941. https://doi.org/10.1007/s11252-020-01077-0

- Caizergues, A. E., Grégoire, A., & Charmantier, A. (2018). Urban versus forest ecotypes are not explained by divergent reproductive selection. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20180261. https://doi.org/10.1098/rspb.2018.0261
- Caizergues, A. E., Grégoire, A., Choquet, R., Perret, S., & Charmantier, A. (2022). Are behaviour and stress-related phenotypes in urban birds adaptive? *Journal of Animal Ecology*, *91*(8), 1627–1641. https://doi.org/10.1111/1365-2656.13740
- Caizergues, A. E., Le Luyer, J., Grégoire, A., Szulkin, M., Senar, J., Charmantier, A., & Perrier, C. (2022). Epigenetics and the city: Non-parallel DNA methylation modifications across pairs of urban-forest Great tit populations. *Evolutionary Applications*, 15(1), 149–165. https://doi.org/10.1111/eva.13334
- Camacho, C., & Hendry, A. P. (2020). Matching habitat choice: It's not for everyone. *Oikos*, 689–699. https://doi.org/10.1111/oik.06932
- Camacho, C., Sanabria-Fernández, A., Baños-Villalba, A., & Edelaar, P. (2020). Experimental evidence that matching habitat choice drives local adaptation in a wild population. *Proceedings of the Royal Society B: Biological Sciences*, 287, 20200721. https://doi.org/10.1098/rspb.2020.0721
- Campbell-Staton, S. C., Velotta, J. P., & Winchell, K. M. (2021). Selection on adaptive and maladaptive gene expression plasticity during thermal adaptation to urban heat islands. *Nature Communications*, *12*(1), 6195. https://doi.org/10.1038/s41467-021-26334-4
- Candolin, U., Tukiainen, I., & Bertell, E. (2016). Environmental change disrupts communication and sexual selection in a stickleback population. *Ecology*, 97(4), 969–979. https://doi.org/10.1890/15-1090.1
- Candolin, U., & Wong, B. B. M. (2019). Mate choice in a polluted world: Consequences for individuals, populations and communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1781), 20180055. https://doi.org/10.1098/rstb.2018.0055
- Capilla-Lasheras, P., Dominoni, D. M., Babayan, S. A., O'Shaughnessy, P. J., Mladenova, M., Woodford, L., Pollock, C. J., Barr, T., Baldini, F., & Helm, B. (2017). Elevated immune gene expression is associated with poor reproductive success of urban blue tits. *Frontiers in Ecology and Evolution*, 5, 1–13. https://doi.org/10.3389/fevo.2017.00064
- Capilla-Lasheras, P., Thompson, M. J., Sánchez-Tójar, A., Haddou, Y., Branston, C. J., Réale, D., Charmantier, A., & Dominoni, D. M. (2022). A global meta-analysis reveals higher variation in breeding phenology in urban birds than in their non-urban neighbours. *Ecology Letters*, 25(11), 2552–2570. https://doi.org/10.1111/ele.14099
- Carere, C., & van Oers, K. (2004). Shy and bold great tits (*Parus major*): Body temperature and breath rate in response to handling stress. *Physiology and Behavior*, 82(5), 905–912. https://doi.org/10.1016/j.physbeh.2004.07.009
- Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M. A., Guo, J., Li, P., & Riddell, A. (2017). Stan: A probabilistic programming language. *Journal of Statistical Software*, 76. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9788645/
- Carrete, M., Martínez-Padilla, J., Rodríguez-Martínez, S., Rebolo-Ifrán, N., Palma, A., & Tella, J. L. (2016). Heritability of fear of humans in urban and rural populations of a bird species. *Scientific Reports*, 6, 1–6. https://doi.org/10.1038/srep31060
- Carvalho, C. da S., Lucas, M. S., & Côrtes, M. C. (2021). Rescuing intraspecific variation in humanimpacted environments. *Journal of Applied Ecology*, 58(2), 350–359. https://doi.org/10.1111/1365-2664.13764
- Casasole, G., Raap, T., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., & Eens, M. (2017). Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*, 210(April 2017), 14–21. https://doi.org/10.1016/j.cbpa.2017.05.003
- Caspi, T., Johnson, J. R., Lambert, M. R., Schell, C. J., & Sih, A. (2022). Behavioral plasticity can facilitate evolution in urban environments. *Trends in Ecology & Evolution*, *37*(12), 1092–1103. https://doi.org/10.1016/j.tree.2022.08.002
- Cauchard, L., Dubois, F., Doligez, B., Boogert, N. J., & Lefebvre, L. (2012). Problem-solving performance is correlated with reproductive success in a wild bird population. *Animal Behaviour*, 85, 19–26. https://doi.org/10.1016/j.anbehav.2012.10.005
- Chapman, S., Watson, J. E. M., Salazar, A., Thatcher, M., & McAlpine, C. A. (2017). The impact of urbanization and climate change on urban temperatures: A systematic review. *Landscape Ecology*, 32(10), 1921–1935. https://doi.org/10.1007/s10980-017-0561-4
- Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S., & Grégoire, A. (2017). Urbanization is associated with divergence in pace-of-life in great tits. *Frontiers in Ecology and Evolution*, *5*, 1–13. https://doi.org/10.3389/fevo.2017.00053
- Charmantier, A., Garant, D., & Kruuk, L. E. (2014). Quantitative genetics in the wild. OUP Oxford.
- Cheptou, P.-O., Carrue, O., Rouifed, S., & Cantarel, A. (2008). Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. *Proceedings of the National Academy of Sciences*, 105(10), 3796–3799. https://doi.org/10.1073/pnas.0708446105
- Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biology*, 8(4), e1000357. https://doi.org/10.1371/journal.pbio.1000357
- Chick, L. D., Strickler, S. A., Perez, A., Martin, R. A., & Diamond, S. E. (2019). Urban heat islands advance the timing of reproduction in a social insect. *Journal of Thermal Biology*, 80, 119–125. https://doi.org/10.1016/j.jtherbio.2019.01.004
- Chick, L. D., Waters, J. S., & Diamond, S. E. (2021). Pedal to the metal: Cities power evolutionary divergence by accelerating metabolic rate and locomotor performance. *Evolutionary Applications*, 14(1), 36–52. https://doi.org/10.1111/eva.13083
- Christians, J. K. (2002). Avian egg size: Variation within species and inflexibility within individuals. *Biological Reviews*, 77(1), 1–26. https://doi.org/10.1017/S1464793101005784

- Cleasby, I. R., Nakagawa, S., & Schielzeth, H. (2015). Quantifying the predictability of behaviour: Statistical approaches for the study of between-individual variation in the within-individual variance. *Methods in Ecology and Evolution*, 6(1), 27–37. https://doi.org/10.1111/2041-210X.12281
- Clutton-Brock, T., & Sheldon, B. C. (2010). Individuals and populations: The role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution*, 25(10), 562–573. https://doi.org/10.1016/j.tree.2010.08.002
- Cole, E. F., Morand-Ferron, J., Hinks, A. E., & Quinn, J. L. (2012). Cognitive ability influences reproductive life history variation in the wild. *Current Biology*, 22(19), 1808–1812. https://doi.org/10.1016/j.cub.2012.07.051
- Cole, E. F., Regan, C. E., & Sheldon, B. C. (2021). Spatial variation in avian phenological response to climate change linked to tree health. *Nature Climate Change*, 11(10), 872–878. https://doi.org/10.1038/s41558-021-01140-4
- Collier, C. G. (2006). The impact of urban areas on weather. *Quarterly Journal of the Royal Meteorological Society*, 132(164), 1–25. https://doi.org/10.1256/qj.05.199
- Conover, D. O., Duffy, T. A., & Hice, L. A. (2009). The covariance between genetic and environmental influences across ecological gradients. *The Year in Evolutionary Biology 2009, Volume 1168, 28,* 100. https://doi.org/10.1111/j.1749-6632.2009.04575.x
- Cook, M. O., Weaver, M. J., Hutton, P., & McGraw, K. J. (2017). The effects of urbanization and human disturbance on problem solving in juvenile house finches (*Haemorhous mexicanus*). *Behavioral Ecology and Sociobiology*, 71, 1–10. https://doi.org/10.1007/s00265-017-2304-6
- Coomes, J. R., Davidson, G. L., Reichert, M. S., Kulahci, I. G., Troisi, C. A., Quinn, J. L., & Quinn, J. L. (2021). Inhibitory control, exploration behaviour and manipulated ecological context are associated with foraging flexibility in the great tit. *Journal of Animal Ecology*, 00, 1–14. https://doi.org/10.1111/1365-2656.13600
- Corsini, M., Marrot, P., & Szulkin, M. (2019). Quantifying human presence in a heterogeneous urban landscape. *Behavioral Ecology*, *30*(6), 1632–1641. https://doi.org/10.1093/beheco/arz128
- Corsini, M., Schöll, E. M., Di Lecce, I., Chatelain, M., Dubiec, A., & Szulkin, M. (2021). Growing in the city: Urban evolutionary ecology of avian growth rates. *Evolutionary Applications*, *14*(1), 69–84. https://doi.org/10.1111/eva.13081
- Croston, R., Branch, C. L., Kozlovsky, D. Y., Dukas, R., & Pravosudov, V. V. (2015). Heritability and the evolution of cognitive traits. *Behavioral Ecology*, *26*(6), 1447–1459. https://doi.org/10.1093/beheco/arv088
- Crouchet, T. (2023). Foraging plasticity variation in wild great tits along contrasted ecological habitats. *Thesis: Université de Toulouse*.
- Culina, A., Adriaensen, F., Bailey, L. D., Burgess, M. D., Charmantier, A., Cole, E. F., Eeva, T., Matthysen, E., Nater, C. R., Sheldon, B. C., Sæther, B. E., Vriend, S. J. G., Zajkova, Z., Adamík, P., Aplin, L. M., Angulo, E., Artemyev, A., Barba, E., Barišić, S., ... Visser, M. E. (2020). Connecting the data landscape of long-term ecological studies: The SPI-Birds data hub. *Journal of Animal Ecology 90*(9), 1–14. https://doi.org/10.1111/1365-2656.13388

- Culina, A., Bailey, L. D., Nater, C. R., Vriend, S. J. G., & Visser, M. E. (2020). *Standard protocol for the collection of individual level data*. SPI-Birds; version 1.1. https://github.com/SPI-Birds/documentation/blob/master/standard_protocol/SPI_Birds_Protocol_v1.1.0.pdf
- Czaczkes, T. J., Bastidas-Urrutia, A. M., Ghislandi, P., & Tuni, C. (2018). Reduced light avoidance in spiders from populations in light-polluted urban environments. *The Science of Nature*, *105*(11), 1–5. https://doi.org/10.1007/s00114-018-1589-2
- Davidson, G. L., Reichert, M. S., Coomes, J. R., Kulahci, I. G., de la Hera, I., & Quinn, J. L. (2022). Inhibitory control performance is repeatable over time and across contexts in a wild bird population. *Animal Behaviour*, 187, 305–318. https://doi.org/10.1016/j.anbehav.2022.02.007
- De León, L. F., Sharpe, D. M. T., Gotanda, K. M., Raeymaekers, J. A. M., Chaves, J. A., Hendry, A. P., & Podos, J. (2019). Urbanization erodes niche segregation in Darwin's finches. *Evolutionary Applications*, 12(7), 1329–1343. https://doi.org/10.1111/eva.12721
- De Meester, G., Pafilis, P., Vasilakis, G., & Van Damme, R. (2022). Exploration and spatial cognition show long-term repeatability but no heritability in the Aegean wall lizard. *Animal Behaviour*, *190*, 167–185. https://doi.org/10.1016/j.anbehav.2022.06.007
- De Meester, L., Brans, K. I., Govaert, L., Souffreau, C., Mukherjee, S., Vanvelk, H., Korzeniowski, K., Kilsdonk, L., Decaestecker, E., Stoks, R., & Urban, M. C. (2019). Analysing eco-evolutionary dynamics—The challenging complexity of the real world. *Functional Ecology*, 33(1), 43–59. https://doi.org/10.1111/1365-2435.13261
- de Villemereuil, P. (2018). Quantitative genetic methods depending on the nature of the phenotypic trait. Annals of the New York Academy of Sciences, 1422(1), 29–47. https://doi.org/10.1111/nyas.13571
- de Villemereuil, P., Charmantier, A., Arlt, D., Bize, P., Brekke, P., Brouwer, L., Cockburn, A., Côté, S. D., Dobson, F. S., & Evans, S. R. (2020). Fluctuating optimum and temporally variable selection on breeding date in birds and mammals. *Proceedings of the National Academy of Sciences*, 117(50), 31969–31978. https://doi.org/10.1073/pnas.2009003117
- de Villemereuil, P., Gaggiotti, O. E., & Goudet, J. (2020). Common garden experiments to study local adaptation need to account for population structure. *Journal of Ecology*, *110*(5), 1005-1009. https://doi.org/10.1111/1365-2745.13528
- de Villemereuil, P., Morrissey, M. B., Nakagawa, S., & Schielzeth, H. (2018). Fixed-effect variance and the estimation of repeatabilities and heritabilities: Issues and solutions. *Journal of Evolutionary Biology*, *31*(4), 621–632. https://doi.org/10.1111/jeb.13232
- de Villemereuil, P., Mouterde, M., Gaggiotti, O. E., & Till-Bottraud, I. (2018). Patterns of phenotypic plasticity and local adaptation in the wide elevation range of the alpine plant *Arabis alpina*. *Journal of Ecology*, *106*(5), 1952–1971. https://doi.org/10.1111/1365-2745.12955
- de Villemereuil, P., Schielzeth, H., Nakagawa, S., & Morrissey, M. (2016). General methods for evolutionary quantitative genetic inference from generalized mixed models. *Genetics*, 204(3), 1281–1294. https://doi.org/10.1534/genetics.115.186536
- DeAngelis, D. L., & Grimm, V. (2014). Individual-based models in ecology after four decades. *F1000prime Reports*, 6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4047944/

