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EFFET DES INTERACTIONS ENTRE LE GÉNOTYPE ET L'ENVIRONNEMENT SUR LES TRAITS SEXUELS ET L'IMMUNITÉ

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CAM	Cumulative Assessment Model
GxE	Genotype by environment
h^2	Narrow-sense heritability
m^2	Maternal effect
RHP	Resource-Holding Potential
SAM	Sequential Assessment Model
V_D	Variability attributed to the dam
V_P	Phenotypic variation
V_R	Residual variation
V_S	Variability attributed to the sire

RÉSUMÉ

La valeur sélective d'un individu, aussi appelé "fitness", est généralement dictée par la quantité de ressources qui peut être acquise. En effet, les individus ayant plus de ressources peuvent allouer celles-ci à des traits liés à leur valeur sélective. La quantité de ressources qu'un individu peut acquérir est déterminée par son génotype et son environnement. De plus, il peut y avoir une interaction entre le génotype et l'environnement afin d'établir un phénotype. La prédiction que les individus élevés sous haute densité de population investissent plus dans leurs traits sexuels et leur immunité que les individus en basse densité de population a été testée dans ce projet. L'interaction entre le génotype et l'environnement sur les traits sexuels et l'immunité a également été testée en élevant des grillons (*Gryllus firmus*) en haute et basse densité en utilisant une approche de génétique quantitative. La réponse immunitaire d'un tiers des grillons a été mesuré. La taille des harpes et des mandibules en plus du poids des gonades ont servi d'indice d'investissement en reproduction. Afin d'avoir un meilleur indice de l'investissement précopulatoire, nous avons organisé des combats de grillons. Ceux-ci ont permis d'établir si la taille des mandibules et le niveau d'agressivité augmentent les chances de gagner les combats et si celles-ci varient selon la densité de population lors du développement.

Contrairement à notre hypothèse initiale, aucune interaction entre le génotype et la densité de population sur les traits sexuels ou l'immunité n'a été trouvée. Les grillons d'une même famille ne réagissaient pas de la même façon à la densité de population et au test d'immunité. Les traits qui sont fortement corrélés avec la taille corporelle étaient positivement influencés par la densité. Le seul trait affecté par la densité de population tel que prédit était la taille des harpes, mais pas la taille des testicules et la taille des mandibules. La densité de population n'influençait aucunement l'investissement des grillons dans leur immunité. Bien que la densité de population n'influençât pas la taille des mandibules, ces dernières influençaient l'intensité du combat. Ainsi, l'environnement dans lequel un individu grandit ne semble pas influencer son "fitness" de la manière qui était prédite et l'interaction entre le génotype et l'environnement est moins forte qu'attendu. Pour ce qui est des règlements de conflits entre les grillons, il semblerait que leur mode de combats soit mieux décrit par le CAM ("cumulative assessment model"), bien que les combats de grillons possèdent des éléments des modèles d'auto-évaluation. La théorie ne correspond donc pas parfaitement à ce qui est observé dans la nature.

Mots-clés: Génétique quantitative, interaction génétique x environnement, densité de population, traits sexuels, immunité, combats, grillons

INTRODUCTION

0.1 Effet de l'environnement sur les traits sexuels

La valeur sélective d'un individu, aussi appelé « fitness », est généralement dictée par la quantité de ressources qui peut être acquise. En effet, les individus ayant plus de ressources (c'est-à-dire en meilleure forme; Rowe & Houle, 1996) peuvent allouer plus de ressources à des traits liés à leur valeur sélective (Van Noordwijk & De Jong, 1986). La quantité de ressources qu'un individu peut acquérir est déterminée par son génotype et son environnement (Reznick, Nunney, & Tessier, 2000). Certains génotypes sont meilleurs pour acquérir et assimiler les ressources. Bien que certains individus vont acquérir plus de ressources que d'autres, les individus devront toujours faire un compromis entre un trait d'histoire de vie (ex. : la reproduction) et un autre (ex. : l'immunité) (Adamo, Jensen, & Younger, 2001; Cox *et al.*, 2010; Gustafsson, Qvarnström, & Sheldon, 1995; Stearns, 1989; Van Noordwijk & De Jong, 1986). Il est important de noter que la quantité totale de ressources qui est acquise peut ultimement être dictée par l'environnement. Donc, en théorie, les individus ayant de meilleurs génotypes et étant élevés dans des environnements riches en ressources devraient être en meilleure condition et être en mesure d'allouer plus de ressources à des traits liés à leur valeur sélective (Van Noordwijk & De Jong, 1986).

La qualité de la diète, et donc la quantité de ressources qu'un individu peut acquérir, va affecter plusieurs traits d'histoire de vie. En effet, chez les coléoptères, une diète pauvre chez la mère augmente le temps de développement et diminue le taux de survie de leur progéniture (Fox & Dingle, 1994). Il a aussi été observé que la variété de la diète influençait la vitesse de croissance, ainsi que l'âge et le poids à la métamorphose chez la rainette du Pacifique (Kupferberg, Marks, & Power, 1994). Une restriction de diète diminue la fertilité chez les papillons (Boggs & Ross, 1993); le succès reproducteur, la taille de la couvée, la taille des oisillons, la taille à maturité et le statut social chez la corneille noire (Richner, 1992; Richner, Schneiter, & Stirnimann, 1989); ainsi que la longévité et les capacités immunitaires chez les grillons (Kelly & Tawes, 2013; Lyn *et al.*, 2011).

Bien que les ressources alimentaires soient importantes pour déterminer le phénotype adulte, l'environnement biotique d'un individu influence grandement la physiologie des individus à leur maturité ainsi que leurs traits sexuels (Griffith, Owens, & Burke, 1999; Rowe & Houle, 1996). Les traits sexuels peuvent être sous-divisés en traits sexuels pré-copulatoires et traits sexuels post-copulatoires. Les traits sexuels pré-copulatoires sont les traits qui permettent à un individu d'acquérir des partenaires sexuels (Andersson & Iwasa, 1996). Les mâles acquièrent leurs partenaires de plusieurs manières. Ils peuvent acquérir des territoires nécessaires pour les femelles, chanter, avoir des traits visuellement manifestes, se battre entre eux, intercepter les femelles sur le territoire d'autres mâles, etc. (Andersson & Iwasa, 1996; Cade, 1980).

Les mâles peuvent rester en compétition les uns contre les autres après la copulation. Lorsque les femelles se reproduisent avec deux ou plusieurs mâles lors d'un épisode reproducteur, leurs spermes vont être en compétition pour féconder les œufs (Parker, 1970; Parker, 1998). Les traits sexuels post-copulatoires sont par conséquent les traits permettant à un individu un meilleur succès reproducteur après la copulation et donc lors de la compétition spermatique. Le succès d'un mâle lors de la compétition spermatique va être déterminé par la quantité de spermatozoïdes ou bien leur qualité (Parker, 1998; Simmons, 1987; Snook, 2005). La taille des testicules et leur masse sont de bons indicateurs de l'investissement du mâle dans ses traits post-copulatoires. Ceci a été démontré, par exemple, chez les coléoptères (*Callosobruchus spp.*; Katvala, Rönn, & Arnqvist, 2008), les scatophages du fumier (*Scathophaga stercoraria*; Hosken

& Ward, 2001), les grillons (Gage & Barnard, 1996), les oiseaux (Moller, 1988), et les grenouilles (*Crinia georgiana*; Buzatto, Roberts, & Simmons, 2015).

Comme mentionné plus haut, l'environnement biotique et abiotique lors du développement affecte fortement l'expression des traits sexuels. Un des facteurs environnementaux influençant le développement des individus est la densité de population (Whitehouse & Lewis, 1973). Il a été montré qu'une haute densité de population lors du développement pouvait diminuer la taille corporelle et augmenter la durée de développement chez les moustiques (Gimnig *et al.*, 2002). Celle-ci affecte la proportion de différents morphes au sein d'une population de salamandre (Collins & Cheek, 1983) et augmente le nombre de portées ainsi que la taille de la portée chez les cyclopes (Whitehouse & Lewis, 1973). Alonso-Pimnetel et Papaj (1996) ont montré qu'une haute densité de population entraine une augmentation de la durée de copulation chez mouche du brou de la noix (*Rhagoletis juglis*). Aussi, Grant et Foam (2002) ont montré qu'un ratio sexuel plus fort pour les mâles cause une augmentation du taux d'agression intrasexuelle et Clark et Grant (2010) ont montré que cela cause une augmentation des comportements de séduction chez le médaka *Oryzias latipes* et De Jong *et al.* (2009) chez les gobbies *Gobiusculus flavescens*.

L'environnement social lors du développement peut, entre autres, apporter des indices de l'intensité de la compétition future pour la fécondation des femelles (Emlen & Oring, 1977; He & Tsubaki, 1991; Knell, 2009; Kokko & Rankin, 2006). En effet, il a été démontré qu'une haute densité de population amène les mâles à augmenter leur investissement dans leurs traits sexuels (Kasumovic, Hall, & Brooks, 2012). Chez les mâles, la densité de population lors du développement affecte les ornements qui aident dans la séduction (Vergara *et al.*, 2012). Aussi, un mâle développe de plus grandes armes en fonction du niveau de compétition qu'il connait lors de son développement (Pomfret & Knell, 2008; Radwan, Unrug, & Tomkins, 2002). La densité de population influence ce niveau de compétition et peut donc modifier la taille des armes qui aideront dans la résolution de conflits entre les mâles. La densité de population peut influencer la sélection sexuelle, car elle affecte plus particulièrement les mâles, qui verront une augmentation du risque de compétition pour des partenaires sexuels avec une augmentation de la densité de population dans un système polygame. En réponse à cette augmentation de la compétition, les mâles investiront plus dans leurs traits sexuels. En concordance, Kasumovic & Brooks (2011) montrent qu'une haute densité de population mène à un investissement dans les traits pré-copulatoires chez plusieurs espèces.

Aussi, la densité de population étant un indice indirect du niveau de compétition spermatique dans le futur, elle influence également la taille des testicules (Hosken & Ward, 2001). En effet, un haut taux de compétition perçu lors du développement amènera les individus à investir dans la taille de leur testicule afin de produire plus de spermatozoïdes et d'être en mesure de rivaliser avec le sperme d'autres mâles pour la fécondation des femelles. Par exemple, Buzatto *et al.* (2015) ont montré que la densité de population affectait l'investissement dans la taille des testicules et la qualité de l'éjaculat chez la grenouille *Crinia georgiana* et Hosken & Ward (2001) ont montré un résultat similaire chez la mouche *Scathophaga stercoraria.*. Gage (1995) montre qu'une haute densité de population amène les mâles à investir dans leurs éjaculats en raison de l'augmentation du risque de compétition spermatique chez le papillon de nuit (*Plodia interpunctella*), mais Gay *et al.* (2009) n'a trouvé aucun effet de la densité de population lors du développement sur la taille des testicules chez un coléoptère (*Callosobruchus maculatus*). Les femelles des systèmes polygames n'étant pas soumises aux mêmes pressions de sélection puisqu'elles ne sont pas en compétition pour des partenaires, leurs traits sexuels ne devraient pas être autant influencés par la densité de population (Córdoba-Aguilar, 2009).

Lorsqu'il y a une haute densité de population, il n'est pas clair dans quels traits sexuels sélectionnés les mâles vont investir. Parker *et al.* (1998) et plusieurs autres suggèrent qu'il y a un compromis entre l'investissement dans les traits sexuels pré et post-copulatoire (Buzatto, Tomkins, & Simmons, 2012; Dines *et al.*, 2015; Klaus *et al.*, 2011; Parker, 1998; Parker, Lessells, & Simmons, 2012; Parker, Pizzari, & Road, 2010).

Aussi, le problème avec une haute densité de population est que cela peut aussi entrainer un manque de nourriture et de la compétition pour les ressources. En effet, Sasson, Munoz, Gezan, & Miller (2016) ont montré que la taille des armes et des testicules est également affectée par la diète chez les coreidés (*Narnia femorata*). La plupart des études qui manipulent la densité s'assurent donc d'offrir de la nourriture en abondance.

0.2 Effet de l'environnement sur l'immunité

L'immunité est un trait lié au « fitness » qui impose un épuisement significatif des ressources énergétiques d'un individu, lorsqu'activé. Les individus vont peut-être compromettre leur reproduction pour leur immunité, mais ceux en meilleure condition auront plus de ressources à allouer aux deux fonctions. Par conséquent, nous devrions voir une relation positive entre les traits sexuels et la réponse immunitaire parmi les individus. En effet, la théorie de sélection sexuelle médiée par parasite prédit que l'immunocompétence est héritable et que le niveau d'attirance sexuel du mâle est un signalement honnête de son immunocompétence (Clayton, 1991; Hamilton & Zuk, 1982). Par conséquent, si un mâle est capable de supporter et maintenir des traits sexuels secondaires coûteux, alors il doit être en santé, car il serait trop coûteux de combattre un parasite ou une infection en même temps. L'immunocompétence d'un individu adulte peut être significativement influencée par son environnement lors du développement (Brinkhof *et al.*, 1999; Gross & Siegel, 1988; Lindström, 1999). Par exemple, il a été démontré que le stress environnemental pouvait influencer les compétences immunitaires du poulet (*Gallus gallus domesticus*; Gross & Siegel, 1973). Il a aussi été montré que la diète lors du développement influence l'immunocompétence chez les grillons *Gryllus texensis* (Kelly & Tawes, 2013).