- del Hoyo, J., Elliot, A., & Christie, D. A. (2007). *Handbook of the Birds of the World: Picathartes to Tits and Chickadees* (Vol. 12). Lynx Edicions.
- Demeyrier, V., Lambrechts, M. M., Perret, P., & Grégoire, A. (2016). Experimental demonstration of an ecological trap for a wild bird in a human-transformed environment. *Animal Behaviour*, 118, 181– 190. https://doi.org/10.1016/j.anbehav.2016.06.007
- Des Roches, S., Pendleton, L. H., Shapiro, B., & Palkovacs, E. P. (2021). Conserving intraspecific variation for nature's contributions to people. *Nature Ecology and Evolution*, *5*(5), 574–582. https://doi.org/10.1038/s41559-021-01403-5
- Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology and Evolution*, 2(1), 57–64. https://doi.org/10.1038/s41559-017-0402-5
- Dhondt, A. A. (1982). Heritability of blue tit tarsus length from normal and cross-fostered broods. *Evolution*, *36*(2), 418–419. https://doi.org/10.1111/j.1558-5646.1982.tb05061.x
- Diamond, S. E., Chick, L. A., Perez, A., Strickler, S. A., & Zhao, C. (2018). Evolution of plasticity in the city: Urban acorn ants can better tolerate more rapid increases in environmental temperature. *Conservation Physiology*, 6(1), 1–12. https://doi.org/10.1093/conphys/coy030
- Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., & Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: Parallel and non-parallel responses to urban heat islands across three cities. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), 20180036. https://doi.org/10.1098/rspb.2018.0036
- Diamond, S. E., Chick, L., Perez, A. B. E., Strickler, S. A., & Martin, R. A. (2017). Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biological Journal of the Linnean Society*, 121(2), 248–257. https://doi.org/10.1093/biolinnean/blw047
- Diamond, S. E., & Martin, R. A. (2021). Evolution in cities. *Annual Review of Ecology, Evolution, and Systematics*, 52, 519–540. https://doi.org/10.1146/annurev-ecolsys-012021-021402
- Diamond, S. E., Martin, R. A., Bellino, G., Crown, K. N., & Prileson, E. G. (2022). Urban evolution of thermal physiology in a range-expanding, mycophagous fruit fly, *Drosophila tripunctata*. *Biological Journal of the Linnean Society*, 137(3), 409–420. https://doi.org/10.1093/biolinnean/blac094
- Dibble, C. J., & Rudolf, V. H. W. (2019). Phenotype-environment matching predicts both positive and negative effects of intraspecific variation. *American Naturalist*, 194(1), 47–58. https://doi.org/10.1086/703483
- Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal Dispersal and Personalities in Great Tits. *Proceedings of the Royal Society B: Biological Sciences*, 270(1516), 741–747. https://doi.org/10.1098/rspb.2002.2300
- Dingemanse, N. J., & Wolf, M. (2013). Between-individual differences in behavioural plasticity within populations: Causes and consequences. *Animal Behaviour*, 85(5), 1031–1039. https://doi.org/10.1016/j.anbehav.2012.12.032

- Dirienzo, N., Johnson, J. C., & Dornhaus, A. (2019). Juvenile social experience generates differences in behavioral variation but not averages. *Behavioral Ecology*, 30(2), 455–464. https://doi.org/10.1093/beheco/ary185
- Dominoni, D. M., Kjellberg Jensen, J., De Jong, M., Visser, M. E., & Spoelstra, K. (2020). Artificial light at night, in interaction with spring temperature, modulates timing of reproduction in a passerine bird. *Ecological Applications*, 30(3), e02062. https://doi.org/10.1002/eap.2062
- Donihue, C. M., & Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio*, 44(3), 194–203. https://doi.org/10.1007/s13280-014-0547-2
- Dougherty, L. R., & Guillette, L. M. (2018). Linking personality and cognition: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373, 1–12. https://doi.org/10.1098/rstb.2017.0282
- Dubuc-Messier, G., Caro, S. P., Perrier, C., van Oers, K., Réale, D., & Charmantier, A. (2018). Gene flow does not prevent personality and morphological differentiation between two blue tit populations. *Journal of Evolutionary Biology*, 31(8), 1127–1137. https://doi.org/10.1111/jeb.13291
- Dubuc-Messier, G., Réale, D., Perret, P., & Charmantier, A. (2016). Environmental heterogeneity and population differences in blue tits personality traits. *Behavioral Ecology*, 28, arw148. https://doi.org/10.1093/beheco/arw148
- Ducatez, S., Sol, D., Sayol, F., & Lefebvre, L. (2020). Behavioural plasticity is associated with reduced extinction risk in birds. *Nature Ecology & Evolution*, 4(6), 788–793. https://doi.org/10.1038/s41559-020-1168-8
- Duckworth, R. A., & Aguillon, S. M. (2015). Eco-evolutionary dynamics: Investigating multiple causal pathways linking changes in behavior, population density and natural selection. *Journal of Ornithology*, 156, 115–124. https://doi.org/10.1007/s10336-015-1239-9
- Dunlop, E. S., Shuter, B. J., & Dieckmann, U. (2007). Demographic and Evolutionary Consequences of Selective Mortality: Predictions from an Eco-Genetic Model for Smallmouth Bass. *Transactions* of the American Fisheries Society, 136(3), 749–765. https://doi.org/10.1577/T06-126.1
- Edelaar, P., & Bolnick, D. I. (2012). Non-random gene flow: An underappreciated force in evolution and ecology. *Trends in Ecology and Evolution*, 27(12), 659–665. https://doi.org/10.1016/j.tree.2012.07.009
- Eeva, T., Belskii, E., & Kuranov, B. (2006). Environmental pollution affects genetic diversity in wild bird populations. *Mutation Research*, 608(1), 8–15. https://doi.org/10.1016/j.mrgentox.2006.04.021
- Eeva, T., & Lehikoinen, E. (1996). Growth and mortality of nestling great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. *Oecologia*, 108(4), 631– 639. https://doi.org/10.1007/BF00329036
- Eeva, T., Rainio, M., Berglund, Å., Kanerva, M., Stauffer, J., Stöwe, M., & Ruuskanen, S. (2014). Experimental manipulation of dietary lead levels in great tit nestlings: Limited effects on growth, physiology and survival. *Ecotoxicology*, 23(5), 914–928. https://doi.org/10.1007/s10646-014-1235-5

- Eggenberger, H., Frey, D., Pellissier, L., Ghazoul, J., Fontana, S., & Moretti, M. (2019). Urban bumblebees are smaller and more phenotypically diverse than their rural counterparts. *Journal of Animal Ecology*, 88(10), 1522–1533. https://doi.org/10.1111/1365-2656.13051
- Eisenhauer, J. G. (2021). Meta-analysis and mega-analysis: A simple introduction. *Teaching Statistics*, 43(1), 21–27. https://doi.org/10.1111/test.12242
- El-Sabaawi, R. (2018). Trophic structure in a rapidly urbanizing planet. *Functional Ecology*, 32(7), 1718–1728. https://doi.org/10.1111/1365-2435.13114
- Eötvös, C. B., Magura, T., & Lövei, G. L. (2018). A meta-analysis indicates reduced predation pressure with increasing urbanization. *Landscape and Urban Planning*, 180, 54–59. https://doi.org/10.1016/j.landurbplan.2018.08.010
- European Environment Agency. (2020). *Imperviousness Density 2018* (2.2) [dataset]. Copernicus Land Monitoring Service. https://land.copernicus.eu/user-corner/technical-library/imperviousness-2018user-manual.pdf
- European Environment Agency. (2021). *CORINE Land Cover* (1.0) [Spatial]. Copernicus Land Monitoring Service. https://land.copernicus.eu/user-corner/technical-library/clc-product-usermanual
- Faeth, S. H., Warren, P. S., Shochat, E., & Marussich, W. A. (2005). Trophic dynamics in urban communities. *BioScience*, 55(5), 399–407. https://doi.org/10.1641/0006-3568(2005)055[0399:TDIUC]2.0.CO;2
- Falconer, D. S. (1996). Introduction to quantitative genetics. Pearson Education India.

Falconer, D. S., & Mackay, T. F. (1983). *Quantitative genetics*. Longman London.

- Falvey, C. H., Aviles-Rodriguez, K. J., Hagey, T. J., & Winchell, K. M. (2020). The finer points of urban adaptation: Intraspecific variation in lizard claw morphology. *Biological Journal of the Linnean Society*, 131(2), 304–318. https://doi.org/10.1093/biolinnean/blaa123
- Farine, D. R., Montiglio, P. O., & Spiegel, O. (2015). From individuals to groups and back: The evolutionary implications of group phenotypic composition. *Trends in Ecology and Evolution*, 30(10), 609–621. https://doi.org/10.1016/j.tree.2015.07.005
- Fisher, J., & Hinde, R. A. (1949). The opening of milk bottles by birds. *British Birds*, 42, 347–357. https://www.cabidigitallibrary.org/doi/full/10.5555/19520402565
- Fisher, R. A. (1919). XV.—The correlation between relatives on the supposition of Mendelian inheritance. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 52(2), 399–433. https://doi.org/10.1017/S0080456800012163
- Fisogni, A., Hautekèete, N., Piquot, Y., Brun, M., Vanappelghem, C., Michez, D., & Massol, F. (2020). Urbanization drives an early spring for plants but not for pollinators. *Oikos*, 129(11), 1681–1691. https://doi.org/10.1111/oik.07274
- Forman, R. T. T. (2014). Urban ecology: Science of cities. Cambridge University Press.

- Forsman, A. (2014). Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. *Proceedings of the National Academy of Sciences of the United States of America*, 111(1), 302–307. https://doi.org/10.1073/pnas.1317745111
- Fox, J., Weisberg, S., & Bates, D. M. (2010). *car: Companion to Applied Regression*. [Computer software].
- Fudickar, A. M., Greives, T. J., Abolins-Abols, M., Atwell, J. W., Meddle, S. L., Friis, G., Stricker, C. A., & Ketterson, E. D. (2017). Mechanisms associated with an advance in the timing of seasonal reproduction in an urban songbird. *Frontiers in Ecology and Evolution*, 5, 85. https://doi.org/10.3389/fevo.2017.00085
- Fugère, V., & Hendry, A. P. (2018). Human influences on the strength of phenotypic selection. Proceedings of the National Academy of Sciences of the United States of America, 115(40), 10070–10075. https://doi.org/10.1073/pnas.1806013115
- Fukano, Y., Guo, W., Uchida, K., & Tachiki, Y. (2020). Contemporary adaptive divergence of plant competitive traits in urban and rural populations and its implication for weed management. *Journal of Ecology*, 108(6), 2521–2530. https://doi.org/10.1111/1365-2745.13472
- Gaertner, M., Wilson, J. R. U., Cadotte, M. W., MacIvor, J. S., Zenni, R. D., & Richardson, D. M. (2017). Non-native species in urban environments: Patterns, processes, impacts and challenges. *Biological Invasions*, 19(12), 3461–3469. https://doi.org/10.1007/s10530-017-1598-7
- Garant, D., Hadfield, J. D., Kruuk, L. E. B., & Sheldon, B. C. (2008). Stability of genetic variance and covariance for reproductive characters in the face of climate change in a wild bird population. *Molecular Ecology*, 17(1), 179–188. https://doi.org/10.1111/j.1365-294X.2007.03436.x
- Garant, D., Kruuk, L. E. B., Wilkin, T. A., McCleery, R. H., & Sheldon, B. C. (2005). Evolution driven by differential dispersal within a wild bird population. *Nature*, *433*(7021), 60–65. https://doi.org/10.1038/nature03051
- Gause, G. F. (1947). Problems of evolution. *Transactions of the Conneticut Academy of Arts and Science* 37, 17-68. https://www.cabidigitallibrary.org/doi/full/10.5555/19521600904
- Gebhardt-Henrich, S. G., & Van Noordwijk, A. J. (1991). Nestling growth in the great tit I. Heritability estimates under different environmental conditions. *Journal of Evolutionary Biology*, 4(3), 341–362. https://doi.org/10.1046/j.1420-9101.1991.4030341.x
- Gebhardt-Henrich, S. G., & Van Noordwijk, A. J. (1994). The genetical ecology of nestling growth in the great tit. Environmental influences on the expression of genetic variances during growth. *Functional Ecology*, 469–476. https://doi.org/10.2307/2390071
- Geffroy, B., Sadoul, B., Putman, B. J., Berger-Tal, O., Garamszegi, L. Z., Møller, A. P., & Blumstein, D. T. (2020). Evolutionary dynamics in the anthropocene: Life history and intensity of human contact shape antipredator responses. *PLoS Biology*, *18*(9), e3000818. https://doi.org/10.1371/journal.pbio.3000818
- Géron, C., Lembrechts, J. J., Hamdi, R., Berckmans, J., Nijs, I., & Monty, A. (2022). Phenotypic variation along urban-to-rural gradients: An attempt to disentangle the mechanisms at play using the alien species *Matricaria discoidea (Asteraceae)*. *Plant Ecology*, 223(10), 1219–1231. https://doi.org/10.1007/s11258-022-01269-y

- Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21(3), 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x
- Gibb, R., Redding, D. W., Chin, K. Q., Donnelly, C. A., Blackburn, T. M., Newbold, T., & Jones, K. E. (2020). Zoonotic host diversity increases in human-dominated ecosystems. *Nature*, 584(7821), 398–402. https://doi.org/10.1038/s41586-020-2562-8
- Gibert, J. P. (2016). The effect of phenotypic variation on metapopulation persistence. *Population Ecology*, 58(3), 345–355. https://doi.org/10.1007/s10144-016-0548-z
- Gibert, J. P., & Brassil, C. E. (2014). Individual phenotypic variation reduces interaction strengths in a consumer-resource system. *Ecology and Evolution*, 4(18), 3703–3713. https://doi.org/10.1002/ece3.1212
- Gilbert, S. F., Bosch, T. C. G., & Ledón-Rettig, C. (2015). Eco-Evo-Devo: Developmental symbiosis and developmental plasticity as evolutionary agents. *Nature Reviews Genetics*, 16(10), 611–622. https://doi.org/10.1038/nrg3982
- Gómez-Benitez, A., Walker, J. M., López-Moreno, A. E., & Hernández-Gallegos, O. (2021). The influence of urbanization on morphological traits in the Balsas Basin Whiptail lizard (*Aspidoscelis costatus costatus*). Urban Ecosystems, 24(2), 327–333. https://doi.org/10.1007/s11252-020-01038-7
- González-Suárez, M., & Revilla, E. (2013). Variability in life-history and ecological traits is a buffer against extinction in mammals. *Ecology Letters*, *16*(2), 242–251. https://doi.org/10.1111/ele.12035
- Gorton, A. J., Moeller, D. A., & Tiffin, P. (2018). Little plant, big city: A test of adaptation to urban environments in common ragweed (*Ambrosia artemisiifolia*). Proceedings of the Royal Society B: Biological Sciences, 285(1881), 20180968. https://doi.org/10.1098/rspb.2018.0968
- Gosler, A. G., & Harper, D. G. C. (2000). Assessing the heritability of body condition in birds: A challenge exemplified by the great tit *Parus major* L. (*Aves*). *Biological Journal of the Linnean Society*, *71*(1), 103–117. https://doi.org/10.1111/j.1095-8312.2000.tb01245.x
- Griffin, A. S., Netto, K., & Peneaux, C. (2017). Neophilia, innovation and learning in an urbanized world: A critical evaluation of mixed findings. *Current Opinion in Behavioral Sciences*, 16, 15–22. https://doi.org/10.1016/j.cobeha.2017.01.004
- Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm Package. *Journal of Statistical Software*, 33(2), 1–22. https://doi.org/10.18637/jss.v033.i02
- Hahs, A. K., Fournier, B., Aronson, M. F., Nilon, C. H., Herrera-Montes, A., Salisbury, A. B., Threlfall, C. G., Rega-Brodsky, C. C., Lepczyk, C. A., & La Sorte, F. A. (2023). Urbanisation generates multiple trait syndromes for terrestrial animal taxa worldwide. *Nature Communications*, 14(1), 4751. https://doi.org/10.1038/s41467-023-39746-1
- Hale, J. D., Davies, G., Fairbrass, A. J., Matthews, T. J., Rogers, C. D. F., & Sadler, J. P. (2013). Mapping Lightscapes: Spatial Patterning of Artificial Lighting in an Urban Landscape. *PLoS ONE*, 8(5), e61460. https://doi.org/10.1371/journal.pone.0061460

- Halfwerk, W., Blaas, M., Kramer, L., Hijner, N., Trillo, P. A., Bernal, X. E., Page, R. A., Goutte, S., Ryan, M. J., & Ellers, J. (2019). Adaptive changes in sexual signalling in response to urbanization. *Nature Ecology and Evolution*, 3(3), 374–380. https://doi.org/10.1038/s41559-018-0751-8
- Hall, S. J., Learned, J., Ruddell, B., Larson, K. L., Cavender-Bares, J., Bettez, N., Groffman, P. M., Grove, J. M., Heffernan, J. B., Hobbie, S. E., Morse, J. L., Neill, C., Nelson, K. C., O'Neil-Dunne, J. P. M., Ogden, L., Pataki, D. E., Pearse, W. D., Polsky, C., Chowdhury, R. R., ... Trammell, T. L. E. (2016). Convergence of microclimate in residential landscapes across diverse cities in the United States. *Landscape Ecology*, *31*(1), 101–117. https://doi.org/10.1007/s10980-015-0297-y
- Hanmer, H. J., Dadam, D., & Siriwardena, G. M. (2022). Evidence that rural wintering bird populations supplement suburban breeding populations. *Bird Study*, 69(1–2), 12–27. https://doi.org/10.1080/00063657.2022.2139814
- Hansen, T. F., & Houle, D. (2008). Measuring and comparing evolvability and constraint in multivariate characters. *Journal of Evolutionary Biology*, 21(5), 1201–1219. https://doi.org/10.1111/j.1420-9101.2008.01573.x
- Hart, S. P., Schreiber, S. J., & Levine, J. M. (2016). How variation between individuals affects species coexistence. *Ecology Letters*, 19(8), 825–838. https://doi.org/10.1111/ele.12618
- Harten, L., Gonceer, N., Handel, M., Dash, O., Fokidis, H. B., & Yovel, Y. (2021). Urban bat pups take after their mothers and are bolder and faster learners than rural pups. *BMC Biology*, *19*(1), 190. https://doi.org/10.1186/s12915-021-01131-z
- Hartig, F. (2019). DHARMa: Residual Diagnostics for Hierarchical (Muli-Level / Mixed) Regression Models. R Package version 0.2.3. [Computer software].
- Hendry, A. P. (2017). *Eco-evolutionary Dynamics*. Princeton University Press. https://doi.org/10.1515/9781400883080
- Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology*, 17(1), 20–29. https://doi.org/10.1111/j.1365-294X.2007.03428.x
- Hertel, A. G., Royauté, R., Zedrosser, A., & Mueller, T. (2021). Biologging reveals individual variation in behavioural predictability in the wild. *Journal of Animal Ecology*, 90(3), 723–737. https://doi.org/10.1111/1365-2656.13406
- Hinks, A. E., Cole, E. F., Daniels, K. J., Wilkin, T. A., Nakagawa, S., & Sheldon, B. C. (2015). Scaledependent phenological synchrony between songbirds and their caterpillar food source. *The American Naturalist*, 186(1), 84–97. https://doi.org/10.1086/681572
- Hoffman, A. A., & Hercus, M. J. (2000). Environmental stress as an evolutionary force. *BioScience*, 50(3), 217–226. https://doi.org/10.1641/0006-3568(2000)050[0217:esaaef]2.3.co;2
- Hoffmann, A. A., & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, 14(3), 96–101. https://doi.org/10.1016/S0169-5347(99)01595-5

- Honda, T., Iijima, H., Tsuboi, J., & Uchida, K. (2018). A review of urban wildlife management from the animal personality perspective: The case of urban deer. *Science of the Total Environment*, 644, 576–582. https://doi.org/10.1016/j.scitotenv.2018.06.335
- Hõrak, P. (1994). Effect of nestling history on adult size and reproduction in the Great Tit. *Ornis Fennica*, *71*(2), 47–54.
- Hõrak, P., Mänd, R., Ots, I., & Leivits, A. (1995). Egg size in the Great Tit Parus major: Individual, habitat and geographic differences. *Ornis Fennica*, 72(3), 97–114. https://ornisfennica.journal.fi/article/view/133392
- Huisman, J. (2017). Pedigree reconstruction from SNP data: Parentage assignment, sibship clustering and beyond. *Molecular Ecology Resources*, 17(5), 1009–1024. https://doi.org/10.1111/1755-0998.12665
- Hwang, C. C., & Turner, B. D. (2009). Small-scaled geographical variation in life-history traits of the blowfly *Calliphora vicina* between rural and urban populations. *Entomologia Experimentalis et Applicata*, 132(3), 218–224. https://doi.org/10.1111/j.1570-7458.2009.00891.x
- Ichikawa, I., & Kuriwada, T. (2023). The combined effects of artificial light at night and anthropogenic noise on life history traits in ground crickets. *Ecological Research*, *38*(3), 446–454. https://doi.org/10.1111/1440-1703.12380
- Irwin, R. E., Warren, P. S., Carper, A. L., & Adler, L. S. (2014). Plant–animal interactions in suburban environments: Implications for floral evolution. *Oecologia*, 174, 803–815. https://doi.org/10.1007/s00442-013-2797-2
- Isaksson, C. (2009). The chemical pathway of carotenoids: From plants to birds. *Ardea*, 97(1), 125–128. https://doi.org/10.5253/078.097.0116
- Isaksson, C., Andersson, M. N., Nord, A., Von Post, M., & Wang, H. L. (2017). Species-dependent effects of the urban environment on fatty acid composition and oxidative stress in birds. *Frontiers in Ecology and Evolution*, 5, 1–13. https://doi.org/10.3389/fevo.2017.00044
- Isaksson, C., & Andersson, S. (2007). Carotenoid diet and nestling provisioning in urban and rural great tits *Parus major*. *Journal of Avian Biology*, *38*(5), 564–572. https://doi.org/10.1111/j.2007.0908-8857.04030.x
- Jacquier, L., Doums, C., Four-Chaboussant, A., Peronnet, R., Tirard, C., & Molet, M. (2021). Urban colonies are more resistant to a trace metal than their forest counterparts in the ant *Temnothorax nylanderi*. Urban Ecosystems, 24, 561–570. https://doi.org/10.1007/s11252-020-01060-9
- Jacquier, L., Molet, M., Bocquet, C., & Doums, C. (2021). Hibernation conditions contribute to the differential resistance to cadmium between urban and forest ant colonies. *Animals*, 11(4), 1050. https://doi.org/10.3390/ani11041050
- Jarrett, C., Powell, L. L., McDevitt, H., Helm, B., & Welch, A. J. (2020). Bitter fruits of hard labour: Diet metabarcoding and telemetry reveal that urban songbirds travel further for lower-quality food. *Oecologia*, 193(2), 377–388. https://doi.org/10.1007/s00442-020-04678-w

- Jensen, J. K., Ekroos, J., Watson, H., Salmón, P., Olsson, P., & Isaksson, C. (2023). Urban tree composition is associated with breeding success of a passerine bird, but effects vary within and between years. *Oecologia*, 201(3), 585–597. https://doi.org/10.1007/s00442-023-05319-8
- Jensen, J. K., Jayousi, S., von Post, M., Isaksson, C., & Persson, A. S. (2022). Contrasting effects of tree origin and urbanization on invertebrate abundance and tree phenology. *Ecological Applications*, 32(2), e2491. https://doi.org/10.1002/eap.2491
- Jochner, S., & Menzel, A. (2015). Urban phenological studies—Past, present, future. *Environmental Pollution*, 203, 250–261. https://doi.org/10.1016/j.envpol.2015.01.003
- Johnson, M. T. J., & Munshi-South, J. (2017). Evolution of life in urban environments. *Science*, 358(6363), 1–11. https://doi.org/10.1126/science.aam8327
- Kabadayi, C., Bobrowicz, K., & Osvath, M. (2018). The detour paradigm in animal cognition. *Animal Cognition*, 21(1), 21–35. https://doi.org/10.1007/s10071-017-1152-0
- Kaiser, A., Merckx, T., & Van Dyck, H. (2016). The urban heat island and its spatial scale dependent impact on survival and development in butterflies of different thermal sensitivity. *Ecology and Evolution*, 6(12), 4129–4140. https://doi.org/10.1002/ece3.2166
- Kaiser, A., Merckx, T., & Van Dyck, H. (2018). Urbanisation and sex affect the consistency of butterfly personality across metamorphosis. *Behavioral Ecology and Sociobiology*, 72(12), 1–11. https://doi.org/10.1007/s00265-018-2616-1
- Kaiser, A., Merckx, T., & Van Dyck, H. (2020). An experimental test of changed personality in butterflies from anthropogenic landscapes. *Behavioral Ecology and Sociobiology*, 74(7), 1–8. https://doi.org/10.1007/s00265-020-02871-8
- Kaushal, S. S., Delaney-Newcomb, K., Findlay, S. E. G., Newcomer, T. A., Duan, S., Pennino, M. J., Sivirichi, G. M., Sides-Raley, A. M., Walbridge, M. R., & Belt, K. T. (2014). Longitudinal patterns in carbon and nitrogen fluxes and stream metabolism along an urban watershed continuum. *Biogeochemistry*, 121(1), 23–44. https://doi.org/10.1007/s10533-014-9979-9
- Kaushal, S. S., McDowell, W. H., & Wollheim, W. M. (2014). Tracking evolution of urban biogeochemical cycles: Past, present, and future. *Biogeochemistry*, 121(1), 1–21. https://doi.org/10.1007/s10533-014-0014-y
- Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x
- Kempenaers, B., Verheyen, G. R., den Broeck, M. V., Burke, T., Broeckhoven, C. V., & Dhondt, A. (1992). Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature*, 357(6378), 494–496. https://doi.org/10.1038/357494a0
- Kern, E., & Langerhans, R. B. (2019). Urbanization alters swimming performance of a stream fish. *Frontiers in Ecology and Evolution*, *6*, 229. https://doi.org/10.3389/fevo.2018.00229
- Kern, E. M. A., & Langerhans, R. B. (2018). Urbanization drives contemporary evolution in stream fish. Global Change Biology, 24(8), 3791–3803. https://doi.org/10.1111/gcb.14115