Un autre facteur environnemental important est encore une fois la densité de population. Celle-ci affecte le risque d'infection de plusieurs manières. Bien entendu, plus la densité de population est grande, plus la fréquence de rencontre avec d'autres individus pouvant être infectés augmente et donc aussi le risque de contamination et le risque de transmission des pathogènes (Barnes & Siva-Jothy, 2000; Steinhaus, 1958; Tella *et al.*, 2001). Le risque d'infection d'un individu (Steinhaus, 1958) et le risque d'exposition aux pathogènes chez les adultes (Anderson & May, 1981) est donc directement proportionnel à la densité de population dans laquelle il se trouve (Ebert, 1995). Chez les insectes, une haute densité de population peut activer un virus ou modifier les mécanismes de défense des individus afin de les rendre plus susceptibles à l'infection (Steinhaus, 1958). Une surpopulation peut également augmenter la concentration de matière infectée rejetée par les insectes malades et donc augmenter la chance qu'un individu en santé entre en contact avec de la nourriture contaminée ou autre (Steinhaus, 1958). Ainsi, bien que le mécanisme par lequel la densité de population affecte le risque d'infection d'un individu n'est pas encore clair, il semblerait que les capacités immunitaires d'un individu peuvent être influencées par la densité de population lors du développement. En effet, Reeson *et al.* (1998) ont trouvé qu'une haute densité de population peut amener les individus à investir dans leur immunité et dans leur résistance aux pathogènes. On parle alors de prophylaxies densité-dépendante (Wilson *et al.*, 2001). Calsbeek *et al.* (2008) ont trouvé que la densité de population lors du développement affectait la proportion des différents morphes dans la population, et que celles-ci avaient différentes réponses immunitaires.

En particulier chez les vertébrés et les insectes sociaux, la première source d'infection provient des conspécifiques (Freeland, 1983). Ainsi, l'investissement en immunité dépend des probabilités de rencontrer un défi immunitaire qui est directement lié à la densité de population (Wilson & Reeson, 1998). Investir dans des mécanismes de défense aux pathogènes est coûteux, alors un individu devrait ajuster son investissement dans son immunité en fonction des risques d'infections et donc en fonction de la densité de population chez les insectes (Wilson & Cotter, 2008).

Investir dans son immunité enlève des ressources à d'autres fonctions, telles que la reproduction, puisque toutes les fonctions doivent se partager la même source d'énergie et de ressources (Adamo *et al.*, 2001; Cox *et al.*, 2010; Kelly, 2011). En effet, les mâles ne peuvent pas augmenter leur investissement dans tous leurs traits. Moller, Christe, & Lux (1999) ont montré un compromis entre les fonctions immunitaires et l'expression des traits sexuels secondaires chez les oiseaux. Donc, lesquels favorisent-ils? Hosken (2001) a montré que les mouches mâles favorisent de plus gros testicules aux dépens de leur immunité. Le système immunitaire est donc plastique et peut s'adapter aux conditions environnementales pour allouer ses ressources efficacement (Adamo, Jensen, & Younger, 2001).

0.3 Importance de la génétique pour les traits sexuels et l'immunité

Une autre possibilité est que l'environnement joue un très petit rôle dans la décision des phénotypes exprimés et que ceux-ci sont plus largement influencés par les gènes. Pour qu'il y ait sélection sexuelle sur un trait, le trait sexuel favorisé par les femelles ou celui conférant un avantage dans la compétition intrasexuelle, devra être héritable, et conférer un avantage aux mâles concernés ou à leurs progénitures (Kodric-Brown & Brown, 1984). Par exemple, les traits sexuels secondaires semblent être fortement héritables par le chromosome Y chez les guppies (Karino & Haijima, 2001). Il a aussi été établi que la taille des testicules et la morphologie des spermatozoïdes sont partiellement héritables chez la Teigne des fruits secs (*Onthophagus taurus*; Simmons & Otiaho, 2002). De plus, Hosken & Ward (2001) ont démontré que la taille des testicules peut évoluer selon le niveau de compétition spermatique chez *Scathophaga stercoraria*. Okada & Miyatake (2009) ont montré que les armes étaient un trait génétique chez les coléoptères (*Gnatocerus cornutus*) et Kruuk *et al.* (2002) chez les cerfs élaphes *Cervus elaphus*. Chez les oiseaux (*Ficedula albicollis*), Qvarnström, Brommer, & Gustafsson (2006) ont montré que les ornements étaient aussi un trait héritable. Finalement, la taille des testicules est également un trait héritable chez la drosophile (*Drosophila simulans*) (Joly *et al.*, 1997; Reinhold, 1994).

Les traits liés aux compétences immunitaires peuvent aussi être influencées par la génétique. En effet, Tinsley *et al.* (2006) et plusieurs autres ont montré qu'il y a une variation génétique associée à la résistance aux parasites chez la drosophile (Lazzaro, Sceurman, & Clark, 2004). Même la théorie ou l'hypothèse du handicap d'immunocompétence est basé sur l'héritabilité des traits immunitaires. Cette théorie établit qu'une femelle va montrer une préférence pour un mâle ayant un phénotype dont l'expression est possible uniquement si le mâle est en bonne santé et a une bonne résistance aux pathogènes (Hamilton & Zuk, 1982). Ce choix de la femelle lui permet par la suite d'avoir une progéniture ayant hérité des caractéristiques immunitaires de leur père, et donc ayant eux-mêmes une bonne résistance aux pathogènes (Kurtz & Sauer, 1999; Zhang & He, 2014). La génétique serait de ce fait un important déterminant de la qualité immunitaire d'un individu.

0.4 Effet des interactions génétique-par-environnement

Plusieurs traits sont affectés par une combinaison de la génétique et de l'environnement. Des génotypes différents peuvent réagir de différentes manières à une variation dans l'environnement. Ainsi, certains traits de divers génotypes ne performeront pas de la même manière dans différents environnements (Bowman, 1972). Contrairement à la plasticité phénotypique, où un génotype peut exprimer plusieurs phénotypes en fonction de l'environnement, le phénotype exprimé dans ce cas-ci ne varie pas en fonction de l'environnement, mais certains génotypes sont avantagés dans un environnement, alors que d'autres génotypes sont avantagés dans un autre environnement (Lewis *et al.*, 2012). Cette interaction entre l'environnement et le génotype permet le maintien de plusieurs phénotypes lorsqu'il y a sélection sexuelle (Ingleby, Hunt, & Hosken, 2010; Lewontin, 1974). Par exemple, Lewis *et al.* (2012) ont montré une interaction entre les gènes et l'environnement, plus précisément la qualité de la diète, chez les coléoptères concernant l'habileté de défense du sperme et la longévité. En d'autres termes, les mâles de certaines familles avaient une meilleure défense spermatique lorsque la qualité de la diète était base alors que les mâles de d'autres famille avaient une meilleure défense spermatique lorsque la qualité de la diète était haute. Engqvist (2008) a aussi montré qu'une telle interaction existe pour la vitesse de transfert de sperme chez la mouche scorpion *Panorpa cognata*. Dans la littérature, l'interaction entre le génotype et la densité de population lors du développement a été très peu étudiée (Pérez-Rostro & Ibarra, 2003; Ingleby, Hunt, & Hosken, 2010). Nous ne connaissons pas l'impact de cette interaction sur la sélection sexuelle pré-copulatoire et post-copulatoire (sélection mâle-mâle et sélection mâle-femelle). Encore beaucoup de recherche reste à faire afin de déterminer l'effet de cette interaction sur les traits sexuels tels que les armes, les ornements et la taille des testicules.

Il a aussi été démontré qu'une interaction entre le génotype et l'environnement affecte l'immunocompétence chez le poulet (*Gallus gallus domesticus*; Gross & Siegel, 1988). Plus précisément, Svensson, Sinervo, & Comendant (2001) ont trouvé qu'il y avait une interaction entre le génotype et la densité de population sur l'immunocompétence chez les lézards. Cependant, la façon dont l'interaction entre le génotype et la densité de population lors du développement affecte la réponse immunitaire chez un individu n'est toujours pas comprise. Il n'est pas clair si certains génotypes sont avantagés au niveau immunitaire dans un environnement à haute densité de population et si d'autres génotypes sont plus avantagés lors de basse densité de population ou bien si tous les génotypes réagissent de la même manière à la variation environnementale (plasticité phénotypique). Plus encore, nous ne savons pas l'influence de l'interaction entre la génétique et l'environnement sur le compromis entre les traits sexuels (pré- et post-copulatoires) et l'immunité.

0.5 Armes et règlement de combats

Dans le règne animal, les mâles se battent souvent entre eux afin d'acquérir le droit à des partenaires sexuelles, ou bien à des territoires leur étant utiles pour la séduction des femelles. Ainsi, les traits des mâles leur donnant un avantage lors des combats, tels que la taille des armes, seront des traits pré-copulatoires liés à la valeur sélective du mâle. Ces traits seront influencés par l'interaction entre le génotype et l'environnement de la même manière que ceux mentionnés plus haut.

Les combats peuvent n'impliquer aucun contact physique dans le cas de manifestations visuelles. Souvent, les combats impliqueront un contact physique. Lors des combats physiques, le résultat sera influencé par le RHP (Resource-Holding Potential, Parker, 1974) respectif de chaque individu. De manière générale, le RHP d'un individu est influencé par sa capacité offensive, sa capacité défensive ainsi que son endurance (Palaoro & Briffa, 2016). La théorie démontre chez plusieurs animaux que le RHP d'un individu sera déterminé plus précisément par sa taille, ses armes, son endurance (Briffa

& Sneddon, 2007; Parker, 1974), son expérience et son état physiologique (Arnott & Elwood, 2008; Khazraïe & Campan, 1999) et cela est soutenu plus précisément chez les grillons (Briffa, 2008; Judge & Bonanno, 2008; Khazraïe & Campan, 1999). Il a été observé que l'expérience des individus affecte le résultat d'un combat puisque les gagnants ont plus de chance de gagner à nouveau et les perdants ont plus de chance de perdre (Condon, Lailvaux, & Patek, 2016; Hsu, Earley, & Wolf, 2006; Judge, Ting, Schneider, & Fitzpatrick, 2010; Kelly, 2006a; Khazraïe & Campan, 1999; Simmons, 1986).

Dans le cas où les deux adversaires accordent la même valeur à la ressource contestée, le combat devrait être gagné par le candidat ayant le plus haut RHP (Arnott & Elwood, 2009; Brown, Chimenti, & Siebert, 2007; Vieira & Peixoto, 2013). Si un combattant accorde une plus grande valeur à la ressource que son adversaire, sa motivation sera alors plus grande et le seuil de coût qu'il sera prêt à tolérer sera plus grand (Arnott & Elwood, 2008; Enquist & Leimar, 1987; Judge *et al.*, 2010). Le coût engendré par les combats agressifs peut être en termes de dépense énergétique et parfois en termes de blessures (Briffa & Sneddon, 2007; Briffa & Elwood, 2009). La théorie prédit donc qu'un animal devrait se retirer d'un combat lorsque les coûts dépassent les bénéfices ou lorsqu'il détermine que ses chances de gagner sont inférieures à celles de son adversaire (Palaoro & Briffa, 2016). Le coût d'un combat est principalement déterminé en termes de consommation d'oxygène (Briffa & Sneddon, 2007; Hack, 1997) et très peu en termes de blessures. Le coût énergétique est donc directement lié à la durée du combat (Prenter, Elwood, & Taylor, 2006).

Il existe plusieurs modèles qui tentent de prédire les stratégies de combat. On peut les classer en deux grandes catégories : ceux basés sur une auto-évaluation du combattant et ceux basés sur une évaluation mutuelle. Dans le premier cas, l'individu n'a d'information que sur son propre RHP et le seuil maximum de coût qu'il est prêt à investir dans le combat (Arnott & Elwood, 2009; Enquist & Leimar, 1983; Enquist *et* *al.*, 1990). Dans le second cas, il est présumé que les deux combattants ont des fonctions cognitives assez développées pour comparer leur capacité combative respective (RHP relatif) et que les combats servent à obtenir de plus en plus d'information précise afin de permettre cette évaluation (ex : modèle d'évaluation séquentielle (SAM); Enquist & Leimar, 1983, 1987; Hsu *et al.*, 2006; Junior & Peixoto, 2013; Palaoro & Briffa, 2016). Selon le SAM, les perdants devraient abandonner dès qu'ils perçoivent qu'ils sont plus faibles que leur adversaire. Ainsi, le combat devrait se dérouler en plusieurs phases (c'est-à-dire avec des interruptions) avec des changements de comportement (Kelly, 2006b). Ce modèle prédit aussi qu'il n'y a pas de relation entre la taille corporelle et la durée du combat (Palaoro & Briffa, 2016), mais qu'il y a une relation négative entre la durée du combat et le RHP du gagnant (Gammell & Hardy, 2003).

Le modèle EWOA (« Energetic war of attrition ») est un modèle d'auto-évaluation qui n'implique que des performances visuelles et aucun contact physique. Les combats escaladent en performances de plus en plus coûteuses énergétiquement et se terminent lorsque le perdant a atteint sa limite de temps et d'énergie dépensée (Kelly, 2006b; Payne & Pagel, 1996; Taylor & Elwood, 2003). Selon le EWOA, la relation entre la taille corporelle et la durée du « combat » est positive (Palaoro & Briffa, 2016).

Le modèle d'évaluation cumulative (Cumulative Assessment Model; CAM) développé par Payne (1998) entre dans la première catégorie et propose que les combattants persistent jusqu'à ce qu'ils atteignent un seuil de coût qu'ils sont prêts à payer (Hsu *et al.,* 2006; Judge & Bonanno, 2008; Kelly, 2006b; Payne, 1998). Selon le CAM, les décisions prises lors d'un combat seront basées sur la somme des actions de son adversaire. Ainsi, le CAM prédit que les adversaires persisteront dans le combat jusqu'à ce que le total des coûts infligés par un adversaire dépasse la limite que l'autre individu peut supporter. Encore, si l'adversaire a le choix entre deux stratégies d'attaque et qu'il utilise la plus coûteuse, sa limite sera atteinte plus vite que s'il utilise la stratégie moins coûteuse. Les individus sont incapables de déterminer l'effet de leur propre attaque sur leur adversaire. Les combats se dérouleront donc en une seule phase (donc aucune interruption) qui augmente en intensité (Kelly, 2006b). Selon ce modèle, la relation entre la taille corporelle et la durée du combat sera donc positive (Palaoro & Briffa, 2016).

Afin de prédire le gagnant de chaque combat, il faut regarder la capacité offensive des individus (habilité à infliger des blessures), la capacité de supporter des dommages et des blessures, mais aussi l'endurance de chaque individu (Palaoro & Briffa, 2016). L'endurance d'un individu peut être manipulée en contrôlant sa condition corporelle par l'alimentation. La capacité à infliger des blessures dépend du RHP (ex.: taille des armements). Il se pourrait que cette dernière soit proportionnelle à la taille de l'individu, mais il a été proposé que la taille des armements varie de manière exponentielle en fonction de la taille corporelle (Palaoro & Briffa, 2016). Si tel est le cas, cela modifiera la relation entre les RHP des deux individus. La durée du combat et la résolution du combat étant dépendantes de la différence de RHP entre les individus, elles seront donc affectées si la relation est non linéaire (Palaoro & Briffa, 2016). L'effet de l'allométrie des armes sur la résolution des conflits nécessite plus de recherche.