- Kerstes, N. A., Breeschoten, T., Kalkman, V. J., & Schilthuizen, M. (2019). Snail shell colour evolution in urban heat islands detected via citizen science. *Communications Biology*, 2(1), 264. https://doi.org/10.1038/s42003-019-0511-6
- Khalid, N., Hussain, M., Ashraf, M., Masood, A., & Akhtar, Y. (2018). Spatio-temporal variation in cadmium released by automobiles along two roads in Pakistan. *Pakistan Journal of Botany*, 50(2), 529–536. http://www.pakbs.org/pjbot/papers/1518733201.pdf
- Kheirbek, I., Ito, K., Neitzel, R., Kim, J., Johnson, S., Ross, Z., Eisl, H., & Matte, T. (2014). Spatial variation in environmental noise and air pollution in New York City. *Journal of Urban Health*, 91(3), 415–431. https://doi.org/10.1007/s11524-013-9857-0
- Kluijver, H. N. (1951). The population ecology of the great tit, Parus m. Major. Adrea, 39, 1–135.
- Klump, B. C., Martin, J. M., Wild, S., Hörsch, J. K., Major, R. E., & Aplin, L. M. (2021). Innovation and geographic spread of a complex foraging culture in an urban parrot. *Science*, 373(6553), 456–460. https://doi.org/10.1126/science.abe7808
- Koile, E., & Cristia, A. (2021). Toward cumulative cognitive science: A comparison of meta-analysis, mega-analysis, and hybrid approaches. *Open Mind*, 5, 154–173. https://doi.org/10.1162/opmi_a_00048
- Kolbe, J. J., Kearney, M., & Shine, R. (2010). Modeling the consequences of thermal trait variation for the cane toad invasion of Australia. *Ecological Applications*, 20(8), 2273–2285. https://doi.org/10.1890/09-1973.1
- Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., & Palanza, P. (2011). Stress revisited: A critical evaluation of the stress concept. *Neuroscience & Biobehavioral Reviews*, 35(5), 1291–1301.
- Koolhaas, J. M., De Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*, 31(3), 307–321. https://doi.org/10.1016/j.neubiorev.2011.02.003
- Kostanecki, A., Gorton, A. J., & Moeller, D. A. (2021). An urban–rural spotlight: Evolution at small spatial scales among urban and rural populations of common ragweed. *Journal of Urban Ecology*, 7(1), juab004. https://doi.org/10.1093/jue/juab004
- Krams, I. A., Vrublevska, J., Sepp, T., Abolins-Abols, M., Rantala, M. J., Mierauskas, P., & Krama, T. (2014). Sex-specific associations between nest defence, exploration and breathing rate in breeding pied flycatchers. *Ethology*, 120(5), 492–501. https://doi.org/10.1111/eth.12222
- Krauze-Gryz, D., Żmihorski, M., & Gryz, J. (2017). Annual variation in prey composition of domestic cats in rural and urban environment. *Urban Ecosystems*, 20(4), 945–952. https://doi.org/10.1007/s11252-016-0634-1
- Kruuk, L. E. B., Slate, J., & Wilson, A. J. (2008). New Answers for Old Questions: The Evolutionary Quantitative Genetics of Wild Animal Populations. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 525–548. https://doi.org/10.1146/annurev.ecolsys.39.110707.173542

- Kuriwada, T. (2023). Differences in male calling song and female mate location behaviour between urban and rural crickets. *Biological Journal of the Linnean Society*, *139*(3), 275–285. https://doi.org/10.1093/biolinnean/blad027
- Kyba, C. C. M., & Hölker, F. (2013). Do artificially illuminated skies affect biodiversity in nocturnal landscapes? *Landscape Ecology*, 28(9), 1637–1640. https://doi.org/10.1007/s10980-013-9936-3
- Lack, D. (1955). British tits (Parus spp.) in nestboxes. Adrea, 43, 50-84.
- Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., Coss, R. G., Donohue, K., & Foster, S. A. (2009). Relaxed selection in the wild. *Trends in Ecology and Evolution*, 24(9), 487–496. https://doi.org/10.1016/j.tree.2009.03.010
- Lambert, M. R., Brans, K. I., Roches, S. D., Donihue, C. M., & Diamond, S. E. (2021). Adaptive evolution in cities: Progress and misconceptions. *Trends in Ecology & Evolution*, 36(3), 239–257. https://doi.org/10.1016/j.tree.2020.11.002
- Lambert, M. R., & Donihue, C. M. (2020). Urban biodiversity management using evolutionary tools. *Nature Ecology and Evolution*, 4(7), 903–910. https://doi.org/10.1038/s41559-020-1193-7
- Lambrecht, S. C., Mahieu, S., & Cheptou, P.-O. (2016). Natural selection on plant physiological traits in an urban environment. *Acta Oecologica*, 77, 67–74. https://doi.org/10.1016/j.actao.2016.09.002
- Lampe, U., Reinhold, K., & Schmoll, T. (2014). How grasshoppers respond to road noise: Developmental plasticity and population differentiation in acoustic signalling. *Functional Ecology*, 28(3), 660–668. https://doi.org/10.1111/1365-2435.12215
- Lande, R., & Arnold, S. J. (1983). The Measurement of Selection on Correlated Characters. *Evolution*, 37(6), 1210–1226. https://doi.org/10.2307/2408842
- Langley, E. J., Adams, G., Beardsworth, C. E., Dawson, D. A., Laker, P. R., van Horik, J. O., Whiteside, M. A., Wilson, A. J., & Madden, J. R. (2020). Heritability and correlations among learning and inhibitory control traits. *Behavioral Ecology*, 31(3), 798–806. https://doi.org/10.1093/beheco/araa029
- Lazić, M. M., Carretero, M. A., Crnobrnja-Isailović, J., & Kaliontzopoulou, A. (2015). Effects of environmental disturbance on phenotypic variation: An integrated assessment of canalization, developmental stability, modularity, and allometry in lizard head shape. *The American Naturalist*, 185(1), 44–58. https://doi.org/10.1086/679011
- Lee, V. E., & Thornton, A. (2021). Animal cognition in an urbanised world. *Frontiers in Ecology and Evolution*, 9, 633947. https://doi.org/10.3389/fevo.2021.633947
- Leinonen, T., Mccairns, R. J. S., Hara, R. B. O., & Merilä, J. (2013). QST FST comparisons: Evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics*. https://doi.org/10.1038/nrg3395
- Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology and Evolution*, 17(4), 183–189. https://doi.org/10.1016/S0169-5347(02)02497-7
- Lepczyk, C. A., Aronson, M. F. J., Evans, K. L., Goddard, M. A., Lerman, S. B., & Macivor, J. S. (2017). Biodiversity in the city: Fundamental questions for understanding the ecology of urban green

spaces for biodiversity conservation. *BioScience*, 67(9), 799–807. https://doi.org/10.1093/biosci/bix079

- Leroi, A. M., Chippindale, A. K., & Rose, M. R. (1994). Long-term laboratory evolution of a genetic lifehistory trade-off in *Drosophila melanogaster*. 1. the role of genotype-by-environment interaction. *Evolution*, 48(4), 1244–1257. https://doi.org/10.1111/j.1558-5646.1994.tb05309.x
- Leveau, L. M. (2018). Urbanization, environmental stabilization and temporal persistence of bird species: A view from Latin America. *PeerJ*, *6*, e6056. https://doi.org/10.7717/peerj.6056
- Littleford-Colquhoun, B. L., Clemente, C., Whiting, M. J., Ortiz-Barrientos, D., & Frère, C. H. (2017). Archipelagos of the Anthropocene: Rapid and extensive differentiation of native terrestrial vertebrates in a single metropolis. *Molecular Ecology*, 26(9), 2466–2481. https://doi.org/10.1111/mec.14042
- Lohr, V. I., Kendal, D., & Dobbs, C. (2014). Urban trees worldwide have low species and genetic diversity, posing high risks of tree loss as stresses from climate change increase. XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014), 1108, 263–270. https://10.17660/ActaHortic.2016.1108.34
- Loss, S. R., & Marra, P. P. (2017). Population impacts of free-ranging domestic cats on mainland vertebrates. *Frontiers in Ecology and the Environment*, 15(9), 502–509. https://doi.org/10.1002/fee.1633
- Lush, J. L. (1937). Animal breeding plans. Iowa State Press.
- Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits* (Vol. 1). Sinauer Sunderland, MA.
- MacLean, E. L., Hare, B., Nunn, C. L., Addessi, E., Amici, F., Anderson, R. C., Aureli, F., Baker, J. M., Bania, A. E., Barnard, A. M., Boogert, N. J., Brannon, E. M., Bray, E. E., Bray, J., Brent, L. J. N., Burkart, J. M., Call, J., Cantlon, J. F., Cheke, L. G., ... Zhao, Y. (2014). The evolution of selfcontrol. *Proceedings of the National Academy of Sciences*, *111*(20). https://doi.org/10.1073/pnas.1323533111
- Macphie, K. H., Samplonius, J. M., Pick, J. L., Hadfield, J. D., & Phillimore, A. B. (2023). Modelling thermal sensitivity in the full phenological distribution: A new approach applied to the spring arboreal caterpillar peak. *Functional Ecology*, 37(12), 3015–3026. https://doi.org/10.1111/1365-2435.14436
- Madden, J. R., Langley, E. J. G., Whiteside, M. A., Beardsworth, C. E., & Van Horik, J. O. (2018). The quick are the dead: Pheasants that are slow to reverse a learned association survive for longer in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1756), 20170297. https://doi.org/10.1098/rstb.2017.0297
- Magle, S. B., Fidino, M., Lehrer, E. W., Gallo, T., Mulligan, M. P., Ríos, M. J., Ahlers, A. A., Angstmann, J., Belaire, A., Dugelby, B., Gramza, A., Hartley, L., MacDougall, B., Ryan, T., Salsbury, C., Sander, H., Schell, C., Simon, K., St Onge, S., & Drake, D. (2019). Advancing urban wildlife research through a multi-city collaboration. *Frontiers in Ecology and the Environment*, 17(4), 232–239. https://doi.org/10.1002/fee.2030

- Marko, P. B., & Hart, M. W. (2011). The complex analytical landscape of gene flow inference. *Trends in Ecology and Evolution*, *26*(9), 448–456. https://doi.org/10.1016/j.tree.2011.05.007
- Marques, P. S., Manna, L. R., Mazzoni, R., & El-Sabaawi, R. (2019). Intraspecific trait variation in urban stream ecosystems: Toward understanding the mechanisms shaping urban stream communities. *Freshwater Science*, 38(1), 1–11. https://doi.org/10.1086/701652
- Marrot, P., Charmantier, A., Blondel, J., & Garant, D. (2018). Current spring warming as a driver of selection on reproductive timing in a wild passerine. *Journal of Animal Ecology*, 87(3), 754–764. https://10.1111/1365-2656.12794
- Martin, J. G., Pirotta, E., Petelle, M. B., & Blumstein, D. T. (2017). Genetic basis of between-individual and within-individual variance of docility. *Journal of Evolutionary Biology*, *30*(4), 796–805. https://doi.org/10.1111/jeb.13048
- Martin, R. A., Chick, L. D., Yilmaz, A. R., & Diamond, S. E. (2019). Evolution, not transgenerational plasticity, explains the adaptive divergence of acorn ant thermal tolerance across an urban–rural temperature cline. *Evolutionary Applications*, 12(8), 1678–1687. https://doi.org/10.1111/eva.12826
- Matthysen, E., Adriaensen, F., Van de Kerckhove, P., & Vandekerkhove, K. (2021). Great and blue tit laying dates vary with fine-scale variation in local tree composition but not tree budburst. *Journal of Ornithology*, *162*, 709–722. https://doi.org/10.1007/s10336-021-01872-2
- McCallum, E., & Shaw, R. C. (2023). Repeatability and heritability of inhibitory control performance in wild toutouwai (*Petroica longipes*). *Royal Society Open Science*, 10(11), 231476. https://doi.org/10.1098/rsos.231476
- McDonnell, M. J., & Hahs, A. K. (2015). Adaptation and Adaptedness of Organisms to Urban Environments. *Annual Review of Ecology, Evolution, and Systematics*, 46(1), 261–280. https://doi.org/10.1146/annurev-ecolsys-112414-054258
- McElreath, R. (2020). *Statistical rethinking: A Bayesian course with examples in R and Stan*. CRC press New York. https://doi.org/10.1201/9781315372495
- McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, 127(3), 247–260. https://doi.org/10.1016/j.biocon.2005.09.005
- McLean, M. A., Angilletta Jr, M. J., & Williams, K. S. (2005). If you can't stand the heat, stay out of the city: Thermal reaction norms of chitinolytic fungi in an urban heat island. *Journal of Thermal Biology*, 30(5), 384–391. https://doi.org/10.1016/j.jtherbio.2005.03.002
- McNew, S. M., Beck, D., Sadler-Riggleman, I., Knutie, S. A., Koop, J. A. H., Clayton, D. H., & Skinner, M. K. (2017). Epigenetic variation between urban and rural populations of Darwin's finches. BMC Evolutionary Biology, 17(1), 1–14. https://doi.org/10.1186/s12862-017-1025-9
- Merckx, T., Nielsen, M. E., Heliölä, J., Kuussaari, M., Pettersson, L. B., Pöyry, J., Tiainen, J., Gotthard, K., & Kivelä, S. M. (2021). Urbanization extends flight phenology and leads to local adaptation of seasonal plasticity in Lepidoptera. *Proceedings of the National Academy of Sciences*, 118(40), e2106006118. https://doi.org/10.1073/pnas.2106006118