Chez les grillons, les mâles se battent pour des terriers d'où appeler les femelles (Alexander, 1961; Brown, Chimenti, & Siebert, 2007; Otte & Cade, 1976; Tachon *et al.*, 1999). Un terrier est considéré comme meilleur lorsqu'il offre plus de protection contre les prédateurs et les parasites (Simmons, 1986). Les mâles ayant les plus grands RHP acquièrent de meilleurs territoires, ce qui leur permet d'avoir un meilleur succès reproducteur (Kelly, 2008; Nelson & Nolen, 1997; Simmons, 1986). Le RHP des mâles est directement influencé par leur taille corporelle ainsi que la taille des mandibules, dont ils se servent comme armes lors des combats.

Puisque les combats servent indirectement à acquérir des opportunités de reproduction, les mâles matures sexuellement sont beaucoup plus agressifs que les mâles immatures pour qui les risques seraient inutiles (Dixon & Cade, 1986).

Rillich *et al.* (2007) ont trouvé que les grillons *Gryllus bimaculatus* n'évaluent pas leur propre qualité ou celle de leur adversaire, mais que la décision d'abandonner semble basée purement sur les actions de leur adversaire et au coût total qu'ils peuvent tolérer. Ces résultats sont en accord avec le modèle d'évaluation cumulative, qui est particulièrement approprié pour les combats ritualisés avec des blessures physiques limitées (Rillich, Schildberger, & Stevenson, 2007) et soutenu chez les grillons (Briffa, 2008). Ce que nous ne savons toujours pas est la manière dont l'interaction entre la condition nutritionnelle et la densité de population lors du développement affecte le RHP d'un mâle, ni comment les conflits entre mâles rivaux sont résolus. Plus précisément, nous ne savons pas comment la différence entre la taille des armes affecte la capacité de combat des mâles et les conditions sous lesquels les grillons auront de plus grandes armes.

0.6 Espèce modèle : *Gryllus firmus*

Pour mes expériences, j'utiliserai comme système d'étude le grillon des champs (*Gryllus firmus*). Cette espèce est originaire de Floride. Nous avons acquis les œufs de G. firmus d'un laboratoire à l'université de MacEwan (Edmonton, Alberta, Canada) qui ont maintenu les grillons en hautes densités depuis qu'ils ont récoltés 35 individus adultes (13 mâles et 22 femelles) à l'automne 2010 près de Gainesville, Floride par Kevin Judge. La colonie utilisée pour les expériences de notre laboratoire a été envoyée en novembre 2015 et a été gardée à une température constante de 28°C et environ 60% d'humidité avec un cycle de lumière inversée de 12 heures jour/nuit jusqu'à ce jour. La colonie a été maintenue dans des bacs de 70 L en nombre d'environ 50 à 100 individus (mâles et femelles) par bac. Nous leur avons fourni de l'eau sous forme de tubes de plastiques bloqués par des boules de coton et de la nourriture pour chat adulte Iams™ Proactive Health™ (32% protéines, 15% gras, 3% fibres, 372.33kcal/tasse) ad libitum. Chaque bac contenait plusieurs épaisseurs de cartons d'œufs pour fournir des abris. Chez G. firmus, les œufs prennent environ 21 jours à éclore. Les juvéniles prennent ensuite environ 60 jours pour devenir adultes. Ensuite, ils nécessitent de 7 à 14 jours pour devenir matures sexuellement. Les mâles se reproduisent avec plusieurs femelles et les femelles peuvent également se reproduire avec plusieurs mâles. Les accouplements ne sont donc pas contrôlés d'une génération à une autre. Pour chaque génération, les œufs sont obtenus de plusieurs femelles libres de se reproduire avec plusieurs mâles. Les femelles pondent leurs œufs dans un substrat humide pendant environ 5 jours.

Les grillons sont un très bon système pour l'étude des combats, car les mâles se battent spontanément, sans nécessiter une ressource à acquérir (Briffa, 2008; Judge *et al.*, 2010). Il n'est donc pas nécessaire d'introduire les mâles à une femelle avant l'expérience (Adamo & Hoy, 1995; Alexander, 1961; Rillich et al., 2007; Simmons, 1986). De plus, ils sont faciles à élever et garder dans le laboratoire. On peut facilement distinguer les sexes à l'âge adulte et même à la dernière phase avant la maturité. Finalement, une grande quantité de mâles de même âge et de même poids sont assez faciles à obtenir afin de minimiser l'effet des différences entre les grillons pour l'expérience et se concentrer sur les différences dans les tailles de mandibules et les différents traitements (Briffa, 2008).

Chez les grillons, la capacité à acquérir des partenaires sexuels est affectée par leur aptitude à remporter des combats pour acquérir les meilleurs territoires d'où appeler les femelles, ainsi que leur aptitude à chanter afin d'attirer les femelles sur ces territoires (Alexander, 1961; Judge & Bonanno, 2008; Shackleton & Jennions, 2005).

Leur aptitude au chant est affectée par la taille de leurs harpes, qui est une structure située sur les ailes des mâles (Bentley & Hoy, 1974; Bertram & Rook, 2012; Mowles, 2014; Simmons & Ritchie, 1996). De plus grandes mandibules leur sont avantageuses afin de remporter les combats avec d'autres mâles puisqu'elles leur servent d'armes (Adamo & Hoy, 1995). La façon dont l'interaction entre le génotype et la densité de population au cours du développement affecte la taille des harpes, des testicules et des mandibules n'est pas connue.

Aussi, plusieurs analyses ont été développées chez les grillons afin de quantifier la réponse immunitaire, incluant le taux d'encapsulation. Lorsqu'une substance est reconnue comme pathogène, une des réponses possibles de l'organisme est de former une couche de mélanine. Pour ce faire, des hémocytes spécialisés se regroupent et forment des capsules autour du corps étrangé (Beckage, 1998). Afin de former cette capsule, les enzymes phénoloxidase convertissent des molécules précurseures composées de tyrosine en mélanine par une cascade de réactions (Siva-Jothy, Moret, & Rolff, 2005; Beckage, 1998). Il est ensuite possible de quantifier la force de la réponse immunitaire en calculant le niveau de mélanisation. En effet, en insérant un microfilament de nylon dans l'abdomen pour une durée donnée, il sera possible de déterminer le niveau de mélanisation en calculant la noirceur de la capsule de mélanine entourant le microfilament après extraction (Kelly & Jennions, 2009; Lawniczak *et al.*, 2006; Siva-Jothy *et al.*, 2005). Le niveau de mélanisation reflète bien la réponse immunitaire d'un individu puisqu'elle est souvent corrélée avec d'autres mesures d'immunité comme l'activité lytique, l'activité des phénoloxidases et le nombre d'hémocytes chez les Orthoptères (Fedorka, Zuk, & Mousseau, 2004; Kelly & Jennions, 2009; Rantala & Roff, 2005; Rantala & Kortet, 2004; Wilson, 2001). De plus, le niveau de mélanisme est associé positivement à la résistance aux pathogènes (Wilson *et al.*, 2001).

0.7 Hypothèses

Ce mémoire est composé de deux chapitres sous forme de manuscrits. Dans mon premier chapitre, j'ai utilisé un plan expérimental de demi-frères/sœurs pour comparer l'héritabilité de traits sexuels pré-copulatoire (harpes, mandibules) et post-copulatoire (testicules), ainsi qu'un trait représentant la fonction immunitaire (ex. : le taux de mélanisation). Une approche expérimentale de génétique quantitative m'a permis d'examiner les interactions génotypes-par-environnement (densité de population lors du développement).

Si l'environnement social lors du développement est un indice important du niveau de compétition sexuelle futur perçu par le mâle, alors les mâles élevés à haute densité de population devraient investir significativement plus dans leurs traits sexuels pré-copulatoire (harpes, mandibules). Alternativement, si l'environnement social lors du développement est un indice important du niveau de compétition spermatique perçu par le mâle, alors les mâles élevés à haute densité devraient investir significativement plus dans leurs traits sexuels post-copulatoire et donc produire de plus grands testicules que les mâles élevés à basse densité. De plus, si l'environnement social lors du développement permet aux mâles de prédire leur risque d'exposition à des pathogènes à l'âge adulte et si la survie tôt dans la vie est plus importante que la fécondation, alors les mâles élevés à haute densité devraient investir significativement plus dans leur immunité que dans leurs traits sexuels. Ainsi, ils devraient produire de plus grandes réponses de mélanisation sous de hautes densités de population que sous de basses densités de population lors du développement. Finalement, certains individus issus de la même famille devraient être avantagé en basse densité de population alors que d'autres devraient être avantagé en haute densité de population, autant pour les traits sexuels que pour l'immunité. Je prédis un compromis entre les traits sexuels et l'immunité.

Dans mon second chapitre, j'ai testé l'hypothèse que les mâles élevés sous haute densité produisent des mandibules significativement plus grandes (pour une certaine taille de corps), car de plus grandes mandibules vont permettre aux mâles d'augmenter leur chance de gagner les combats, cette dernière étant corrélé avec le succès reproducteur. De manière alternative, de plus grandes mandibules pourraient être sélectionnées sous haute densité de population, car elles permettent, par exemple, une meilleure acquisition de nourriture. J'ai testé cette éventualité en comparant des mâles et des femelles élevés sous différentes densités de population. Si l'acquisition de nourriture est la raison pour la différence dans la taille des mandibules, alors les deux sexes devraient avoir de plus grandes mandibules lors d'une haute densité de population. S'il s'agit de sélection sexuelle, nous observerons une différence seulement chez les mâles.

J'ai testé l'hypothèse que les mâles élevés sous haute densité de population vont produire de plus grosses mandibules et que de plus grosses mandibules entrainent un plus grand succès pour gagner les combats avec les mâles rivaux. Les mandibules étant utilisés comme armes dans les combats chez les grillons, leur taille donne un indice direct de la capacité offensive de l'individus, un des éléments importants dans les règlements de conflits selon Palaoro et Briffa (2016). Un autre aspect important de la résolution de conflits est l'endurance. Pour cette raison, j'ai testé si les mâles en meilleure condition nutritionnelle ont plus de succès lors des combats que des adversaires de même taille en mauvaise condition, indépendamment de la densité de population lors de leur développement. Ici, la prédiction est qu'un mâle en meilleure condition nutritionnelle aura plus d'endurance et donc devrait gagner plus souvent les combats. Je teste explicitement comment les combats sont réglés chez cette espèce en fonction de la capacité offensive et de l'endurance, et les facteurs influençant ces traits.

Les deux chapitres permettent une synthèse des points importants de chacune des expériences. Ils seront suivis d'une conclusion faisant le retour sur l'ensemble du projet.

CHAPTER I

EFFECT OF GENOTYPE AND REARING DENSITY ON INVESTMENT IN SEXUAL AND LIFE HISTORY TRAITS IN THE SAND FIELD CRICKET (*GRYLLUS FIRMUS*)

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1.1 Abstract

An individual's fitness is dictated by the amount of resources acquired, which is, in turn, dictated by the individual's genotype and its environment. The social environment can also determine how individuals will invest in their morphological and life history traits and their sexual traits. The hypothesis we wanted to test was whether investment in sexual traits would be influenced by the individual's environment and if, under high population density, individuals would invest more in their immune system and their reproductive traits than under low population density. To test this hypothesis, crickets (*Gryllus firmus*) were reared in high and low population density in a half-sib/full-sib design, after which the immune response of a third (N=233) of the crickets was measured. Harp and mandible size, in addition to gonad mass were used as indicators of reproductive investment. Population density did not influence testis size or immune response. Mandibles were bigger in males than females but were not influenced by rearing density. Traits that were strongly correlated with body size were smaller under high population density and these traits were most heritable. There was no genotype by environment (GXE) interaction on any traits measured. From our study, there is no reason to believe families within the *Gryllus firmus* species respond differently to population density during development. However, environments can influence morphology in many ways other than via population density. It is thus possible that crickets are more sensitive to other cues in the environment and prioritizing resource allocation accordingly.

Key words: allocation, heritability, rearing density, GxE interaction

1.2 Introduction

An individual's abiotic and biotic rearing environment can profoundly affect its adult phenotype (Dmitriew, 2011; Griffith *et al.*, 1999; Kelly, 2018) with individuals growing in nutritionally depauperate environments likely experiencing high fitness costs when sexually mature. For example, adult *Speyeria mormonia* butterflies fed a poor diet during their larval stage have a smaller body size and wing length, which reduces their flight performance and survivorship (Boggs & Freeman, 2005). Other studies show that poor larval diet negatively affects adult dominance rank in the carrion crow *Corvus corone* (Richner *et al.*, 1989; Richner, 1992), tarsus size in the pheasant *Phasianus colchicus* (Ohlsson & Smith, 2001), adult body size in the seed beetle *Stator limbatus* (Fox, Thakar, & Mousseau, 1997), in addition to many other fitness-related traits (De Kogel, 1997; Fox *et al.*, 1997; Richner, 1992; Godfray, 1994). Kelly & Tawes (2013) also demonstrated that juvenile diet has a sex-specific effect on disease resistance in adult *Gryllus texensis* crickets with females in poor condition having better disease resistance than females in good condition.