- Merckx, T., Nielsen, M. E., Kankaanpää, T., Kadlec, T., Yazdanian, M., & Kivelä, S. M. (2023). Dim light pollution prevents diapause induction in urban and rural moths. *Journal of Applied Ecology*, 60(6), 1022–1031. https://doi.org/10.1111/1365-2664.14373
- Merckx, T., Nielsen, M. E., Kankaanpää, T., Kadlec, T., Yazdanian, M., & Kivelä, S. M. (2024). Continent-wide parallel urban evolution of increased heat tolerance in a common moth. *Evolutionary Applications*, 17(1), e13636. https://doi.org/10.1111/eva.13636
- Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J. M. T., Fontaneto, D., Gianuca, A. T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., ... Van Dyck, H. (2018). Body-size shifts in aquatic and terrestrial urban communities. *Nature*, 558(7708), 113–116. https://doi.org/10.1038/s41586-018-0140-0
- Merilä, J., & Fry, J. D. (1998). Genetic variation and causes of genotype-environment interaction in the body size of blue tit (*Parus caeruleus*). *Genetics*, 148(3), 1233–1244. https://doi.org/10.1093/genetics/148.3.1233
- Mertens, J. A. L. (1977). Thermal conditions for successful breeding in great tits (*Parus major* L.) I. Relation of growth and development of temperature regulation in nestling great tits. *Oecologia*, 28(1), 1–29. https://doi.org/10.1007/BF00346834
- Miles, L. S., Rivkin, L. R., Johnson, M. T., Munshi-South, J., & Verrelli, B. C. (2019). Gene flow and genetic drift in urban environments. *Molecular Ecology*, 28, 4138–4151. https://doi.org/10.1111/mec.15221
- Miller, R., Boeckle, M., Jelbert, S. A., Frohnwieser, A., Wascher, C. A. F., & Clayton, N. S. (2019). Selfcontrol in crows, parrots and nonhuman primates. WIREs Cognitive Science, 10(6), e1504. https://doi.org/10.1002/wcs.1504
- Mimura, M., Yahara, T., Faith, D. P., Vázquez-Domínguez, E., Colautti, R. I., Araki, H., Javadi, F., Núñez-Farfán, J., Mori, A. S., Zhou, S., Hollingsworth, P. M., Neaves, L. E., Fukano, Y., Smith, G. F., Sato, Y. I., Tachida, H., & Hendry, A. P. (2017). Understanding and monitoring the consequences of human impacts on intraspecific variation. *Evolutionary Applications*, 10(2), 121– 139. https://doi.org/10.1111/eva.12436
- Miranda, A. C., Schielzeth, H., Sonntag, T., & Partecke, J. (2013). Urbanization and its effects on personality traits: A result of microevolution or phenotypic plasticity? *Global Change Biology*, 19(9), 2634–2644. https://doi.org/10.1111/gcb.12258
- Miranda-Moreno, L. F., & Lahti, A. C. (2013). Temporal trends and the effect of weather on pedestrian volumes: A case study of Montreal, Canada. *Transportation Research Part D: Transport and Environment*, 22, 54–59. https://doi.org/10.1016/j.trd.2013.02.008
- Mitchell-Olds, T., Willis, J. H., & Goldstein, D. B. (2007). Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nature Reviews*, 8, 845–856. https://doi.org/10.1038/nrg2207
- Mohajerani, A., Bakaric, J., & Jeffrey-Bailey, T. (2017). The urban heat island effect, its causes, and mitigation, with reference to the thermal properties of asphalt concrete. *Journal of Environmental Management*, 197, 522–538. https://doi.org/10.1016/j.jenvman.2017.03.095

- Monniez, E., Jiguet, F., Vignal, C., & Biard, C. (2022). Differential effects of anthropogenic noise and vegetation cover on the breeding phenology and success of two urban passerines. *Frontiers in Ecology and Evolution*, 10, 1058584. https://doi.org/10.3389/fevo.2022.1058584
- Moran, E. V., Hartig, F., & Bell, D. M. (2016). Intraspecific trait variation across scales: Implications for understanding global change responses. *Global Change Biology*, 22(1), 137–150. https://doi.org/10.1111/gcb.13000
- Morand-Ferron, J., Cole, E. F., & Quinn, J. L. (2016). Studying the evolutionary ecology of cognition in the wild: A review of practical and conceptual challenges. *Biological Reviews*, 91(2), 367–389. https://doi.org/10.1111/brv.12174
- Mueller, J. C., Kuhl, H., Boerno, S., Tella, J. L., Carrete, M., & Kempenaers, B. (2018). Evolution of genomic variation in the burrowing owl in response to recent colonization of urban areas. *Proceedings of the Royal Society B: Biological Sciences*, 285(1878), 20180206. https://doi.org/10.1098/rspb.2018.0206
- Mühlenhaupt, M., Baxter-Gilbert, J., Makhubo, B. G., Riley, J. L., & Measey, J. (2022). No evidence for innate differences in tadpole behavior between natural, urbanized, and invasive populations. *Behavioral Ecology and Sociobiology*, 76(1), 11. https://doi.org/10.1007/s00265-021-03121-1
- Multini, L. C., Wilke, A. B. B., & Marrelli, M. T. (2019). Urbanization as a driver for temporal wingshape variation in *Anopheles cruzii* (Diptera: Culicidae). *Acta Tropica*, 190(September 2018), 30– 36. https://doi.org/10.1016/j.actatropica.2018.10.009
- Munshi-South, J., Zolnik, C. P., & Harris, S. E. (2016). Population genomics of the Anthropocene: Urbanization is negatively associated with genome-wide variation in white-footed mouse populations. *Evolutionary Applications*, 9(4), 546–564. https://doi.org/10.1111/eva.12357
- Murray, M. H., Sánchez, C. A., Becker, D. J., Byers, K. A., Worsley-Tonks, K. E. L., & Craft, M. E. (2019). City sicker? A meta-analysis of wildlife health and urbanization. *Frontiers in Ecology and the Environment*, 17(10), 575–583. https://doi.org/10.1002/fee.2126
- Nager, R. G., & van Noordwijk, A. J. (1995). Proximate and ultimate aspects of phenotypic plasticity in timing of great tit breeding in a heterogeneous environment. *The American Naturalist*, 146(3), 454–474. https://doi.org/10.1086/285809
- Nakagawa, S., Lagisz, M., O'Dea, R. E., Rutkowska, J., Yang, Y., Noble, D. W. A., & Senior, A. M. (2021). The orchard plot: Cultivating a forest plot for use in ecology, evolution, and beyond. *Research Synthesis Methods*, 12(1), 4–12. https://doi.org/10.1002/jrsm.1424
- Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M., & Senior, A. M. (2015). Meta-analysis of variation: Ecological and evolutionary applications and beyond. *Methods in Ecology and Evolution*, 6(2), 143–152. https://doi.org/10.1111/2041-210X.12309
- Neumann, W., Ericsson, G., Dettki, H., Bunnefeld, N., Keuler, N. S., Helmers, D. P., & Radeloff, V. C. (2012). Difference in spatiotemporal patterns of wildlife road-crossings and wildlife-vehicle collisions. *Biological Conservation*, 145(1), 70–78. https://doi.org/10.1016/j.biocon.2011.10.011
- Nicolaus, M., & Edelaar, P. (2018). Comparing the consequences of natural selection, adaptive phenotypic plasticity, and matching habitat choice for phenotype–environment matching, population genetic

structure, and reproductive isolation in meta-populations. *Ecology and Evolution*, 8(8), 3815–3827. https://doi.org/10.1002/ece3.3816

- Nicolaus, M., Tinbergen, J. M., Ubels, R., Both, C., & Dingemanse, N. J. (2016). Density fluctuations represent a key process maintaining personality variation in a wild passerine bird. *Ecology Letters*, 19, 478–486. https://doi.org/10.1111/ele.12584
- Niemelä, J., Breuste, J. H., Guntenspergen, G., McIntyre, N. E., Elmqvist, T., & James, P. (2011). Urban ecology: Patterns, processes, and applications. OUP Oxford.
- Nussey, D. H., Postma, E., Gienapp, P., & Visser, M. E. (2005). Selection on heritable phenotypic plasticity in a wild bird population. *Science*, 310(5746), 304–306. https://doi.org/10.1126/science.1117004
- Oddie, K. R. (2000). Size matters: Competition between male and female great tit offspring. *Journal of Animal Ecology*, 69(5), 903–912. https://doi.org/10.1046/j.1365-2656.2000.00438.x
- O'Dea, R. E., Lagisz, M., Hendry, A. P., & Nakagawa, S. (2019). Developmental temperature affects phenotypic means and variability: A meta-analysis of fish data. *Fish and Fisheries*, 20(5), 1005–1022. https://doi.org/10.1111/faf.12394
- O'Dea, R. E., Noble, D. W., & Nakagawa, S. (2022). Unifying individual differences in personality, predictability and plasticity: A practical guide. *Methods in Ecology and Evolution*, *13*(2), 278–293. https://doi.org/10.1111/2041-210X.13755
- Oliveira Hagen, E., Hagen, O., Ibáñez-álamo, J. D., Petchey, O. L., & Evans, K. L. (2017). Impacts of urban areas and their characteristics on avian functional diversity. *Frontiers in Ecology and Evolution*, 5, 1–15. https://doi.org/10.3389/fevo.2017.00084
- Olliff-Yang, R. L., Gardali, T., & Ackerly, D. D. (2020). Mismatch managed? Phenological phase extension as a strategy to manage phenological asynchrony in plant–animal mutualisms. *Restoration Ecology*, 28(3), 498–505. https://doi.org/10.1111/rec.13130
- Pagani-Núñez, E., Liang, D., He, C., Zhou, X., Luo, X., Liu, Y., & Goodale, E. (2019). Niches in the anthropocene: Passerine assemblages show niche expansion from natural to urban habitats. *Ecography*, 42(8), 1360–1369. https://doi.org/10.1111/ecog.04203
- Palomar, G., Wos, G., Stoks, R., & Sniegula, S. (2023). Latitude-specific urbanization effects on life history traits in the damselfly *Ischnura elegans*. *Evolutionary Applications*, 16(8), 1503–1515. https://doi.org/10.1111/eva.13583
- Papp, S., Vincze, E., Preiszner, B., Liker, A., & Bókony, V. (2015). A comparison of problem-solving success between urban and rural house sparrows. *Behavioral Ecology and Sociobiology*, 69(3), 471–480. https://doi.org/10.1007/s00265-014-1859-8
- Paquette, A., Sousa-Silva, R., Maure, F., Cameron, E., Belluau, M., & Messier, C. (2021). Praise for diversity: A functional approach to reduce risks in urban forests. *Urban Forestry and Urban Greening*, 62, 127157. https://doi.org/10.1016/j.ufug.2021.127157
- Partecke, J., & Gwinner, E. (2007). Increased sedentariness in European blackbirds following urbanization: A consequence of local adaptation? *Ecology*, 88(4), 882–890. https://doi.org/10.1890/06-1105

- Partecke, J., Schwabl, I., & Gwinner, E. (2006). Stress and the city: Urbanization and its effects on the stress physiology in European blackbirds. *Ecology*, 87(8), 1945–1952. https://doi.org/10.1890/0012-9658(2006)87[1945:SATCUA]2.0.CO;2
- Pearse, W. D., Cavender-Bares, J., Hobbie, S. E., Avolio, M. L., Bettez, N., Roy Chowdhury, R., Darling, L. E., Groffman, P. M., Grove, J. M., & Hall, S. J. (2018). Homogenization of plant diversity, composition, and structure in North American urban yards. *Ecosphere*, 9(2), e02105. https://doi.org/10.1002/ecs2.2105
- Pélabon, C., Hilde, C. H., Einum, S., & Gamelon, M. (2020). On the use of the coefficient of variation to quantify and compare trait variation. *Evolution Letters*, 4(3), 180–188. https://doi.org/10.1002/evl3.171
- Pelletier, F., Clutton-Brock, T., Pemberton, J., Tuljapurkar, S., & Coulson, T. (2007). The evolutionary demography of ecological change: Linking trait variation and population growth. *Science*, 315(5818), 1571–1574. https:// 10.1126/science.1139024
- Perrier, C., Caizergues, A., & Charmantier, A. (2020). Adaptation genomics in urban environments. In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), *Urban Evolutionary Biology* (pp. 74–90). Oxford University Press.
- Perrier, C., Lozano del Campo, A., Szulkin, M., Demeyrier, V., Gregoire, A., & Charmantier, A. (2018). Great tits and the city: Distribution of genomic diversity and gene–environment associations along an urbanization gradient. *Evolutionary Applications*, 11(5), 593–613. https://doi.org/10.1111/eva.12580
- Perrins, C. M. (1979). British Tits. William Collins Sons and Company Limited.
- Pfennig, D. W. (2021). *Phenotypic plasticity & evolution: Causes, consequences, controversies*. Taylor & Francis. https://library.oapen.org/handle/20.500.12657/47881
- Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology and Evolution*, 25(8), 459–467. https://doi.org/10.1016/j.tree.2010.05.006
- Pickett, S. T. A., Cadenasso, M. L., Rosi-Marshall, E. J., Belt, K. T., Groffman, P. M., Grove, J. M., Irwin, E. G., Kaushal, S. S., LaDeau, S. L., Nilon, C. H., Swan, C. M., & Warren, P. S. (2017). Dynamic heterogeneity: A framework to promote ecological integration and hypothesis generation in urban systems. *Urban Ecosystems*, 20(1), 1–14. https://doi.org/10.1007/s11252-016-0574-9
- Pipoly, I., Preiszner, B., Sándor, K., Sinkovics, C., Seress, G., Vincze, E., Bókony, V., & Liker, A. (2022). Extreme hot weather has stronger impacts on Avian reproduction in forests than in cities. *Frontiers in Ecology and Evolution*, 10. https://doi.org/10.3389/fevo.2022.825410
- Pisman, M., Bonte, D., & De La Peña, E. (2020). Urbanization alters plastic responses in the common dandelion *Taraxacum officinale*. *Ecology and Evolution*, 10(9), 4082–4090. https://doi.org/10.1002/ece3.6176
- Pollock, C. J., Capilla-Lasheras, P., McGill, R. A. R., Helm, B., & Dominoni, D. M. (2017). Integrated behavioural and stable isotope data reveal altered diet linked to low breeding success in urban-

dwelling blue tits (*Cyanistes caeruleus*). *Scientific Reports*, 7(1), 1–14. https://doi.org/10.1038/s41598-017-04575-y

- Prasher, S., Evans, J. C., Thompson, M. J., & Morand-Ferron, J. (2019). Characterizing innovators: Ecological and individual predictors of problem-solving performance. *PLoS ONE*, 14(6), 1–19. https://doi.org/10.1371/journal.pone.0217464
- Prentice, P. M., Thornton, A., Kolm, N., & Wilson, A. J. (2023). Genetic and context-specific effects on individual inhibitory control performance in the guppy (*Poecilia reticulata*). Journal of Evolutionary Biology, 36(12), 1796–1810. https://doi.org/10.1111/jeb.14241
- Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1523), 1433–1440. https://doi.org/10.1098/rspb.2003.2372
- QGIS Development Team. (2023). *QGIS Geographic Information System* (3.22.0) [Computer software]. QGIS Association. https://www.qgis.org
- Qiu, T., Song, C., & Li, J. (2017). Impacts of urbanization on vegetation phenology over the past three decades in Shanghai, China. *Remote Sensing*, 9(9), 1–16. https://doi.org/10.3390/rs9090970
- Qu, J., Bonte, D., & Vandegehuchte, M. L. (2022). Phenotypic and genotypic divergence of plant– herbivore interactions along an urbanization gradient. *Evolutionary Applications*, 15(5), 865–877. https://doi.org/10.1111/eva.13376
- Quinn, J. L., Cole, E. F., Reed, T. E., Morand-ferron, J., & Quinn, J. L. (2016). Environmental and genetic determinants of innovativeness in a natural population of birds. *Philosophical Transactions: Biological Sciences*, 371, 20150184. https://doi.org/10.1098/rstb.2015.0184
- R Core Team. (2024). *R: A language and environment for statistical computing* [Computer software]. R Foundation for Statistical Computing.
- Raap, T., Casasole, G., Costantini, D., Abdelgawad, H., Asard, H., Pinxten, R., & Eens, M. (2016).
 Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: An experimental study. *Scientific Reports*, 6, 1–8. https://doi.org/10.1038/srep35626
- Radchuk, V., Reed, T., Teplitsky, C., van de Pol, M., Charmantier, A., Hassall, C., Adamík, P.,
 Adriaensen, F., Ahola, M. P., Arcese, P., Miguel Avilés, J., Balbontin, J., Berg, K. S., Borras, A.,
 Burthe, S., Clobert, J., Dehnhard, N., de Lope, F., Dhondt, A. A., ... Kramer-Schadt, S. (2019).
 Adaptive responses of animals to climate change are most likely insufficient. *Nature Communications*, *10*(1), 1–14. https://doi.org/10.1038/s41467-019-10924-4
- Rasner, C. A., Yeh, P., Eggert, L. S., Hunt, K. E., Woodruff, D. S., & Price, T. D. (2004). Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi. Molecular Ecology*, 13(3), 671–681. https://doi.org/10.1046/j.1365-294X.2004.02104.x
- Reichard, D. G., Atwell, J. W., Pandit, M. M., Cardoso, G. C., Price, T. D., & Ketterson, E. D. (2020). Urban birdsongs: Higher minimum song frequency of an urban colonist persists in a common garden experiment. *Animal Behaviour*, 170, 33–41. https://doi.org/10.1016/j.anbehav.2020.10.007
- Reinhold, K., & Engqvist, L. (2013). The variability is in the sex chromosomes. *Evolution*, 67(12), 3662–3668. https://doi.org/10.1111/evo.12224

- Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., de Keyzer, C. W., Diamond, S. E., Fortin, M. J., Frazee, L. J., Gorton, A. J., Hendry, A. P., Liu, Y., Losos, J. B., MacIvor, J. S., Martin, R. A., McDonnell, M. J., Miles, L. S., Munshi-South, J., Ness, R. W., Newman, A. E. M., ... Johnson, M. T. J. (2019). A roadmap for urban evolutionary ecology. *Evolutionary Applications*, *12*(3), 384–398. https://doi.org/10.1111/eva.12734
- Riyahi, S., Björklund, M., Mateos-Gonzalez, F., & Senar, J. C. (2017). Personality and urbanization: Behavioural traits and DRD4 SNP830 polymorphisms in great tits in Barcelona city. *Journal of Ethology*, 35(1), 101–108. https://doi.org/10.1007/s10164-016-0496-2
- Riyahi, S., Sánchez-Delgado, M., Calafell, F., Monk, D., & Senar, J. C. (2015). Combined epigenetic and intraspecific variation of the DRD4 and SERT genes influence novelty seeking behavior in great tit *Parus major. Epigenetics*, 10(6), 516–525. https://doi.org/10.1080/15592294.2015.1046027
- Rizwan, A. M., Dennis, L. Y. C., & Liu, C. (2008). A review on the generation, determination and mitigation of Urban Heat Island. *Journal of Environmental Sciences*, 20(1), 120–128. https://doi.org/10.1016/S1001-0742(08)60019-4
- Rodewald, A. D., & Arcese, P. (2017). Reproductive contributions of cardinals are consistent with a hypothesis of relaxed selection in urban landscapes. *Frontiers in Ecology and Evolution*, *5*, 1–7. https://doi.org/10.3389/fevo.2017.00077
- Salmón, P., Jacobs, A., Ahrén, D., Biard, C., Dingemanse, N. J., Dominoni, D. M., Helm, B., Lundberg, M., Senar, J. C., Sprau, P., Visser, M. E., & Isaksson, C. (2021). Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe. *Nature Communications*, 12(1), 2983. https://doi.org/10.1038/s41467-021-23027-w
- Samocha, Y., & Scharf, I. (2020). Comparison of wormlion behavior under man-made and natural shelters: Urban wormlions more strongly prefer shaded, fine-sand microhabitats, construct larger pits and respond faster to prey. *Current Zoology*, 66(1), 91–98. https://doi.org/10.1093/cz/zoz006
- Samplonius, J. M., Atkinson, A., Hassall, C., Keogan, K., Thackeray, S. J., Assmann, J. J., Burgess, M. D., Johansson, J., Macphie, K. H., Pearce-Higgins, J. W., Simmonds, E. G., Varpe, Ø., Weir, J. C., Childs, D. Z., Cole, E. F., Daunt, F., Hart, T., Lewis, O. T., Pettorelli, N., ... Phillimore, A. B. (2020). Strengthening the evidence base for temperature-mediated phenological asynchrony and its impacts. *Nature Ecology and Evolution*. https://doi.org/10.1038/s41559-020-01357-0
- Sánchez-Tójar, A., Moran, N. P., O'Dea, R. E., Reinhold, K., & Nakagawa, S. (2020). Illustrating the importance of meta-analysing variances alongside means in ecology and evolution. *Journal of Evolutionary Biology*, 33(9), 1216–1223. https://doi.org/10.1111/jeb.13661
- Sanderson, S., Bolnick, D. I., Kinnison, M. T., O'Dea, R. E., Gorné, L. D., Hendry, A. P., & Gotanda, K. M. (2023). Contemporary changes in phenotypic variation, and the potential consequences for eco-evolutionary dynamics. *Ecology Letters*, 26. https://doi.org/10.1111/ele.14186
- Santangelo, J. S., Ness, R. W., Cohan, B., Fitzpatrick, C. R., Innes, S. G., Koch, S., Miles, L. S., Munim, S., Peres-Neto, P. R., & Prashad, C. (2022). Global urban environmental change drives adaptation in white clover. *Science*, 375(6586), 1275–1281. https:// 10.1126/science.abk0989

- Santangelo, J. S., Rivkin, L. R., Advenard, C., & Thompson, K. A. (2020). Multivariate phenotypic divergence along an urbanization gradient. *Biology Letters*, 16(9), 20200511. https://doi.org/10.1098/rsbl.2020.0511
- Sato, A., & Takahashi, Y. (2022). Responses in thermal tolerance and daily activity rhythm to urban stress in *Drosophila suzukii*. *Ecology and Evolution*, *12*(12), e9616. https://doi.org/10.1002/ece3.9616
- Saulnier, A., Bleu, J., Boos, A., Millet, M., Zahn, S., Ronot, P., El Masoudi, I., Rojas, E. R., Uhlrich, P., & Del Nero, M. (2023). Inter-annual variation of physiological traits between urban and forest great tits. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 279, 111385. https://doi.org/10.1016/j.cbpa.2023.111385
- Schlaepfer, M. A., Runge, M. C., & Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, 17(10), 474–480. https://doi.org/10.1016/S0169-5347(02)02580-6
- Schmidt, C., Domaratzki, M., Kinnunen, R. P., Bowman, J., & Garroway, C. J. (2020). Continent-wide effects of urbanization on bird and mammal genetic diversity. *Proceedings of the Royal Society B*, 287(1920), 20192497. https://doi.org/10.1098/rspb.2019.2497
- Schmitz, G., Linstädter, A., Frank, A. S. K., Dittberner, H., Thome, J., Schrader, A., Linne von Berg, K., Fulgione, A., Coupland, G., & De Meaux, J. (2024). Environmental filtering of life-history trait diversity in urban populations of *Arabidopsis thaliana*. *Journal of Ecology*, *112*(1), 14–27. https://doi.org/10.1111/1365-2745.14211
- Senar, J. C., & Björklund, M. (2021). Recent spread of blue tits into the Barcelona urban environment: Morphological differences and the role of balanced dispersal. *Evolutionary Ecology*, 35, 83–99. https://doi.org/10.1007/s10682-020-10087-5
- Sepp, T., McGraw, K. J., Kaasik, A., & Giraudeau, M. (2017). A review of urban impacts on avian lifehistory evolution: Does city living lead to slower pace of life? *Global Change Biology*, 24, 1452– 1469. https://doi.org/10.1111/gcb.13969
- Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., Pipoly, I., Sinkovics, C., Evans, K. L., & Liker, A. (2018). Impact of urbanization on abundance and phenology of caterpillars and consequences for breeding in an insectivorous bird. *Ecological Applications*, 28(5), 1143–1156. https://doi.org/10.1002/eap.1730
- Seress, G., Sándor, K., Evans, K. L., & Liker, A. (2020). Food availability limits avian reproduction in the city: An experimental study on great tits *Parus major. Journal of Animal Ecology*, 89(7), 1570– 1580. https://doi.org/10.1111/1365-2656.13211
- Shaw, R. C. (2016). Testing cognition in the wild: Factors affecting performance and individual consistency in two measures of avian cognition. *Behavioural Processes*. https://doi.org/10.1016/j.beproc.2016.06.004
- Shirihai, H., & Svensson, L. (2018). Handbook of Western Palearctic Birds: Passerines, Flycatchers to Buntings (Vol. 2). Bloomsbury Publishing Limited.
- Shochat, E. (2004). Credit or debit? Resource input changes population dynamics of city-slicker birds. *Oikos*, 106(3), 622–626. https://doi.org/10.1111/j.0030-1299.2004.13159.x

- Shochat, E., Lerman, S. B., Katti, M., & Lewis, D. B. (2004). Linking optimal foraging behavior to bird community structure in an urban-desert landscape: Field experiments with artificial food patches. *American Naturalist*, 164(2), 232–243. https://doi.org/10.1086/422222
- Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., & Hope, D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology and Evolution*, 21(4), 186–191. https://doi.org/10.1016/j.tree.2005.11.019
- Shutt, J. D., Cabello, I. B., Keogan, K., Leech, D. I., Samplonius, J. M., Whittle, L., Burgess, M. D., & Phillimore, A. B. (2019). The environmental predictors of spatio-temporal variation in the breeding phenology of a passerine bird. *Proceedings of the Royal Society B*, 286(1908), 20190952.
- Simmonds, E. G., Cole, E. F., Sheldon, B. C., & Coulson, T. (2020). Phenological asynchrony: A ticking time-bomb for seemingly stable populations? *Ecology Letters*, 23(12), 1766–1775. https://doi.org/10.1111/ele.13603
- Sinkovics, C., Seress, G., Pipoly, I., Vincze, E., & Liker, A. (2023). Comparison of nestling diet between first and second broods of great tits Parus major in urban and forest habitats. *Animal Biodiversity and Conservation*, *46*(2), 199–212.
- Snell, R. S., Beckman, N. G., Fricke, E., Loiselle, B. A., Carvalho, C. S., Jones, L. R., Lichti, N. I., Lustenhouwer, N., Schreiber, S. J., Strickland, C., Sullivan, L. L., Cavazos, B. R., Giladi, I., Hastings, A., Holbrook, K. M., Jongejans, E., Kogan, O., Montaño-Centellas, F., Rudolph, J., ... Schupp, E. W. (2019). Consequences of intraspecific variation in seed dispersal for plant demography, communities, evolution and global change. *AoB PLANTS*, *11*(4), 1–19. https://doi.org/10.1093/aobpla/plz016
- Snell-Rood, E. C. (2013). An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour, 85(5), 1004–1011. https://doi.org/10.1016/j.anbehav.2012.12.031
- Snell-Rood, E. C., Kobiela, M. E., Sikkink, K. L., & Shephard, A. M. (2018). Mechanisms of plastic rescue in novel environments. *Annual Review of Ecology, Evolution, and Systematics*, 49, 331– 354. https://doi.org/10.1146/annurev-ecolsys-110617-062622
- Snell-Rood, E., Cothran, R., Espeset, A., Jeyasingh, P., Hobbie, S., & Morehouse, N. I. (2015). Lifehistory evolution in the anthropocene: Effects of increasing nutrients on traits and trade-offs. *Evolutionary Applications*, 8(7), 635–649. https://doi.org/10.1111/eva.12272
- Sol, D., Griffin, A. S., Bartomeus, I., & Boyce, H. (2011). Exploring or Avoiding Novel Food Resources? The Novelty Conflict in an Invasive Bird. *PLoS ONE*, 6(5), e19535. https://doi.org/10.1371/journal.pone.0019535
- Sol, D., Lapiedra, O., & Ducatez, S. (2020). Cognition and adaptation to urban. In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), *Urban Evolutionary Biology* (p. 253–266). Oxford University Press, USA.
- Sol, D., Lapiedra, O., & González-Lagos, C. (2013). Behavioural adjustments for a life in the city. *Animal Behaviour*, 85(5), 1101–1112. https://doi.org/10.1016/j.anbehav.2013.01.023