Juvenile animals might experience a nutritionally-poor environment, not because the available resources are poor or scarce per se, but because there is an abundance of individuals competing for them. This situation will reduce the amount of resource available per individual for allocation to the soma (assuming population-wide resource abundance and competitive ability remains constant). For example, Gimnig *et al.* (2002) found that a high population density of mosquitoes *Anopheles gambiae* resulted in lower adult body size in both sexes. High population density also causes *Culex pipiens quinquefasciatus* mosquitoes to develop smaller body sizes and shorter wings (Agnew, Haussy, & Michalakis, 2000) and *Ambystoma opacum* marbled salamanders to develop smaller body sizes, lower lipid stores and lower lifespan (Scott, 1994). A more complex effect of demography - and one that is independent of resource availability - is that the juvenile environment cues the developing individual to life in the eventual adult environment (Gilbert, 2001, 2005; Monaghan, 2008; Weaver, 2009). For example, high rearing population density can cue the individual to high competition for mates in the future. If the present environment provides reliable cues of the future environment, then individuals should adaptively allocate resources to produce trait values that maximize fitness in the future (adult) environment. Density-dependent prophylaxis is a classic example of how rearing density can act as an informative cue that affects resource allocation strategies. The density-dependent prophylaxis hypothesis posits that individuals invest more in immunity as population density increases because higher densities increase contact frequency with other individuals who might be infected by disease or pathogens (Barnes & Siva-Jothy, 2000; Steinhaus, 1958; Wilson *et al.*, 2001). For example, crowded *Spodoptera exempta* moth larvae survive longer during viral infection than solitary larvae because they invest more in their immune system than their solitary counterparts *(*Reeson *et al.*, 1998). Also, the solitary armyworm larvae, *Pseudaletia separata*, is more susceptible to viral infection than those reared under crowded conditions (Kunimi & Yamada, 1990).

Another aspect of an animal's biology that the juvenile environment can foreshadow is the intensity of sexual competition (He & Tsubaki, 1991; Kasumovic & Brooks, 2011; Radwan, 1993). When placed under high-densities, large adult male *Asellus aquaticus* isopods will have greater mating success (Bertin & Cézilly, 2005). Intensity of mate competition is directly proportional to population density and high population densities will cause individuals, usually males, to invest in traits that make them more successful in sexual competition (Bertin & Cézilly, 2005). Higher levels of perceived competition can lead males to invest in testis size in order to increase their sperm competitiveness (Buzatto *et al.*, 2015). For example, despite unlimited food resources in all density treatments, high population densities can cause *Pseudaletia separata* armyworm to produce larger spermatophores (He & Tsubaki, 1991), *Scathophaga stercoraria* yellow dung fly, *Plodia interpunctella* moths and *Crinia georgiana* chorusing frogs to produce larger testes (Buzatto *et al.*, 2015; Gage, 1995; Stockley & Seal, 2001, Hosken & Ward, 2001). Male *Sancassania berlesei* mites and several species of South African dung beetles develop bigger weapons with increasing competition for mates during their development (Pomfret & Knell, 2008; Radwan *et al.*, 2002).

It is unclear how males invest in sexually selected and life history traits when reared under high densities. Several scenarios are possible. First, the density-dependent prophylaxis hypothesis suggests that individuals reared under high densities should invest more heavily in immunity than those reared under low densities. Males, however, are expected to accrue fitness via mating success rather than longevity (Rolff, 2001). Because investment in immunity is expected to trade-off with investment in sexual selection, males should allocate resources to sexually selected traits at the expense of immunity. The next problem faced by males is how to partition resources amongst pre- and post-copulatory traits (Fitzpatrick *et al.*, 2012; Parker *et al.*, 2012). This will likely depend on the animal's mating system, that is, how males interact with females to acquire fertilizations (Harcourt, Purvist, & Liles, 1995). Therefore, if pre-copulatory sexual selection is more important for mate acquisition then males reared under high density are expected to invest more heavily in weaponry and sexual signaling structures. However, if males gain fitness by being better sperm competitors, then males reared under high density should allocate resources to testis size. Females, on the other hand, are expected to invest in survival and longevity (Rolff, 2001) and should thus increase their investment in immunity under high density rearing conditions.

Beyond the straightforward direct effects of the rearing environment on allocation decisions, resource allocation strategies and eventual expression of the adult phenotype can often depend on the interaction between the individual's rearing environment and its genotype (Ingleby *et al.*, 2010). Many traits are affected by genetics in addition to being affected by the environment. For example, Okada & Miyatake (2009) showed that weapon size has significant heritable variation in *Gnatocerus cornutus* beetles. Joly *et al.* (1997) and Reinhold (1994) demonstrated that testis size is heritable in *Drosophila simulans* fruit flies. However, little is known about how different genotypes respond to different environments during development and how the interaction affects trade-offs between various fitness-related traits, such as sexual (e.g. weaponry, testes) and life history (e.g. immunity) traits. Most studies investigating the effect genotype-by-rearing environment interactions on fitness-related traits were done in the context of aquaculture (Fishback *et al.*, 2002; Mulder, Arendonk, & Komen, 2013) and very few examined at male sexually selected and reproductive traits (Ingleby *et al.*, 2010). More studies on genotype by environment (GXE) interaction are needed to understand species adaptations and evolution. Without those studies, one might think individuals of a species are not well adapted to their environment when in fact they are better adapted to a changing environment.

In this study, we used a quantitative genetic approach to examine the relative contributions of individual genotype and rearing environment on investment in sexual (including reproductive) and life history traits in male and female sand field crickets (*Gryllus firmus*). Specifically, we predict that males reared under high densities will invest less in immunity (i.e. melanization response) than males reared under low densities. However, high-density males are expected to invest more in sexually selected traits than low-density males. We calculated the heritability of one immune trait (level of melanization), several sexual traits (testis mass, mandible length, harp size, ovary size and average egg length) and multiple somatic traits (body mass, femur length, tibia length, pronotum width and length and eye surface area). We then explore whether male crickets allocate more resources to pre- (i.e. mandibular weaponry, harp size) or post (i.e. testis size) -copulatory sexually selected traits. We predict that females reared under high densities should allocate more resources to immunity than their low-density counterparts because female fitness is likely to be more dependent upon survival and longevity than in males (Rolff, 2001). If trait expression of different genotypes is dependent upon rearing environment then we expect significant genotype-by-environment interactions. In line with other studies (Applebaum & Heifetz, 1999; Niemelä *et al.*, 2012), we also predicted that individuals reared at low-density would take longer to become adults, and eclose at a larger body mass and size than crickets reared under high-density, due to a trade-off between development speed and adult mass. Crickets are expected to exhibit significant sexual dimorphism independent of rearing environment with males having larger mandibles than females but females having larger adult body mass and size (Gwynne, 1984; Judge & Bonanno, 2008; Zajitschek *et al.*, 2009).

1.3 Methods

1.3.1 Animal rearing and breeding design

The crickets used in this experiment were lab-raised descendants of individuals collected in Fall 2010 in Gainesville, FL (USA). Crickets were raised communally in 70-L bins until their penultimate instar in an environmentally-controlled room at constant temperature (28°C), relative humidity (60%), and on a reverse light cycle of 12 hours of day: 12 hours of night. Crickets were provided with cotton-plugged water tubes, Iams™ Proactive Health™ cat food *ad libitum*, and layers of cardboard egg cartons for shelter.

The interactive effects of genotype and rearing density on the behaviour of the crickets used in this study was previously examined (Wey, Réale & Kelly, 2019) and morphological measurements were then taken for this study after the experiment by

Wey, Réale & Kelly (2019). Penultimate instar male and female juvenile crickets were haphazardly selected from the rearing colony and individually placed in a 250-mL plastic deli container to ensure virginity. Each cricket was provided with *ad libitum* food and water so that multiple crickets could eat and drink at the same time, and a piece of cardboard egg carton for shelter. At sexual maturity, we haphazardly placed males with three females in 750 mL container with *ad libitum* food, water, and a piece of egg carton. We created 30 mating groups wherein 30 sires were each paired with 3 dams (i.e. 90 dams in total). The mating groups were given 5 days to mate, after which they were all weighed on a Sartorius Secura 224-1S analytical balance. The females were then individually placed in containers (750 mL) and provided with a small cup of oviposition substrate (damp vermiculite) and allowed to lay eggs for 5 days. Females were then removed from their containers and the vermiculite kept damp until hatching began (approximately 10-14 days after laying). Three days of hatching provided a sufficient number of hatchlings for our experiment; therefore, on day four we removed the cup of oviposition substrate and provided the hatchlings with powdered cat food (cat food was ground in a coffee grinder), water and egg cartons. We removed from the experiment any mating group that produced no offspring and those in questionable condition (e.g., the sire died early in the mating period). In the end, hatchlings from 24 sires and 68 dams were used for the experiment and subsequently placed into density treatments.

1.3.2 Experimental design

At 7d post-hatch, crickets were randomly assigned to either a low- or high-density treatment. The low-density treatment comprised one individual per 250-mL transparent plastic container, whereas the high-density treatment comprised 10 full siblings per 750 mL transparent plastic container. Individuals in both treatments were provided with *ad* *libitum* food and water, and all were provided with a piece of egg carton for refuge. Food and water were replaced, and containers cleaned, once per week.

Each family was separated into 10 low-density replicates (one cricket/container) and 2 high-density replicates (10 crickets/container). Natural mortality reduced the density in some high-density replicates. We removed from our analyses those replicates having five or fewer crickets; replicates with six or more crickets were retained because these densities should provide a social environment that is markedly different than the low-density treatment (Wey, Réale & Kelly, 2019If both groups from the same maternal family dropped to fewer than 10 individuals in total, the two groups were combined to maintain high density. All containers were haphazardly rotated in the growth chamber to minimize the effects of microhabitat variation.

Eclosion to adulthood was checked daily or every two days. At eclosion to adulthood, crickets were weighed to the nearest 0.001g on a Sartorius Secura 224-1S analytical balance and individually placed into a 250-mL container provisioned with food, water and piece of egg carton for shelter. Eclosion was checked 5 to 6 days a week. Sexually mature crickets (7-14 days post-eclosion) were behaviorally tested as part of another study (Wey, Réale & Kelly, 2019), after which, all individuals were re-weighed (to the nearest 0.001g) with one third (n=233) of them subjected to an immunological assay (described below). The remaining two-thirds (n=470) were immediately euthanized by freezing at -20°C for later morphological analysis. Our immunological assay tested an individual's melanization response, a standard assay in studies of insect immunology. When a substance is recognized as foreign by an insect's immune system, specialized hemocytes regroup and form a capsule around the invader (Beckage, 1998). To form the capsule, phenoloxidase enzymes convert precursor molecules into melanin in a series of reactions (Beckage, 1998; Siva-Jothy *et al.*, 2005). The degree of melanization of a foreign object can then be used to quantify an immune response (Kelly & Jennions, 2009; Lawniczak *et al.*, 2006; Siva-jothy *et al.*, 2005). The immune test consisted of inserting a 3-mm nylon microfilament into the cricket's abdomen at the third posterior segment. After implantation, crickets were returned to their container for 24 hours and kept under the environmental conditions described above. After 24 hours, the crickets were euthanized by freezing at -20°C and kept frozen until later dissection to remove the microfilament. The degree of melanization of the microfilament was measured using ImageJ (Java 1.6.0_65, National Institutes of Health, USA).

All individuals used in this experiment (n=710) were preserved and digitally photographed using a Leica SC170 HD camera (Leica Microsystems Inc., Concord, ON, Canada) connected to a Leica S6D microscope (Leica Microsystems Inc., Concord, ON, Canada). We used LAS V4.5.5 software (Leica Application Suite) to digitally stamp a scale bar on each image. We then used ImageJ (Java 1.6.0_65, National Institutes of Health, USA) to measure femur length, tibia length, pronotum length and width (spanning the ventral margins across the neck membrane and cervical sclerites), eye surface area, mandible length (from the lateral articulation to the distal tip) and harp area (surface area of triangular shape found on male wings used to create sound; Bennett-Clark, 1989). Mandible length and harp surface area were used as measures of investment in pre-copulatory sexual selection because males use them in male-male contest for access to females. For measures of femur length, tibia length, eye surface area, mandible length and harp area, the right and left body parts were measured and we used the average of both in our analysis. All crickets were dissected in order to remove testes and ovaries, and microfilaments where applicable. Gonads were weighed to the nearest 0.00001 g using a microbalance (Mettler Toledo XP26) after being dried in an oven at 60°C for 24 hours. For each female, five eggs were randomly selected, digitally photographed and measured using the same system as the other morphological measurements. The average of the five egg was then used for the analysis.

1.3.3 Statistical analysis

We used univariate linear mixed-models to examine the effect of rearing density and sex, and their interaction on multiple offspring traits (see Table 1.1 for list of traits). Linear measurements were to the nearest 0.001 mm and surface area measurements to the nearest 0.0001 mm^2. Rearing density and sex were entered as fixed effects whereas the term 'family' was entered as a random effect. Body mass was entered as a covariate into each model. We took two measurements independently of each of the traits described above for a subset of individuals (at least 50 individuals) to assess measurement repeatability (Lessells & Boag, 1987; Yezerinac, Lougheed, & Handford, 1992). All measurements were highly repeatable $R>0.95$. All measurements other than body mass were log transformed to satisfy the model assumption of normally distributed residuals.

We first ran a set of models that included random intercepts for dam nested within sire and sires nested within mating block (to identify mating groups that were formed at the same time). We tested for genotype by environment interactions using a reaction norm approach, where we included random slopes for density treatment within sire with correlated intercept and slopes. When these random slopes were significant ($p < 0.05$), we calculated the variance components for the two density treatments separately. Otherwise, we report results from models without random slopes.

For all models, we estimated narrow-sense heritability (h^2) as $V_A /(V_A + V_D + V_{block} + V_R)$, where V_A is the additive genetic variance, V_D is the dam variance (maternal effects), V_{block} is the variance due to differences in mating periods, and V_R is residual variance. In the nested half-sib, full-sib design, the sire component (V_S) is approximately $V_A/4$, thus we used $V_A = 4 \times V_S$ in our calculation of h^2. We also estimated maternal effects (m^2) as the ratio $V_D /(V_A + V_D + V_{block} + V_R)$.

The above set of models included temporal block as a random effect. However, we could not randomize families among blocks with our experimental design, as blocks necessarily represented separate periods during which we selected some individuals to create families and each family is nested within a block. In this situation, variance among blocks may take part of the variance among sires or dams, as blocks may be composed of different genotypes due to uncontrolled factors (e.g., by chance related to the small number of parents selected in each block or for phenological reasons; Wey, Réale & Kelly, 2019). Therefore, including block in the model may lead to underestimation of both V_A and V_D. Also, we included low- and high-density treatments throughout the experiment, so the experimental design should dampen the temporal effect on our results. We thus ran a second set of models on the same dependent variables that differed only in excluding the random intercept for block effect. For this second set of models, we estimated narrow-sense heritability (h^2) as $V_A /(V_A + V_D + V_R)$. We present results for variance components calculated from both sets of models. We assumed that if block really influences the response variable, independent of sires and dams, then we should observe an increase in both V_A and V_D in models without temporal block. On the other hand, changes in either V_A or V_D as a result of removing blocks from the model would suggest that variance among blocks actually represented part of that variance component.