- Sol, D., Trisos, C., Múrria, C., Jeliazkov, A., González-Lagos, C., Pigot, A. L., Ricotta, C., Swan, C. M., Tobias, J. A., & Pavoine, S. (2020). The worldwide impact of urbanisation on avian functional diversity. *Ecology Letters*, 23(6), 962–972. https://doi.org/10.1111/ele.13495
- Somers, C. M., McCarry, B. E., Malek, F., & Quinn, J. S. (2004). Reduction of particulate air pollution lowers the risk of heritable mutations in mice. *Science*, 304(5673), 1008–1010. https://doi.org/10.1126/science.1095815
- Sonnenberg, B. R., Branch, C. L., Pitera, A. M., Bridge, E., & Pravosudov, V. V. (2019). Natural selection and spatial cognition in wild food- caching mountain chickadees. *Current Biology*, 29(4), 1–7. https://doi.org/10.1016/j.cub.2019.01.006
- Soravia, C., Ashton, B. J., Thornton, A., & Ridley, A. R. (2023). High temperatures are associated with reduced cognitive performance in wild southern pied babblers. *Proceedings of the Royal Society B: Biological Sciences*, 290(2011), 20231077. https://doi.org/10.1098/rspb.2023.1077
- Sprau, Philipp., Mouchet, Alexia., & Dingemanse, N. J. (2017). Multidimensional environmental predictors of variation in avian forest and city life histories. *Behavioral Ecology*, 28(1), 59–68. https://doi.org/10.1093/beheco/arw130
- Stan Development Team. (2023). *Stan Modeling Language Users Guide and Reference Manual* [Computer software]. https://mc-stan.org
- Stirling, D. G., Réale, D., & Roff, D. A. (2002). Selection, structure and the heritability of behaviour. *Journal of Evolutionary Biology*, 15(2), 277–289. https://doi.org/10.1046/j.1420-9101.2002.00389.x
- Stofberg, M., Cunningham, S. J., Sumasgutner, P., & Amar, A. (2019). Juggling a "junk-food" diet: Responses of an urban bird to fluctuating anthropogenic-food availability. *Urban Ecosystems*, 22(6), 1019–1026. https://doi.org/10.1007/s11252-019-00885-3
- Strubbe, D., Salleh Hudin, N., Teyssier, A., Vantieghem, P., Aerts, J., & Lens, L. (2020). Phenotypic signatures of urbanization are scale-dependent: A multi-trait study on a classic urban exploiter. *Landscape and Urban Planning*, 197(February), 103767. https://doi.org/10.1016/j.landurbplan.2020.103767
- Sumasgutner, P., Cunningham, S. J., Hegemann, A., Amar, A., Watson, H., Nilsson, J. F., Andersson, M. N., & Isaksson, C. (2023). Interactive effects of rising temperatures and urbanisation on birds across different climate zones: A mechanistic perspective. *Global Change Biology*, 29(9), 2399–2420. https://doi.org/10.1111/gcb.16645
- Sung, Y. J., Schwander, K., Arnett, D. K., Kardia, S. L., Rankinen, T., Bouchard, C., Boerwinkle, E., Hunt, S. C., & Rao, D. C. (2014). An empirical comparison of meta-analysis and mega-analysis of individual participant data for identifying gene-environment interactions. *Genetic Epidemiology*, 38(4), 369–378. https://doi.org/10.1002/gepi.21800
- Svensson, L. (1992). Identification guide to European passerines. Edicao do autor. Stockholm.
- Swan, G. J. F., Redpath, S. M., Bearhop, S., & McDonald, R. A. (2017). Ecology of problem individuals and the efficacy of selective wildlife management. *Trends in Ecology and Evolution*, 32(7), 518– 530. https://doi.org/10.1016/j.tree.2017.03.011

- Szulkin, M., Garroway, C. J., Corsini, M., Kotarba, A. Z., & Dominoni, D. (2020). How to Quantify Urbanization When Testing for Urban Evolution? In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), Urban Evolutionary Biology (p. 13). Oxford University Press, USA.
- Szulkin, M., Munshi-South, J., & Charmantier, A. (2020). Urban evolutionary biology. Oxford University Press, USA.
- Szulkin, M., & Sheldon, B. C. (2008). Dispersal as a means of inbreeding avoidance in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences*, 275(1635), 703–711. https://doi.org/10.1098/rspb.2007.0989
- Taichi, N., & Ushimaru, A. (2024). Trait variation along an urban–rural gradient in Asian dayflower: The contribution of phenotypic plasticity and genetic divergence. *Plant Biology*, 26(1), 74–81. https://doi.org/10.1111/plb.13595
- Talloen, W., Lens, L. U. C., Van Dongen, S., Adriaensen, F., & Matthysen, E. (2010). Mild stress during development affects the phenotype of great tit *Parus major* nestlings: A challenge experiment. *Biological Journal of the Linnean Society*, 100(1), 103–110. https://doi.org/10.1111/j.1095-8312.2010.01422.x
- Templeton, C. N., O'Connor, A., Strack, S., Meraz, F., & Herranen, K. (2023). Traffic noise inhibits inhibitory control in wild-caught songbirds. *Iscience*, 26(5). https://doi.org/10.1016/j.isci.2023.106650
- Tene Fossog, B., Antonio-Nkondjio, C., Kengne, P., Njiokou, F., Besansky, N. J., & Costantini, C. (2013). Physiological correlates of ecological divergence along an urbanization gradient: Differential tolerance to ammonia among molecular forms of the malaria mosquito *Anopheles gambiae*. *BMC Ecology*, 13(1), 1–12. https://doi.org/10.1186/1472-6785-13-1
- Tene Fossog, B., Poupardin, R., Costantini, C., Awono-Ambene, P., Wondji, C. S., Ranson, H., & Antonio-Nkondjio, C. (2013). Resistance to DDT in an urban setting: Common mechanisms implicated in both M and S forms of *Anopheles gambiae* in the city of Yaoundé Cameroon. *PloS* One, 8(4), e61408. https://doi.org/10.1371/journal.pone.0061408
- Thackeray, S. J., Henrys, P. A., Hemming, D., Bell, J. R., Botham, M. S., Burthe, S., Helaouet, P., Johns, D. G., Jones, I. D., Leech, D. I., MacKay, E. B., Massimino, D., Atkinson, S., Bacon, P. J., Brereton, T. M., Carvalho, L., Clutton-Brock, T. H., Duck, C., Edwards, M., ... Wanless, S. (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature*, *535*(7611), 241–245. https://doi.org/10.1038/nature18608
- Theodorou, P., Baltz, L. M., Paxton, R. J., & Soro, A. (2021). Urbanization is associated with shifts in bumblebee body size, with cascading effects on pollination. *Evolutionary Applications*, 14(1), 53– 68. https://doi.org/10.1111/eva.13087
- Therneau, T. M. (2024). *coxme: Mixed Effects Cox Models* (R package version 2.2-20) [Computer software]. https://CRAN.R-project.org/package=coxme>
- Therneau, T. M., & Grambsch, P. M. (2000). *Modeling Survival Data: Extending the Cox Model*. Springer New York. https://doi.org/10.1007/978-1-4757-3294-8_3

- Thompson, K. A., Renaudin, M., & Johnson, M. T. J. (2016). Urbanization drives the evolution of parallel clines in plant populations. *Proceedings of the Royal Society B: Biological Sciences*, 28(1845), 20162180. 10.1098/rspb.2016.2180
- Thompson, K. A., Rieseberg, L. H., & Schluter, D. (2018). Speciation and the city. *Trends in Ecology and Evolution*, *33*(11), 815–826. https://doi.org/10.1016/j.tree.2018.08.007
- Thompson, M. J., Capilla-Lasheras, P., Dominoni, D. M., Réale, D., & Charmantier, A. (2022). Phentoypic variation in urban environments: Mechanisms and implications. *Trends in Ecology & Evolution*, 37, 171–182. https://doi.org/10.1016/j.tree.2021.09.009
- Thorley, J. B., & Lord, A. M. (2015). Laying date is a plastic and repeatable trait in a population of blue tits Cyanistes caeruleus. *Ardea*, 103(1), 69–78. https://doi.org/10.5253/arde.v103i1.a7
- Tryjanowski, P., Skórka, P., Sparks, T. H., Biaduń, W., Brauze, T., Hetmański, T., Martyka, R., Indykiewicz, P., Myczko, Ł., Kunysz, P., Kawa, P., Czyż, S., Czechowski, P., Polakowski, M., Zduniak, P., Jerzak, L., Janiszewski, T., Goławski, A., Duduś, L., ... Wysocki, D. (2015). Urban and rural habitats differ in number and type of bird feeders and in bird species consuming supplementary food. *Environmental Science and Pollution Research*, 22, 15097–15103. https://doi.org/10.1007/s11356-015-4723-0
- Tüzün, N., Debecker, S., de Beeck, L. O., & Stoks, R. (2015). Urbanisation shapes behavioural responses to a pesticide. *Aquatic Toxicology*, 163, 81–88. https://doi.org/10.1016/j.aquatox.2015.04.002
- Tüzün, N., Mueller, S., Koch, K., & Stoks, R. (2017). Pesticide-induced changes in personality depend on the urbanization level. *Animal Behaviour*, 134, 45–55. https://doi.org/10.1016/j.anbehav.2017.10.007
- Tüzün, N., Op de Beeck, L., Brans, K. I., Janssens, L., & Stoks, R. (2017). Microgeographic differentiation in thermal performance curves between rural and urban populations of an aquatic insect. *Evolutionary Applications*, 10(10), 1067–1075. https://doi.org/10.1111/eva.12512
- Tüzün, N., & Stoks, R. (2021). Lower bioenergetic costs but similar immune responsiveness under a heat wave in urban compared to rural damselflies. *Evolutionary Applications*, 14(1), 24–35. https://doi.org/10.1111/eva.13041
- Uchida, K., Blakey, R. V., Burger, J. R., Cooper, D. S., Niesner, C. A., & Blumstein, D. T. (2021). Urban biodiversity and the importance of scale. *Trends in Ecology and Evolution*, 36(2), 123–131. https://doi.org/10.1016/j.tree.2020.10.011
- United Nations. (2019). Department of Economic and Social Affairs, Population Division. World Urbanization Prospects: The 2018 Revision (ST/ESA/SER.A/420). Department of Economic and Social Affairs.
- Urban, M. C., Alberti, M., De Meester, L., Zhou, Y., Verrelli, B. C., Szulkin, M., Schmidt, C., Savage, A. M., Roberts, P., & Rivkin, L. R. (2024). Interactions between climate change and urbanization will shape the future of biodiversity. *Nature Climate Change*, 1–12. https://doi.org/10.1038/s41558-024-01996-2
- Van Balen, J. H. (1973). A comparative sudy of the breeding ecology of the great Tit Parus major in different habitats. *Ardea*, 55(1–2), 1–93. https://doi.org/10.5253/arde.v61.p1