We excluded from these statistical analyses families that experienced irregular rearing conditions such as 50% mortality or above, resulting in 662 offspring for which we had all the information out of the 710 individuals we tested (n=331 crickets reared under high density and n=331 crickets reared under low density; n=318 daughters, n=344 sons) from 23 sires and 59 dams (specific sample sizes varied by analysis due to missing data).

All statistical analyses were conducted using R Studio version 2.1 (v. 1.0.153; 2016). We implemented mixed models with the package *lme4* (Bates *et al.*, 2015) and survival analyses for time period analysis with the package *coxme* (Therneau, 2018). We used the package *boot* (Davison & Hinkley, 1997; Canty & Ripley, 2014) to obtain 95% confidence intervals and *p*-values for fixed effects using 50 000 bootstrap replicates with replacement. Measurement repeatabilities were calculated using the *rptR* package by Stoffel, Nakagawa, & Schielzeth (2017). Unless otherwise stated all tests are two-tailed and data are presented as mean ± standard error.

1.4 Results

1.4.1 Time to eclosion

We found a significant interaction between density and sex on the time required to reach eclosion (GLM: $t = 3.019$, d.f. = 604.2, $p = 0.0027$). Also, low-density crickets matured significantly more slowly than high-density crickets (ANOVA: $F=107.5$, d.f. = 1 and 651, $p < 0.0001$; see Table 1.3). Females matured more quickly than males (ANOVA: $F= 7.727$, d.f. = 1 and 651, $p = 0.0056$; see Table 1.3).

1.4.2 Effect of rearing density and sex on morphological traits, sexual traits and immunity

We did not find significant density x sex interactive effects on any of our measured morphological traits (Table 1.1). Individuals reared at low-density generally had significantly larger body size (both sexes) which was reflected in larger body mass at eclosion, longer pronota, longer femura, longer tibia, and eye surface areas than those reared at high-density (Table 1.1 and Table 1.3). Females also had significantly longer pronota, eye surface areas and body masses at eclosion than males (Table 1.1 and Table 1.3). Sexual traits were less affected by density since mandible length, testis mass, and ovary mass did not significantly differ between density treatments after controlling for body mass (Table 1.1). Only male harp area was bigger for males in low density treatments than males in high density treatments (Table 1.1 and Table 1.3). As expected, we found that males have significantly longer mandibles than females (Table 1.1). As for female reproductive traits, there was no effect of density on ovary mass, but females reared at low density had significantly longer eggs than females reared in high density (Table 1.1 and Table 1.3).

Neither density nor sex had a significant effect on melanization response (mean = 163.9, SE=10.95, range: 105.2-206.4, N = 130) (Table 1.1).

1.4.3 Variance components and heritability

1.4.3.1 Morphological traits

The lack of any significant density x sire interactions for any morphological trait, suggests that all families responded in a similar way to rearing density (Table 1.2).

Significant variance was attributed to sires (V_S) for mass at sexual maturity, eye surface area, pronotum length, femur length and tibia length only when the effect of block (time of the year they mated) was excluded (Table 1.2). Sires explained 26% of the overall phenotypic variation (V_P) for body mass, 20.7% for eye size, 28% for pronotum length, 29.7% for femur length and 29.9% for tibia length when excluding block from the model.

Significant variance was attributed to dams (V_D) for eye size, pronotum length and width, femur length and tibia length. V_D explained 21.1% of V_P for eye size, 15.4% for pronotum length, 15.9% for pronotum width, 13% for femur length and 18.1% for tibia length in models with block (Table 1.2). There was no significant variance attributed to dam (V_D) body mass (Table 1.2). Indeed, dams (V_D) explained very little of the overall phenotypic variation (V_P) in offspring body mass at sexual maturity (12.1% of V_P with block; Table 1.2).

For most morphological traits related to body size (body mass, pronotum length, femur length and tibia length), we found heritability coefficients of 0.4 to 0.54 (Table 1.2). Eye size had a lower heritability of 0.31 (Table 1.2). The heritability of traits was strongest when the effect of block was not included in the model (Table 1.2).

1.4.3.2 Sexually selected traits in males

Significant variance was attributed to sires (V_S) for mandible length when the effect of block (time of the year they mated) was excluded (Table 1.2). Sires explained 17.9% of the overall phenotypic variation (V_P) for mandible length when excluding block from the model.

There was no significant V_S for testis size (with block: 0% of V_P; without block: 15.3% of V_P) or harp size (with block: 20% of V_P) whether block was included or not (Table 1.2). Significant variance was attributed to dams (V_D) for harp size and testis size. V_D explained 23.5% of V_P for harp size and 24.7% for testis size in models with block (Table 1.2).

There was no significant variance attributed to dams (V_D) for mandible length (Table 1.2). Indeed, dams (V_D) explained very little of the overall phenotypic variation (V_P) for mandible length (with block: 13.9% of V_P; Table 1.2).

Traits associated with pre- (mandible length: $h^2 = 0.22$; harp size: $h^2 = 0.23$) and post- (testis size: $h^2 = 0.20$) copulatory sexual selection in males were all similarly heritable (Table 1.2).

1.4.3.3 Females reproductive traits

There was significant V_S for egg length (with block: 25.9% of V_P; without block: 34.5% of V_P) and ovary size (with block: 20.6% of V_P; without block: 21.8% of V_P) whether the effect of block was included or not (Table 1.2).

Significant variance was attributed to dams (V_D) for egg length and ovary size (Table 1.2). V_D explained 15.3% of V_P for egg length in models with block (Table 1.2). There was no significant variance attributed to dams (V_D) for ovary size (Table 1.2). Indeed, dams (V_D) explained very little of the overall phenotypic variation (V_P) in ovary size (with block: 17.2% of V_P; Table 1.2).

For traits associated with female reproduction, egg length was more heritable than ovary mass (egg length: $h^2 = 0.66$; ovary mass: $h^2 = 0.33$; Table 1.2).

1.4.3.4 Immunity

Significant variance was attributed to sires (V_S) for immunity only when the effect of block (time of the year they mated) was excluded (Table 1.2). Sires explained 35.8% of the overall phenotypic variation (V_P) for melanization response when excluding block from the model. Melanization response exhibited the highest heritability of any trait measured in this study ($h^2 = 0.69$; Table 1.2).

1.5 Discussion

Contrary to our prediction, none of the fitness-related traits tested in our quantitative genetic study on *Gryllus firmus* sand field crickets showed significant genotype by environment interactions, despite several being heritable ($h^2 > 0.3$) and influenced by rearing density.

Our data support the findings of other studies across a wide range of insects (e.g. grasshoppers, crickets, butterflies and drosophila; Abrams, Leimar, Nylin, & Wiklund, 1996; Applebaum & Heifetz, 1999; Niemelä *et al.*, 2012; Nunney, 1996; Zonneveld, 1996) that individuals growing under higher densities reach adulthood more quickly, but at a smaller body size, than individuals reared under lower densities. The longer development time of individuals reared under low density presumably permitted individuals to maximize their adult body size. Our results confirm that the environment, more specifically the rearing population density, is important in shaping overall body size in crickets. All traits showed a significant sire component and were heritable when the effect of block was excluded from the model, except testis mass. When block is important, the results will be discussed with and without block.

Block is the moment of the year the crickets hatched, i.e. the batch number. Because of that, the block effect also includes the effect of different sets of sires and dams. There was an effect of block on 6 out of the 12 measurements, showing temporal variation in morphological traits. This temporal variance associated with blocks is most likely caused by genetic variance between the dams and sires from the different mating blocks since V_A and V_D often became significant after removing the effect of block., which means we should consider results of heritability and phenotypic variance from models without the effect of block rather than models including the effect of block.

1.5.1 Pre-copulatory traits

Male crickets use their mandibles as weapons in male-male competition for access to territories where they attract potential mates (Adamo & Hoy, 1995; Alexander, 1961; Dixon & Cade, 1986; Hall *et al.*, 2010; Walker *et al.*, 2008). Mandible size is thus under strong sexual selection in male crickets, with males having bigger mandibles likely accruing greater fitness (Arnott & Elwood, 2008; Emlen, 2008; Hall *et al.*, 2010; Judge & Bonanno, 2008). In females, this trait is not sexually selected and bigger mandibles would simply be costly since they do not use their mouthparts to grapple (Judge & Bonanno, 2008). It was not surprising to find mandibles to be larger in males than females, which is also congruent with the literature (Judge & Bonanno, 2008; Marlowe, Murphy, & Chatzimanolis, 2015; Walker *et al.*, 2008). Contrary to our initial hypothesis, mandible size was not influenced by rearing density. However, mandible length showed a significant sire component and a small heritability. The sire component is only a small component of the narrow-sense heritability. A small heritability but significant sire component indicates an important influence of other factors such as the mating period on the trait.

We predicted high rearing population density would influence perceived level of competition and males would invest more in their mandibles to have a fighting advantage. One possible explanation to the lack of effect of density on mandible size would be that males respond more specifically to the sex ratio in the population than overall population density. Several experiments showed that increasing the number of competitors (males) has a greater impact on sexual traits and mating behavior than increasing overall population density, which could also increase the number of potential mates (Alonso-Pimentel & Papaj, 1996; Grant & Foam, 2002; Head, Lindholm, & Brooks, 2007; Pomfret & Knell, 2008). We could not control sex ratio in our experiment because nymphal crickets cannot be sexed and then could not test the effect of the sex ratio with a post-hoc because we removed females as soon as they matured and some of them matured much earlier than some males. Therefore, the amount of time spent with the females was highly variable.

Harps, which are structures on male wings used to create sound, were affected by density. Under low population density, it was expected that males would invest in structures to help attract females (Stefan, Jin, & Liao, 2017; Tinghitella, Stehle, & Boughman, 2015), since the females are harder to find. There are more competitors for mates under high population density, thus is it possible that males invest more in structures to help them sing such as their harps in order to outcompete the other males in singing contest and attract more females (Stefan *et al.*, 2017). In our study, males reared in low density had significantly bigger harps than males reared in high density after controlling for body mass. Although harp size did not show any sire component (i.e. the identity of the father did not influence harp size), it showed a dam component (i.e. the identity of the mother influenced harp size). Harp size had a similar narrow-sense heritability to mandible size when removing the temporal variation of the block component. We know that harp size is associated with sound frequency and indicates to the female the size of the male, with females preferring low sound frequency associated with bigger males (Nocke, 1972; Simmons & Ritchie, 1996). Males having bigger harps, sing at lower frequency, are thus are more successful at acquiring mates (Simmons, 1995). Harp size is a sexually-selected trait thus it is not surprising that it is heritable. However, the environment, and more specifically population density, definitely plays an important role, since abundance of competitors for mates influences the male's investment in this trait.

1.5.2 Post-copulatory traits

We predicted that if males reared under higher densities expected more intense sperm competition as adults, then they should increase their investment in testis size. Contrary to this prediction, males reared under high density did not develop larger testes than males reared under low density. Studies in other insects showed that males reared under high population density developed larger testes (Gage, 1995; Stockley & Seal, 2001). Males facing greater sperm competition were expected to have larger testis to transfer a greater quantity of sperm at once or to produce a larger quantity of smaller sperm in order to reproduce multiple times (Vahed & Parker, 2012). There is still a possibility that population density affected the investment in other post-copulatory traits that were not measured such as sperm length or velocity (Snook, 2005). We must also consider once again the possibility that the sex ratio in the population is more important than overall population density. Surprisingly, testes size showed no sire component and only a slight heritability compared to the other traits measured, but a significant portion of the variation could be attributed to the dams.

Since female crickets do not compete for mates, their investment in reproduction should not be influenced by population density. As expected, female investment in one aspect of reproduction, ovary mass, was not influenced by density. On the other hand, egg length was affected by density with females in low density producing larger eggs, contrary to what we expected from the literature (Taborsky *et al.*, 2007). More generally, females can adjust their investment in their eggs depending on environmental factors to optimize their fecundity (Blanco *et al.*, 2003; Fox *et al.*, 1997; McGinley, Temme, & Geber, 1987). One possible explanation is that high density was a stressful environment for female crickets, which limited their overall mass and thus their average egg length. Ovary mass and egg length both showed a sire and dam component and were strongly heritable, especially average egg length. This level of heritability for egg size is consistent with studies on *Lagopus lagopus scoticus* red grouse where bigger eggs are associated with higher fitness (Moss & Watson, 1982), *Parus major L.* great tits where there is selection for bigger eggs when resources are limited (Noordwijkl *et al.*, 1981) and *Stator limbatus* seed beetles where egg size trade-offs with lifetime fecundity (Czesak & Fox, 2003; Fox *et al.*, 1999).

1.5.3 Immunity

Counter to expectations, *Gryllus firmus* does not seem to show density-dependent prophylaxis as we found no effect of density on one component of the cricket's immune response. Melanization showed a significant sire component and was the most heritable trait measured, which is consistent with other studies on the heritability of immunity in crickets and other insects (Cotter, Kruuk, & Wilson, 2004; Decanini, Collins, & Evans, 2007; Gershman *et al.*, 2010). High heritability and high genetic variation are important for selection to act on and improve insect health over generations, as demonstrated in the honeybee *Apis mellifera* (Decanini *et al.,* 2007). Also, it has been shown that various traits within the insect immune system trade-off with each other (Cotter *et al.*, 2004). It is possible that increased population density pushes individuals to invest in other traits of their immune system. Furthermore, studies comparing immune function between the sexes found that females had better immune function (Schmid-Hempel, 2011; Nunn *et al.*, 2009), and the same result was found more specifically in crickets (Gershman, 2008; Adamo *et al.*, 2001). Zuk *et al.* (2004) found the opposite, with males *Teleogryllus commodus* crickets having a greater immune response than females. In our study, we found no significant difference in the level of melanization between males and females.