- Van de Schoot, E., Merckx, T., Ebert, D., Wesselingh, R. A., Altermatt, F., & Van Dyck, H. (2024). Evolutionary change in flight-to-light response in urban moths comes with changes in wing morphology. *Biology Letters*, 20(3), 20230486. https://doi.org/10.1098/rsbl.2023.0486
- Van Den Heuvel, K., Quinn, J. L., Kotrschal, A., & Van Oers, K. (2023). Artificial selection for reversal learning reveals limited repeatability and no heritability of cognitive flexibility in great tits (*Parus major*). Proceedings of the Royal Society B: Biological Sciences, 290(2003), 20231067. https://doi.org/10.1098/rspb.2023.1067
- van Dongen, W. F. D., Robinson, R. W., Weston, M. A., Mulder, R. A., & Guay, P. J. (2015). Variation at the DRD4 locus is associated with wariness and local site selection in urban black swans. *BMC Evolutionary Biology*, 15(1), 1–11. https://doi.org/10.1186/s12862-015-0533-8
- van Horik, J. O., Beardsworth, C. E., Laker, P. R., Langley, E. J. G., Whiteside, M. A., & Madden, J. R. (2019). Unpredictable environments enhance inhibitory control in pheasants. *Animal Cognition*, 22(6), 1105–1114. https://doi.org/10.1007/s10071-019-01302-0
- van Horik, J. O., Langley, E. J. G., Whiteside, M. A., Laker, P. R., Beardsworth, C. E., & Madden, J. R. (2018). Do detour tasks provide accurate assays of inhibitory control? *Proceedings of the Royal Society B: Biological Sciences*, 285(1875), 20180150. https://doi.org/10.1098/rspb.2018.0150
- Van Noordwijk, A. J., Keizer, L. C. P., Van Balen, J. H., & Scharloo, W. (1981). Genetic variation in egg dimensions in natural populations of the Great Tit. *Genetica*, 55, 221–232. https://doi.org/10.1007/BF00127206
- Van Noordwijk, A. J., Van Balen, J. H., & Scharloo, W. (1981). Genetic variation in the timing of reproduction in the great tit. *Oecologia*, 49, 158–166. https://doi.org/10.1007/BF00349183
- van Overveld, T., Adriaensen, F., & Matthysen, E. (2011). Postfledging family space use in great tits in relation to environmental and parental characteristics. *Behavioral Ecology*, 22(4), 899–907. https://doi.org/10.1093/beheco/arr063
- Vanden Broeck, A., Cox, K., Melosik, I., Maes, B., & Smets, K. (2018). Genetic diversity loss and homogenization in urban trees: The case of *Tilia × europaea* in Belgium and the Netherlands. *Biodiversity and Conservation*, 27(14), 3777–3792. https://doi.org/10.1007/s10531-018-1628-5
- Vaugoyeau, M., Adriaensen, F., Artemyev, A., Bańbura, J., Barba, E., Biard, C., Blondel, J., Bouslama, Z., Bouvier, J.-C., Camprodon, J., Cecere, F., Charmantier, A., Charter, M., Cichoń, M., Cusimano, C., Czeszczewik, D., Demeyrier, V., Doligez, B., Doutrelant, C., ... Møller, A. P. (2016). Interspecific variation in the relationship between clutch size, laying date and intensity of urbanization in four species of hole-nesting birds. *Ecology and Evolution*, *6*(16), 5907–5920. https://doi.org/10.1002/ece3.2335
- Vehtari, A., Gelman, A., Simpson, D., Carpenter, B., & Bürkner, P.-C. (2021). Rank-normalization, folding, and localization: An improved R^f for assessing convergence of MCMC (with discussion). *Bayesian Analysis*, 16(2), 667–718. https://doi.org/10.1214/20-BA1221
- Verboven, N., Tinbergen, J. M., & Verhulst, S. (2001). Food, reproductive success and multiple breeding in the great tit *Parus major*. *Ardea*, 89(2), 387–406.

- Verrelli, B. C., Alberti, M., Des Roches, S., Harris, N. C., Hendry, A. P., Johnson, M. T., Savage, A. M., Charmantier, A., Gotanda, K. M., & Govaert, L. (2022). A global horizon scan for urban evolutionary ecology. *Trends in Ecology & Evolution*. https://doi.org/10.1016/j.tree.2022.07.012
- Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, *39*(3), 505–522. https://doi.org/10.1111/j.1558-5646.1985.tb00391.x
- Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor. *Journal of Statistical Software*, 36(3), 1–48. https://doi.org/10.18637/jss.v036.i03
- Vincze, E., & Kovács, B. (2022). Urbanization's effects on problem solving abilities: a meta-analysis. *Frontiers in Ecology and Evolution*, 10, 834436. https://doi.org/10.3389/fevo.2022.834436
- Vincze, E., Seress, G., Lagisz, M., Nakagawa, S., Dingemanse, N. J., & Sprau, P. (2017). Does urbanization affect predation of bird nests? A meta-analysis. *Frontiers in Ecology and Evolution*, 5, 1–12. https://doi.org/10.3389/fevo.2017.00029
- Violle, C., Enquist, B. J., McGill, B. J., Jiang, L., Albert, C. H., Hulshof, C., Jung, V., & Messier, J. (2012). The return of the variance: Intraspecific variability in community ecology. *Trends in Ecology and Evolution*, 27(4), 244–252. https://doi.org/10.1016/j.tree.2011.11.014
- Visser, M. E., Adriaensen, F., Van Balen, J. H., Blondel, J., Dhondt, A. A., Van Dongen, S., du Feu Chris, J., Ivankina, E. A., Kerimov, A. B., de Laet, J., Matthysen, E., McCleery, R. H., Orell, M., & Thomson, D. L. (2003). Variable responses to large-scale climate change in European *Parus* populations. *Proceedings of the Royal Society B: Biological Sciences*, 270, 367–372. https://doi.org/10.1098/rspb.2002.2244
- Visser, M. E., & Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nature Ecology and Evolution*, 3(6), 879–885. https://doi.org/10.1038/s41559-019-0880-8
- Visser, M. E., Holleman, L. J., & Caro, S. P. (2009). Temperature has a causal effect on avian timing of reproduction. *Proceedings of the Royal Society B: Biological Sciences*, 276(1665), 2323–2331. https://doi.org/10.1098/rspb.2009.0213
- Visser, M. E., te Marvelde, L., & Lof, M. E. (2012). Adaptive phenological mismatches of birds and their food in a warming world. *Journal of Ornithology*, 153, 75–84. https://doi.org/10.1007/s10336-011-0770-6
- Völter, C. J., Tinklenberg, B., Call, J., & Seed, A. M. (2018). Comparative psychometrics: Establishing what differs is central to understanding what evolves. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1756), 20170283. https://doi.org/10.1098/rstb.2017.0283
- von Merten, S., Oliveira, F. G., Tapisso, J. T., Pustelnik, A., da Luz Mathias, M., & Rychlik, L. (2022). Urban populations of shrews show larger behavioural differences among individuals than rural populations. *Animal Behaviour*, 187, 35–46. https://doi.org/10.1016/j.anbehav.2022.02.012
- Walsh, B., & Blows, M. W. (2009). Abundant genetic variation+ strong selection= multivariate genetic constraints: A geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics*, 40, 41–59. https://doi.org/10.1146/annurev.ecolsys.110308.120232

- Wang Wei, J., Lee, B. P. Y., & Bing Wen, L. (2016). Citizen science and the urban ecology of birds and butterflies—A systematic review. *Plos One*, 11(6), e0156425. https://doi.org/10.1371/journal.pone.0156425
- Waterschoot, B. O. G., Bataille, G., & Van Dyck, H. (2023). Spatial scale-dependent effects of urbanisation on phenotypic traits in a thermophilous grasshopper. *Behavioral Ecology and Sociobiology*, 77(5), 1–20. https://doi.org/10.1007/s00265-023-03325-7
- Watson, H., Powell, D., Salmón, P., Jacobs, A., & Isaksson, C. (2020). Urbanisation is associated with modifications in DNA methylation in a small passerine bird. *Evolutionary Applications*, 14(1), 85–98. https://doi.org/10.1111/eva.13160
- Weismann, A. (1882). *Studies in the theory of descent* (1–2). Sampson, Low, Marston, Searle and Rivington.
- Westby, K. M., & Medley, K. A. (2020). Cold nights, city lights: Artificial light at night reduces photoperiodically induced diapause in urban and rural populations of *aedes albopictus* (Diptera: *Culicidae*). Journal of Medical Entomology, 57(6), 1694–1699. https://doi.org/10.1093/jme/tjaa139
- Weston, L. M., Mattingly, K. Z., Day, C. T. C., & Hovick, S. M. (2021). Potential local adaptation in populations of invasive reed canary grass (*Phalaris arundinacea*) across an urbanization gradient. *Ecology and Evolution*, 11(16), 11457–11476. https://doi.org/10.1002/ece3.7938
- Whitehead, A., Pilcher, W., Champlin, D., & Nacci, D. (2012). Common mechanism underlies repeated evolution of extreme pollution tolerance. *Proceedings of the Royal Society B: Biological Sciences*, 279(1728), 427–433. https://doi.org/10.1098/rspb.2011.0847
- Wilkin, T. A., Garant, D., Gosler, A. G., & Sheldon, B. C. (2006). Density effects on life-history traits in a wild population of the great tit *Parus major*: Analyses of long-term data with GIS techniques. *Journal of Animal Ecology*, 75(2), 604–615. https://doi.org/10.1111/j.1365-2656.2006.01078.x
- Williams, D. M., Nguyen, P.-T., Chan, K., Krohn, M., & Blumstein, D. T. (2019). High human disturbance decreases individual variability in skink escape behavior. *Current Zoology*, 66(May 2019), 63–70. https://doi.org/10.1093/cz/zoz027
- Willmore, K. E., Young, N. M., & Richtsmeier, J. T. (2007). Phenotypic variability: Its components, measurement and underlying developmental processes. *Evolutionary Biology*, 34(3–4), 99–120. https://doi.org/10.1007/s11692-007-9008-1
- Winchell, K. M., Reynolds, R. G., Prado-Irwin, S. R., Puente-Rolón, A. R., & Revell, L. J. (2016). Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, 70(5), 1009– 1022. https://doi.org/10.1111/evo.12925
- Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology and Evolution*, 27(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001
- Wright, J. P., Ames, G. M., & Mitchell, R. M. (2016). The more things change, the more they stay the same? When is trait variability important for stability of ecosystem function in a changing environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1694), 20150272. https://doi.org/10.1098/rstb.2015.0272

- Wright, S. (1921). Systems of mating. I. The biometric relations between parent and offspring. *Genetics*, 6(2), 111. https://doi.org/10.1093/genetics/6.2.111
- Xue, Y., & Schifano, E. D. (2017). Diagnostics for the Cox model. Communications for Statistical Applications and Methods, 24(6), 583–604. https://doi.org/10.29220/CSAM.2017.24.6.583
- y Gomez, G. S. M., & Van Dyck, H. (2012). Ecotypic differentiation between urban and rural populations of the grasshopper *Chorthippus brunneus* relative to climate and habitat fragmentation. *Oecologia*, *169*(1), 125–133. https://doi.org/10.1007/s00442-011-2189-4
- Yakub, M., & Tiffin, P. (2017). Living in the city: Urban environments shape the evolution of a native annual plant. *Global Change Biology*, 23(5), 2082–2089. https://doi.org/10.1111/gcb.13528
- Yauk, C. L., Fox, G. A., McCarry, B. E., & Quinn, J. S. (2000). Induced minisatellite germline mutations in herring gulls (Larus argentatus) living near steel mills. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*, 452(2), 211–218. https://doi.org/10.1016/S0027-5107(00)00093-2
- Yauk, C., Polyzos, A., Rowan-Carroll, A., Somers, C. M., Godschalk, R. W., Van Schooten, F. J., Berndt, M. L., Pogribny, I. P., Koturbash, I., Williams, A., Douglas, G. R., & Kovalchuk, O. (2008). Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *Proceedings of the National Academy of Sciences of the United States of America*, 105(2), 605–610. https://doi.org/10.1073/pnas.0705896105
- Yeh, P. J. (2004). Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution*, 58(1), 166–174. https://doi.org/10.1111/j.0014-3820.2004.tb01583.x
- Yeh, P. J., & Price, T. D. (2004). Adaptive phenotypic plasticity and the successful colonization of a novel environment. *The American Naturalist*, *164*(4), 531–542. https://doi.org/10.1086/423825
- Yilmaz, A. R., Diamond, S. E., & Martin, R. A. (2021). Evidence for the evolution of thermal tolerance, but not desiccation tolerance, in response to hotter, drier city conditions in a cosmopolitan, terrestrial isopod. *Evolutionary Applications*, 14(1), 12–23. https://doi.org/10.1111/eva.13052
- You, W., & Henneberg, M. (2018). Relaxed natural selection contributes to global obesity increase more in males than in females due to more environmental modifications in female body mass. *PLoS* ONE, 13(7), 1–20. https://doi.org/10.1371/journal.pone.0199594
- Young, E. A., & Postma, E. (2023). Low interspecific variation and no phylogenetic signal in additive genetic variance in wild bird and mammal populations. *Ecology and Evolution*, *13*(11), e10693. https://doi.org/10.1002/ece3.10693
- Youngflesh, C., Saracco, J. F., Siegel, R. B., & Tingley, M. W. (2022). Abiotic conditions shape spatial and temporal morphological variation in North American birds. *Nature Ecology & Evolution*, 6(12), 1860–1870. https://doi.org/10.1038/s41559-022-01893-x
- Zajitschek, S. R., Zajitschek, F., Bonduriansky, R., Brooks, R. C., Cornwell, W., Falster, D. S., Lagisz, M., Mason, J., Senior, A. M., & Noble, D. W. (2020). Sexual dimorphism in trait variability and its eco-evolutionary and statistical implications. *Elife*, 9, e63170. https://doi.org/10.7554/eLife.63170

- Zhang, H., He, Y., Yang, J., Mao, H., & Jiang, X. (2022). Contemporary adaptive evolution in fragmenting river landscapes: Evidence from the native waterflea *Ceriodaphnia cornuta*. *Journal* of *Plankton Research*, 44(1), 88–98. https://doi.org/10.1093/plankt/fbab077
- Zhou, B., Rybski, D., & Kropp, J. P. (2017). The role of city size and urban form in the surface urban heat island. *Scientific Reports*, 7(1), 1–9. https://doi.org/10.1038/s41598-017-04242-2
- Zhou, Y., & Levy, J. I. (2007). Factors influencing the spatial extent of mobile source air pollution impacts: A meta-analysis. *BMC Public Health*, 7, 1–11. https://doi.org/10.1186/1471-2458-7-89
- Zwolak, R. (2018). How intraspecific variation in seed-dispersing animals matters for plants. *Biological Reviews*, 93(2), 897–913. https://doi.org/10.1111/brv.12377