Overall, we did not find any genotype by environment interaction on morphological traits. Some traits were affected by rearing density, some by genetics. From our study, there is no reason to believe that *Gryllus firmus* families respond differently to population density during development. Even though we used hundreds of crickets for this experiment, studies of this kind require hundreds of families to test genetic correlations, which makes our sample size relatively small (Lynch & Walsh, 1998; Rodriguez-Munoz, Bretman, Hadfield, & Tregenza, 2008). Our study was not the first to find no GxE interaction. Pérez-Rostro & Ibarra (2003) also found no effect of a genotype by environment interaction on any measured trait in Pacific white shrimp (*Penaeus vannamei)* that were raised under different densities. However, we must also keep in mind that environments can influence morphology in many ways other than population density. We therefore encourage more studies on the matter to shine a light on possible genotype by environment interactions.

1.6 Acknowledgements

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Table 1.1. Results from general linear models (GLMs) showing the effect of rearing density, sex, and their interaction on the expression of each of 12 morphological or life history traits in adult sand field crickets (*Gryllus firmus*) after controlling for body mass. Separate GLMs were run for each trait.

		95% CI				
Factor	**Estimate**	**lower**	**upper**	**t**	**df**	**p-value**
Body mass (mg)						
Intercept	835.7	801.755	869.605	40.84	9.20	<0.001
Density	**51.67**	**29.461**	**73.791**	**3.832**	**620.2**	**<0.001**
Sex	**-191.65**	**-213.262**	**-170.045**	**-14.596**	**614.5**	**<0.001**
Density x Sex	-20.67	-51.454	10.044	-1.110	623.90	0.268
Eye area (mm^2)						
Intercept	0.491	0.4522	0.5285	21.24	98.9	<0.001
Density	**0.026**	**0.0121**	**0.0389**	**3.132**	**613.7**	**0.002**
Sex	**0.057**	**0.0424**	**0.0721**	**6.335**	**619.1**	**<0.001**
Density x Sex	0.012	-0.0066	0.0302	1.055	614.8	0.2856
Pronotum length(mm)						
Intercept	1.148	1.1224	1.1736	74.303	68	<0.001
Density	**0.0159**	**0.0073**	**0.0245**	**3.040**	**615.4**	**0.002**
Sex	-0.0039	-0.0134	0.0056	-0.677	619.8	0.4988
Density x Sex	0.0085	-0.0033	0.0202	1.184	617.1	0.2328
Pronotum width (mm)						
Intercept	1.567	1.5492	1.5854	142.933	195.8	<0.001
Density	**0.015**	**0.0086**	**0.2193**	**3.749**	**618.5**	**0.00028**
Sex	**0.0396**	**0.0322**	**0.0470**	**8.789**	**623.8**	**<0.001**
Density x Sex	0.0048	-0.0044	0.0139	0.858	620.1	0.3942
Femur length (mm)						
Intercept	2.3429	2.3227	2.3632	190.787	78.6	<0.001
Density	**0.0184**	**0.0149**	**0.0253**	**4.380**	**618**	**<0.001**
Sex	0.0077	-0.001	0.0152	1.640	622.9	0.1006
Density x Sex	0.0085	-0.009	0.0180	1.485	620.1	0.1419

Tibia length (mm)						
Intercept	2.126	2.1029	2.1486	152.934	61.8	<0.001
Density	**0.0185**	**0.011**	**0.0261**	**4.016**	**609.6**	**<0.001**
Sex	0.0073	-0.001	0.0157	1.426	613.6	0.156
Density x Sex	0.0034	-0.007	0.0137	0.537	611	0.5929
Mandible length (mm)						
Intercept	0.7455	0.7234	0.7675	55.654	270.5	<0.001
Density	0.0055	-0.0029	0.0141	1.074	621.8	0.2886
Sex	**0.1608**	**0.1515**	**0.1702**	**28.136**	**628.1**	**<0.001**
Density x Sex	0.0121	0.0005	0.0237	1.712	623.6	0.0864
Harp area (mm^2)						
Intercept	2.2380	2.2014	2.2740	102.343	122.8	<0.001
Density	**0.0240**	**0.0130**	**0.034967**	**3.611**	**293.6**	**<0.001**
Testis size (mg)						
Intercept	0.6931	0.5522	0.8326	8.189	61.4	<0.001
Density	0.003	-0.0350	0.0411	0.128	289.3	0.8888
Egg length (mm)						
Intercept	0.8609	0.84	0.8818	67.958	71.5	<0.001
Density	**0.0158**	**0.0103**	**0.0214**	**4.664**	**275.6**	**<0.001**
Ovary size (mg)						
Intercept	2.0106	1.7812	2.2415	14.546	13.82	<0.001
Density	-0.0012	-0.0704	0.0027	-0.028	422.8	0.9786
Immunity						
Intercept	189.2744	171.0760	207.3659	17.289	66.7	<0.001
Density	-4.5783	-10.714	1.535	-1.231	92.52	0.222
Sex	-0.1831	-7.9144	7.5311	-0.039	104.29	0.9719
Density x Sex	1.2712	-7.8436	10.4418	0.230	97.14	0.8209

Statistically significant effects ($\alpha = 0.05$) are in bold. The reference density was ‘high’ and the reference sex was ‘female’.

Table 1.2. Variance components and narrow-sense heritability estimates for measured traits in adult sand field crickets (*Gryllus firmus*).

Variance components	Models with block included					Models with block excluded				
		95% CI					95% CI			
	Predictor	lower	upper	χ^2	p-value	Predictor	lower	upper	χ^2	p-value
Body mass										
V_S	19.660	<0.01	36.779	1.155	0.283	**51.000**	**<0.01**	**38.612**	**13.616**	**< 0.001**
V_D	25.220	0.000	34.169	3.486	0.062	25.860	32.847	67.145	3.629	0.057
V_{block}	**45.060**	**15.154**	**68.974**	**12.54**	**<0.001**					
V_R	118.430	112.736	123.96			118.450	112.81	124.007		
h^2	0.085					0.415				
m^2	0.035					0.027				
Eye area										
V_S	<0.0001	0.017	0.032	0.000	1	**0.025**	**0.017**	**0.034**	**5.438**	**0.02**
V_D	**0.026**	**0.000**	**0.018**	**21.672**	**<0.001**	**0.026**	**0.011**	**0.035**	**21.053**	**<0.001**
V_{block}	**0.027**	**0.007**	**0.041**	**8.148**	**0.004**					
V_R	0.070	0.067	0.074			0.070	0.067	0.074		
h^2	0.000					0.305				
m^2	0.108					0.086				
Pronotum length										
V_S	0.013	0.007	0.018	3.759	0.053	**0.023**	**0.008**	**0.019**	**14.529**	**< 0.001**
V_D	**0.014**	**<0.001**	**0.019**	**9.864**	**0.002**	**0.014**	**0.015**	**0.030**	**10.338**	**0.001**
V_{block}	**0.019**	**0.003**	**0.030**	**7.483**	**0.006**					
V_R	0.045	0.043	0.047			0.045	0.043	0.047		
h^2	0.205					0.489				

m^2	0.056					0.044				
Pronotum width										
V_S	0.009	0.005	0.013	2.827	0.093	**0.013**	**0.005**	**0.014**	**7.883**	**0.005**
V_D	**0.010**	**<0.001**	**0.013**	**8.036**	**0.005**	**0.010**	**0.007**	**0.017**	**8.337**	**0.004**
V_{block}	0.009	0.000	0.015	3.151	0.076					
V_R	0.035	0.034	0.037			0.035	0.034	0.037		
h^2	0.181					0.323				
m^2	0.055					0,049				
Femur length										
V_S	0.009	0.003	0.013	3.240	0.072	**0.019**	**0.003**	**0.013**	**21.673**	**<0.001**
V_D	**0.009**	**<0.001**	**0.014**	**5.387**	**0.020**	**0.009**	**0.013**	**0.025**	**5.501**	**0.019**
V_{block}	**0.015**	**0.004**	**0.024**	**10.411**	**0.001**					
V_R	0.036	0.035	0.038			0.036	0.035	0.038		
h^2	0.172					0.506				
m^2	0.04					0.028				
Tibia length										
V_S	0.010	0.010	0.019	0.999	0.318	**0.023**	**0.009**	**0.019**	**16.342**	**< 0.001**
V_D	**0.015**	**0.000**	**0.016**	**18.724**	**< 0.001**	**0.014**	**0.015**	**0.030**	**17.911**	**< 0.001**
V_{block}	**0.018**	**0.005**	**0.029**	**9.263**	**0.002**					
V_R	0.040	0.038	0.042			0.040	0.038	0.042		
h^2	0.149					0.537				
m^2	0.088					0.053				
Mandible length										
V_S	0.009	<0.001	0.014	2.060	0.151	**0.012**	**<0.001**	**0.015**	**4.494**	**0.034**

V_D	0.010	<0.001	0.015	3.249	0.071	0.010	0.005	0.017	3.422	0.064
V_{block}	0.008	0.000	0.015	1.536	0.215					
V_R	0.045	0.043	0.047			0.045	0.043	0.047		
h^2	0.140					0.222				
m^2	0.036					0.035				
Harp area										
V_S	0.023	0.016	0.036	2.136	0.144	0.023	0.016	0.036	2.381	0.123
V_D	**0.027**	**<0.0001**	**0.033**	**11.261**	**<0.001**	**0.027**	**0.005**	**0.034**	**11.261**	**<0.001**
V_{block}	0.005	0.000	0.022	0.000	1.000					
V_R	0.060	0.056	0.064			0.060	0.056	0.064		
h^2	0.327					0.227				
m^2	0.112					0.034				
Testis size										
V_S	0.000	0.062	0.126	0.000	1.000	0.059	0.081	0.149	0.476	0.490
V_D	**0.102**	**0.000**	**0.071**	**18.584**	**<0.001**	**0.119**	**0.000**	**0.103**	**22.409**	**<0.001**
V_{block}	**0.104**	**0.025**	**0.160**	**6.172**	**0.013**					
V_R	0.207	0.192	0.221			0.207	0.192	0.221		
h^2	0.000					0.198				
m^2	0.162					0.199				
Egg length										
V_S	**0.044**	**0.004**	**0.015**	**12.491**	**<0.001**	**0.016**	**0.004**	**0.015**	**19.269**	**<0.001**
V_D	**0.026**	**0.01**	**0.024**	**4.995**	**0.025**	**0.021**	**0.014**	**0.028**	**5.193**	**0.023**
V_{block}	0.029	0.000	0.022	0.759	0.384					
V_R	0.071	0.027	0.031			0.029	0.027	0.031		

h^2	0.546					0.656				
m^2	0.047					0.041				
Ovaries mass										
V_S	**0.129**	**<0.0001**	**0.165**	**4.054**	**0.044**	**0.131**	**<0.0001**	**0.165**	**4.288**	**0.038**
V_D	0.108	<0.0001	0.182	3.055	0.081	0.108	0.043	0.188	3.055	0.081
V_{block}	0.028	0.000	0.118	0.000	1.000					
V_R	0.362	0.335	0.387			0.362	0.335	0.387		
h^2	0.316					0.325				
m^2	0.056					0.055				
Melanization										
V_S	8.171	1.494	11.777	2.512	0.113	**12.266**	**<0.0001**	**11.559**	**7.821**	**0.005**
V_D	7.700	<0.0001	12.620	3.637	0.057	7.377	7.206	16.769	3.072	0.080
V_{block}	10.937	<0.0001	17.783	2.752	0.097					
V_R	14.45	12.288	16.197			14.602	12.432	16.366		
h^2	0.408					0.692				
m^2	0.091					0.063				

Significant components are in bold. V_S = sire variance component; V_D = dam variance component; V_{block} = block variance component; V_R = residual variance; χ^2 = Likelihood-ratio test statistic; h^2 = narrow-sense heritability; m^2 = maternal effect; CI = 95% confidence intervals from bootstrapping; P = p-value from LRT

Table 1.3 Summary statistics of all morphological traits measured for female and male sand field crickets (*Gryllus firmus*) that were reared at either low or high density in the laboratory.

	Low density						High density					
	Females			Males			Females			Males		
Trait	n	Mean	standard deviation	n	Mean	standard deviation	n	Mean	standard deviation	n	Mean	standard deviation
Development time (days)	159	60.96	0.73	169	65.64	1.01	154	55.76	0.56	171	55.64	0.48
Mass (mg)	161	880.92	12.46	170	672.57	8.57	157	831.93	10.36	174	632.32	8.86
Pronotum length (mm)	161	1.51	0.01	170	1.43	0.01	157	1.48	0.01	174	1.39	0.01
Eye size (mm^2)	161	2.91	0.02	170	2.74	0.02	156	2.75	0.02	174	2.58	0.03
Mandible length (mm)	161	3.04	0.01	170	3.32	0.02	157	2.97	0.01	174	3.23	0.02
Femur length (mm)	161	14.25	0.07	170	13.50	0.06	157	13.76	0.06	173	12.97	0.07
Tibia length (mm)	160	11.46	0.06	169	10.79	0.06	157	11.07	0.05	173	10.43	0.06
Harp size (mm^2)	-	-	-	170	14.81	0.11	-	-	-	174	14.11	0.11
Ovaries mass (mg)	159	73.45	2.95	-	-	-	157	66.51	3.10	-	-	-
Testis mass (mg)												
Egg length (mm)	159	2.45	0.01	-	-	-	157	2.41	0.01	-	-	-

CHAPTER II

WHAT MAKES A WINNER? ANALYSIS OF CONFLICT RESOLUTION IN A FIELD CRICKET (GRYLLUS FIRMUS)

2.1 Abstract

Contests are common among animals whenever a resource is limited. Given the same experience and the same motivation, the contest outcome should be determined by the individuals' physical characteristics. The winner is usually the biggest individual, the one with the biggest weapons, the strongest, etc. Those traits can be shaped by environmental factors and diet. Here, we looked at the environmental effect of rearing density on weapon size and the effect of diet on stamina in the field cricket (*Gryllus firmus*). We tested the hypothesis that males reared in high density would invest more in weapons in preparation for the perceived future competitive environment. We also hypothesized that males given a better-quality diet should have more stamina and last longer in contests. We tested the postulate made by Palaoro and Briffa (2016) that weapons are allometric to body size, which would change predictions of contest settlement of accepted theories on the topic. We reared crickets in high and low population density until maturity. From maturity until experimental day, we allocated crickets to either a low-quality diet or high-quality diet. We size-matched and age-matched crickets, then recorded the fights. Population density did not influence mandible size (weapons), but mandible size had an impact on contest intensity. The treatments did not influence the outcome of the contest. Males with a better diet had more fat but they did not fight longer or more intensely. Although we found an allometric relationship between mandible size and body size, this relationship did not change predictions of contest settlement. It appears that population density and diet are not as important as we would have thought in shaping individuals' morphology or influencing contest resolution. Instead, it might be other environmental factors influencing allocation decisions and contest outcomes in this species; future studies should aim to measure the influence of other factors, such as abiotic factors.

Key words: contest settlement, weapons, density, diet, stamina, RHP, duration, intensity, allometry

2.2 Introduction

Animals often engage in physical fights over access to resources, including food, shelter, and mates (Parker, 1974; Simmons, 1986). The ability to win a contest for resources is generally dependent upon an individual's resource-holding potential (RHP; Parker, 1974), which often comprises a combination of traits including weapon or body size, endurance, and energetic state (Kelly, 2008). RHP can be separated into offensive (ability to inflict damage and injury on the opponent) and defensive ability (ability to withstand a rival's offensive attack; Palaoro & Briffa, 2016).

Fighting is a costly endeavour with respect to injuries, loss of energy, and loss of time that could otherwise be spent doing other fitness-related activities (Briffa & Sneddon, 2007; Briffa & Elwood, 2009). Individuals will not invest in costly fights unless they value the resource they are fighting over. If individuals grant the same value to a contested resource, then the individual with the highest RHP should win the contest for the resource (Arnott & Elwood, 2009; Brown *et al.*, 2007; Vieira & Peixoto, 2013). However, if one of the individuals values the resource more than its opponent, his motivation will be greater and the cost threshold he is willing to tolerate will also be greater (Arnott & Elwood, 2008; Enquist & Leimar, 1987; Judge *et al.*, 2010). Theory predicts that an individual should withdraw from a fight whenever the costs exceed the benefits or whenever they ascertain that their chances of winning are lower than those of their opponent (Palaoro & Briffa, 2016). In many species, the cost of a fight is mostly in the form of oxygen consumption (Briffa & Sneddon, 2007; Hack, 1997) and very little in terms of injuries. Energetic costs are thereby directly linked to contest duration since longer fights result in higher oxygen consumption (Prenter *et al.*, 2006).

Individuals can determine their likelihood of winning a contest by assessing the fighting ability of their rivals. Usually, in male-male competition, bigger traits and bigger RHP confers a greater probability of winning, but that implies combatants can determine their rival's RHP or know when to give up if they have a smaller RHP than their opponent (Judge & Bonanno, 2008; Kelly, 2006b; Parker, 1974). Three theoretical models offer possible explanations as to how individuals gather information on the RHP of their rivals and we can separate these into two categories: those based on self-evaluation and those based on mutual evaluation.

In the first category, the individual only has information about its own RHP and the maximum cost threshold he is willing to invest in the fight (Arnott & Elwood, 2009; Enquist & Leimar, 1983; Enquist *et al.*, 1990). In the second category, it is presumed that both contestants' cognitive functions are developed enough to compare each other's fighting abilities (relative RHP) and that fights are used to gain more precise information to allow this evaluation [e.g. sequential assessment model (SAM); Enquist & Leimar, 1983, 1987; Hsu *et al.*, 2006; Junior & Peixoto, 2013; Palaoro & Briffa, 2016]. The SAM assumes mutual assessment and predicts that losers should give up as soon as they detect that they are weaker than their opponent. Consequently, the contests should proceed in several phases (with interruptions between phases) with each phase escalating in intensity (Kelly, 2006a). Contest duration and contest intensity are thus often highly correlated. This model predicts a negative relationship between contest duration (and intensity) and winner's RHP or a positive relationship between contest duration (and intensity) and loser's RHP (Gammell & Hardy, 2003).

The cumulative assessment model (CAM) also assumes self-assessment and predicts that opponents should persist until they reach a cost threshold and that combatants make decisions based on the sum of their opponent's actions (Hsu *et al.*, 2006; Judge & Bonanno, 2008; Kelly, 2006a; Payne, 1998). The CAM assumes that individuals do not know the effect of their attacks on their opponent. Under this model, contests occur in a single phase (no interruption) that increases in intensity (Kelly, 2006a) and contest duration (and intensity) should positively covary with body size of the focal individual

(Palaoro & Briffa, 2016). An empirical outcome that is common among these models is that contests are generally settled more quickly as the difference in RHP between rivals increases. However, in CAM, contest duration will increase with mean opponent RHP, which is not the case in SAM.

In most taxa, several features of RHP, such as structural body size and weapon size, are fixed at sexual maturation (Calamari, 2016; Koning & Jamieson, 2001; Tedore & Johnsen, 2015). However, there are some features that can change between, or within, fights. For example, motivation and stamina are also important in determining contest outcomes and can vary from one fight to another (Palaoro & Briffa, 2016). Stamina is an individual's endurance or energetic capacity to persist in a contest and sustain demanding physical and metabolic activities (Palaoro & Briffa, 2016). Stamina is generally determined by either energetic reserves (e.g. fat load), metabolic rate, or aerobic capacity (Briffa & Elwood, 2004; Briffa & Lane, 2017; Seebacher & Wilson, 2006). Individuals with lower stamina tend to retreat from contests, or lose more often, than individuals with higher stamina (Briffa & Sneddon, 2007; Briffa & Elwood, 2000; Briffa & Lane, 2017; Fawcett & Mowles, 2013). Therefore, winners of contest are often individuals with higher energetic reserves (i.e. fat load; Marden & Rollins, 1994).

Because an individual's energetic reserves are influenced by its environment, individuals living in an environment with abundant high-quality nutritional resources are likely to have more energetic reserves (and thus stamina) than individuals living in low-quality environments (Droney, 1998). Indeed, experimental studies in several species have shown that lower quality diets reduce the energy reserves of individuals (Uetz, Papke, & Kilinc, 2002; Droney, 1998) but no study has yet examined the effect of diet on stamina in contests over resources.

An important factor in determining environmental quality directly and indirectly is rearing density, as it will directly dictate the amount of resources available per individual (Brown, 1982; Estevez, Andersen, & Naevdal, 2007). For example, in a tropical butterfly (*Bicyclus anynana*), males reared in high density developed faster presumably to avoid resource exhaustion before eclosion to adulthood or because it was a signal of future environmental conditions (Bauerfeind & Fischer, 2005). Also, increasing densities of harlequin flies (*Chironomus riparius*) resulted in higher generation time, and lower fecundity and adult body weight due to lower resource availability (Hooper, Sibly, Hutchinson, & Maund, 2003). However, this is only part of the explanation since genetics also plays a role in determining the quantity of resources acquired with some individuals being better at acquiring resources than others (Reznick *et al.*, 2000; Van Noordwijk & de Jong, 1986).

The rearing environment can also indirectly influence an individual's developmental trajectory by providing cues to the individual regarding the environment that they will likely experience as an adult (Emlen & Oring, 1977; Kasumovic *et al.*, 2012; Kelly, 2018). Therefore, developing individuals should use these cues to adaptively invest in traits that will maximize fitness as an adult. Rearing density could also cue individuals as to the level of competition for resources (i.e. food, shelter, mates) that they will face as adults (Kelly, 2018; Kokko & Rankin, 2006). For example, if individuals perceive a high level of competition for mates in the future due to high population density, males can invest more in RHP-related traits, to enhance their chances for reproductive success (Emlen & Oring, 1977; He & Tsubaki, 1991; Knell, 2009; Kokko & Rankin, 2006; Kasumovic, Hall, & Brooks, 2012). Empirical support for this prediction has been found in species in which males engage in fights for access to mates such as mites (*Sancassania berlesei*) and the Onthophagus dung beetles, where high rearing density cues males to increase their investment in weaponry (Pomfret & Knell, 2008; Radwan, Unrug, & Tomkins, 2002). Also, high rearing density cues male *Forficula auricularia* earwigs to develop more fighter morphs to better compete against other males for mates (Tomkins & Brown, 2004). In contrast, high rearing density causes males in some species to develop into less aggressive non-fighters (Radwan, 1993; Knell, 2009).

It will not pay for males to invest increasingly in weapons or body size and so investment will peak at some intermediate density (Knell, 2009). Knell (2009) argues that the benefits of aggression are low at low population densities because of the unavailability of mates and that males are unlikely to encounter each other, so males should invest in structures to help locate mates rather than invest in traits that promote fighting ability. At the other extreme, the costs of continual fighting (e.g. early mortality) will outweigh the benefits (Knell, 2009). The empirical evidence is equivocal on this front with some studies showing greater, lesser and no change in male investment in RHP. For example male acarid mites (*Caloglyphus berlese*) develop a less aggressive morph at high rearing density (Radwan, 1993) and isolated crickets (*Gryllus bimaculatus*) were more aggressive than those reared under higher density (Iba, Nagao & Urano, 1995). The rosy bitterling *Rhodeus ocellatus* showed no difference in aggression as a function of population density (Casalini, Reichard, & Smith, 2010). The effect of rearing density on allocation to weaponry, aggression and fighting ability is thus unclear.

In this study, we use the sand field cricket *Gryllus firmus* (Orthoptera: Gryllidae Scudder) to investigate the effect of rearing density on investment in male weaponry and fighting ability. In this species, males fight intensely over burrows from which to call females (Alexander, 1961; Brown *et al.*, 2007; Otte & Cade, 1976; Tachon *et al.*, 1999). Crickets fight in a single phase that increases in intensity; at some point the loser retreats from the fight and the winner sings at the end of the contest (Briffa, 2008; Judge & Bonanno, 2008; Reaney, Drayton, & Jennions, 2011). Body size is an important indicator of a male RHP in crickets with bigger males winning more contests against smaller males (Briffa *et al.*, 2013; Judge & Bonanno, 2008). Males with larger mandibles (another primary determinant of RHP in crickets: Judge and Bonanno, 2008) acquire better territories - ones that offer better protection against predator and parasites (Simmons, 1986) - which ultimately translates into higher reproductive success (Kelly, 2008; Nelson & Nolen, 1997; Simmons, 1986). Rillich *et al.* (2007) found that *Gryllus bimaculatus* crickets follow the CAM model of contest settlement with males self-assessing the total costs (i.e. injuries) that they have accrued as a result of their opponents' actions. When the costs surpass a tolerance threshold, males (the loser) will retreat.

Here, we test the hypothesis that rearing density provides developing individuals cues as to the competitive environment that they will likely face as adults and that these conditions should consequently cause developing males to adaptively invest in traits that maximize reproductive success in that environment. We predict that if high-density rearing environments foreshadow future intense male-male competition for mates then males should maximize their RHP by investing in traits such as larger body and mandible size compared with crickets reared in low-density environments. Because high-density individuals should have greater RHP they should win fights against low-density crickets.

We further examined how these contests are settled by testing predictions of the SAM and CAM to determine whether crickets self-assess (CAM) their ability to continue in a fight or decide to give up by mutually-assessing (SAM) their RHP and that of their opponent. We compared our results with the CAM and the SAM models. We predicted that male *G. firmus* crickets will behave as *G. bimaculatus* males (Rillich *et al.*, 2007) and settle contests according to the CAM since the fights occur in a single phase and we believe contest duration to be correlated to mean opponents mass.

We separate the potential effect of rearing density on body condition (stamina) by feeding adult males that were reared under either low or high density a restricted or non-restricted diet. We predict that males fed a non-restricted diet will be more successful in fights due to their greater stamina than males fed a restricted diet, all else being equal.

2.3 Methods

2.3.1 Animal rearing

The crickets used in this experiment were lab-raised descendants of individuals collected in Fall 2010 in Gainesville, FL (USA). Crickets were raised communally in 70-L bins in an environmentally-controlled room at constant temperature (28°C), relative humidity (60%), and on a reverse light cycle of 12 hours of day: 12 hours of night. Crickets were provided with cotton-plugged water tubes, Iams™ Proactive Health™ cat food *ad libitum* so that multiple crickets could eat and drink at the same time, and layers of cardboard egg cartons for shelter.

2.3.2 Experimental design

2.3.2.1 Animal husbandry and density treatment

We randomly assigned crickets that were 10-14 days post-hatch to either a low or high rearing density treatment. The low-density treatment comprised one individual per 250-mL transparent plastic container, whereas the high-density treatment comprised ten individuals per 750-mL transparent plastic container. Individuals in both treatments were provided with *ad libitum* food and water and were all provided with egg carton for refuge. Food and water were replaced, and containers cleaned, twice per week.

Natural mortality reduced the density in all high-density replicates. If two groups dropped to fewer than 5 individuals each, the two groups were combined to maintain a high density; replicates with six or more crickets were retained because these densities should provide a social environment that is markedly different than the low-density treatment. All containers were haphazardly rotated in the growth chamber to minimize the effects of microhabitat variation.

2.3.2.2 Diet treatment

At eclosion to adulthood, crickets were weighed to the nearest 0.0001 g on a Sartorius Secura 224-1S analytical balance and placed individually into a 250-mL container provisioned with water and a piece of egg carton for shelter. We kept only the males for the experiment and discarded the females. We manipulated body condition (i.e. fat load) by altering the amount of food available to adult male crickets from eclosion to their use in an experimental contest. Males were either fed a non-restricted (NR) diet comprising approximately 0.07 g of fish food (NUTRAFIN™ BUG BITES; 32% protein, 10% fat, and 6% fiber) every other day or a restricted (R) diet comprising half as much fish food (0.035 g). Diet should only affect body condition (i.e. body and fat mass) but no other morphological trait because all structural traits (i.e. mandibles and pronotum) are fully developed at this point and will not change.

2.3.2.3 Staged contests

At sexual maturity (8 to 14 days post-eclosion), the crickets were weighed again and size-matched to an opponent (within 5% difference in body mass; Pearson $r = 0.997$, $t = 348.07$, d.f. = 824, $p < 0.001$) to fight the next day. We staged male-male contests in 8 treatment combinations in the dark under infrared light. Once the crickets were age- (maximum 5 days age difference) and size-matched, we uniquely labelled each combatant of a pair with either one or two dots of nail polish on the pronotum to identify crickets during the fight. On the day of a fight, the opponents were placed on opposite sides of a circular plastic arena (11 cm of diameter) and separated by a plastic divider. After a two-minute acclimatization period, the divider was removed and the fight was recorded using a Basler (acA1300-60gm, EV76C560 CM0S) night vision camera until a clear winner could be determined (the winner stridulated and the loser ran away).

The individuals were immediately frozen at the end of each trial for later morphological measurement (see below). We analyzed the videos of contests using Ethovision® XT video tracking software (Noldus, Spink, & Tegelenbosch, 2001) to determine the duration of the contest (time from first antennal contact to the retreat of one male), who initiated the fight and the contest winner (the male that stridulated and chased his opponent). We also determined the level of aggression (contest intensity) for each fight following Hofmann and Schildberger (2001) and Judge and Bonanno, 2008): 1= immediate dominance, 2= mutual antennation, 3= unilateral maxillae/mandible spreading, 4= bilateral maxillae/mandible spreading, and 5=grappling. We did not have any crickets showing mutual avoidance and the lowest level of aggression observed was immediate dominance.

2.3.2.4 Morphological measurements

All preserved specimens were digitally photographed using a Leica SC170 HD camera (Leica Microsystems Inc., Concord, ON, Canada) connected to a Leica S6D microscope (Leica Microsystems Inc., Concord, ON, Canada). We used LAS V4.5.5 software (Leica Application Suite) to digitally stamp a scale bar on each image. We then imported each image into ImageJ™ (National Institutes of Health, USA) to measure pronotum length and width (spanning the ventral margins across the neck membrane and cervical sclerites, to the nearest 0.001 mm; see Figure 2.1), and mandible length (from lateral articulation to the distal tip, to the nearest 0.001 mm; see Figure 2.2) and width (measurement of widest segment to the nearest 0.001 mm; see figure 2.3). After taking measurements we dried crickets at 60°C for 24 hours and weighed them to the nearest 0.0001 g using a Sartorius Secura 224-1S analytical balance. Water mass was measured by taking the difference between the fresh mass (mass of the cricket before being dried) and dry mass (after 24 hours). Body fat was extracted using petroleum ether in a Soxhlet apparatus for 24 hours. The crickets were then dried again for 24 hours and weighed again to acquire lean dry mass. Fat mass was obtained by subtracting lean dry mass from dry mass for each individual. Fat mass was used as a measure of stamina to test the prediction that diet influences the level of stamina and that stamina influences contest outcome as suggested by Palaoro and Briffa (2016). We calculated a scaled mass index (SMI) as a measure of body condition that accounts for the covariation of body size with body mass components (Kelly *et al.*, 2014; Peig & Green, 2009). Fat load was determined post-contest for logistical reasons; thus, we do not know an individual's pre-contest fat load. However, post-contest fat load should accurately indicate the energy reserves available to an individual during a fight because contest duration is generally very short (< 15 s) and so it is unlikely that a cricket would expend all its reserves in one fight. In total, 882 individuals were used for the experiment and 441 fights were recorded (n=390 high density individuals, n=440 low density individuals; n=417 individuals on good diet, n=413 individuals on poor diet).

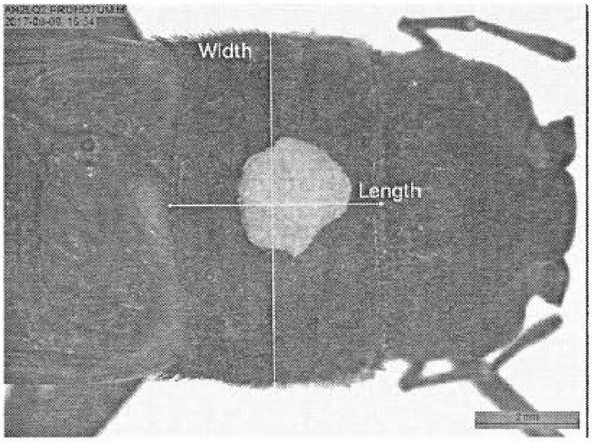

Figure 2.1. Measurement of pronotum width and length of a male cricket *Gryllus firmus*. A system of yellow dots (1 dot or 2 dots) was used to distinguish the crickets in the videos.

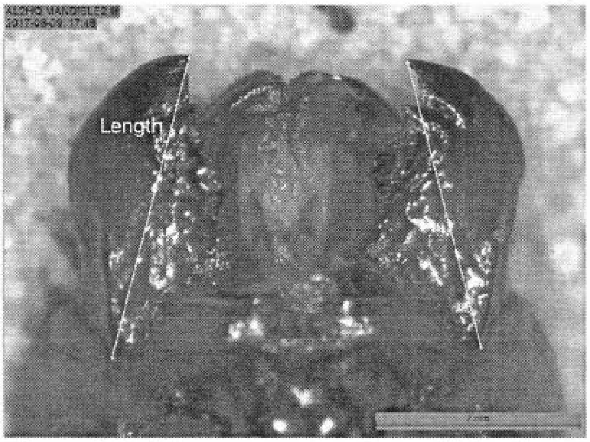

Figure 2.2. Measurements of mandibles length of a male cricket *Gryllus firmus*

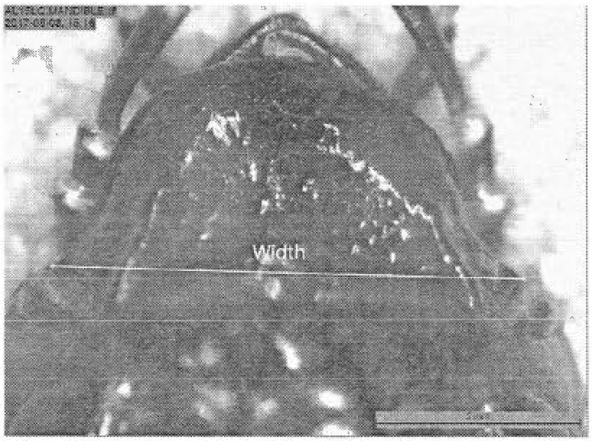

Figure 2.3. Measurement of mandible width of a male cricket *Gryllus firmus*

2.3.3 Statistical analysis

All statistical analyses were conducted using R Studio version 2.1 (v. 1.0.153; 2016). There were significantly more short-winged males in the high-density than in the low-density treatment (Chi-squared test: $X^2 = 12.067$, d.f. = 1, p = 0.0005), but morphotype had no significant effect on contest outcome, duration or intensity (unpublished results) and so we henceforth no longer consider morph differences in our analysis.

Scaled-mass index (Peig and Green, 2009) was calculated as a measure of body condition using the equation:

$$\hat{M}_i = M_i \left[\frac{L_o}{L_i}\right]^{b_{SMA}} \qquad (1)$$

where M_i is the body mass at eclosion and L_i is the pronotum width of individual i. L_o is the mean pronotum length of the population. b_{SMA} is the scaling exponent calculated by the standardized major axis (SMA) regression of ln M on ln L, in our case, b_{SMA} = 2.614.

We used MANOVA to test the prediction that high-density males invested more in RHP-related traits than low-density males and that a post-eclosion NR diet increases body mass, condition, and fat content. We entered the morphological traits into our model as response variables and density and diet as fixed effect factors. We then conducted post-hoc tests on each response variable separately by using two-factor ANOVAs with density and diet as fixed factors.

We tested the probability of winning a contest as a function of rearing density and adult diet by using chi-squared tests. We predict that high-density and NR males should win significantly more than 50% of contests (i.e. better than random) when matched against a low-density or R male. We also tested the probability that the male who initiated the fight also won the fight using a chi-squared test to see if self-assessment of mutual-assessment could start prior to the fight or if it is any indication of motivation. We used separate general linear models to quantify the relationship between the dependent variable contest duration and the independent variables adult diet (R vs. NR) and rearing density (low vs. high). We used generalized linear models with Poisson error distribution for models with contest intensity as the dependent variable. These models will test the predictions that contest duration and intensity will be greater when both combatants: (i) are from the same rearing and diet treatments (i.e. males having nearly identical RHP); (ii) are fed an NR diet versus an R diet; and (iii) were raised under high densities. We consider fights between similar males [i.e. those in (i)] as control treatments and compare other treatments to this group in our analyses. Because we are uncertain as to the predicted effect of diet and rearing density on male RHP and motivation, our experiments are partly exploratory with the goal of identifying the relative importance of these factors on contest variables. For example, it is unclear how high-density/NR vs. high-density/R should differ in duration and intensity from the control compared with high-density/NR vs. low-density/NR.

In addition to examining treatment effects on contest settlement, we examined contests at the individual level because treatment might not have an effect on RHP or motivation but individuals will vary in RHP due to effects unrelated to their treatments, such as genetics. We tested the probability of winning (dependent variable) as a function of various RHP traits (independent variable) using a logistic regression with 0=lost and 1=win. Taylor and Elwood (2003) argue that most mutual- and self-assessment models of contest resolution predict that contest duration will decrease with increasing RHP difference between contestants because a bigger difference between the individuals is more easily detected. However, they also argue that contest duration could be based solely on the contestant's own RHP, with weaker opponents retreating more quickly simply because they are less likely to win. We thus used GLMs to assess the relationship between various (known and putative) components of RHP (i.e. body mass, pronotum size, mandible size and fat mass) and contest duration and intensity. Following Taylor and Elwood (2003), we quantified the direction and magnitude of the relationship between the dependent variable, the RHP of combatants in different roles [i.e. loser, winner, the difference between loser and winner (absolute trait value of the winner minus the loser's trait value), smaller rival, and larger rival], and contest duration (independent variable) using general linear models, or contest intensity (independent variable) using generalized linear models with Poisson error distribution.

Palaoro and Briffa (2016) recently alerted us to an important assumption underlying contest settlement via self-assessment: settlement models traditionally assume a linear relationship between RHP and body size but that is unrealistic since RHP (e.g. weaponry) generally scales allometrically with body size. Their simulations show that positive allometry in weaponry changes the expected relationship between body size (a common indicator of RHP) and contest duration from linear to non-linear (hump-shaped). That is, smaller and larger body size-matched combatants have short contest durations whereas intermediate-sized combatants engage in longer contests. We therefore tested whether mandible size (weapon size) is allometrically related to body mass (i.e. slope > 1.0) at sexual maturity (8-14 d post-eclosion) by using reduced major axis regression (RMA) (see Palaoro and Briffa, 2016). Mandible size was measured using the length (from lateral articulation to the distal tip) of the right mandible, left mandible, and their width (measurement of widest segment, when mandibles are closed).

In addition to the linear tests described above and in light of weapon allometry in *G. firmus* (see Results), we tested Palaoro and Briffa's (2016) predictions that contest duration will be non-linear with respect to the mean body mass (RHP) of a pair of size-matched combatants. We first ran a linear model with contest duration as the response variable and mean body mass of combatants as the independent variable. We then assessed the fit of this model against a quadratic model by comparing the ΔAIC values of the two models. The model with the smallest AIC value was selected as the model of best fit.

Because our general linear models satisfied the assumption of normally distributed residuals, morphological measurements did not need to be transformed. All models including contest intensity used Poisson error distribution (because intensity can take on values of 1, 2, 3, 4 and 5 only) and assumptions of null deviance was verified and met.

All analyses were conducted using R Studio, Version 1.1.453, models used were: *lmodel2* (Legendre, 1998), *lme4* (Bates *et al.*, 2018), *GGally* (Larmarange, 2018), *car* (Fox & Weisberg, 2018), *dplyr* (Wickham *et al.*, 2018), *MASS* (Venables *et al.*, 2002), *reshape2* (Wickham, 2007), *smooth* (Svetunkov *et al.*, 2016), *Mcomp* (Hyndman *et al.*, 2018) and *nlme* (Pinheiro *et al.*, 2018). Means are presented ± 1 standard deviation unless otherwise noted. Statistical tests are at α-level 0.05 and are two-tailed unless otherwise stated.

2.4 Results

2.4.1 Effect of rearing density on male development time and morphology at eclosion

High-density males ($\bar{x}$=64.84 ± 0.43 days, n=390) matured significantly faster than low-density males ($\bar{x}$=74.67 ± 0.54 days, n=442; ANOVA: F = 195.5, d.f. = 1 and 830, $p < 0.0001$). The left and right mandibles were significantly positively related (r=0.981, $p<0.0001$, n=829) and so we used the average of these traits in our analyses. There was an effect of rearing density on morphological traits measured at eclosion (MANOVA: F = 4.511, d.f. = 1 and 826, $p < 0.001$). However, post-hoc ANOVAs showed no significant effect of density on any specific trait (ANOVA: body mass at eclosion: F = 1.548, d.f. = 1 and 823, p = 0.24; pronotum length: F = 1.449, d.f. = 1 and 823, p = 0.23; mandible length: F = 1.173, d.f. = 1 and 823, p = 0.28; scaled mass index: F= 0.0294, d.f. = 1 and 823, p = 0.86; Table 2.1, 2.2, 2.3 and 2.4; Figure 2.4).

2.4.2 Effect of rearing density and diet on morphology at time of contest

A MANOVA showed a significant density * diet interaction (F=4.619, d.f.=826, p=0.01) as well as significant main effects of density (F = 46.001, d.f.=826, $p < 0.0001$) and diet (F = 245.513, d.f.=826, $p<0.0001$) on traits measured post-eclosion. Post-hoc tests showed that the significant MANOVA is likely due to a significant effect of diet on mass at time of contest (ANOVA: F= 21.003, d.f. = 1 and 827, $p < 0.0001$) as well as a significant effect of density (ANOVA: F = 83.845, d.f. = 1 and 827, $p < 0.0001$), diet (F = 465.900, d.f. = 1 and 827, $p< 0.0001$) and their interaction (F = 8.878, d.f. = 1 and 827, $p < 0.003$) on fat mass (Table 2.4). This latter result means that individuals reared at high density or given an NR diet were significantly more fat than individuals reared at low density or given an R diet.

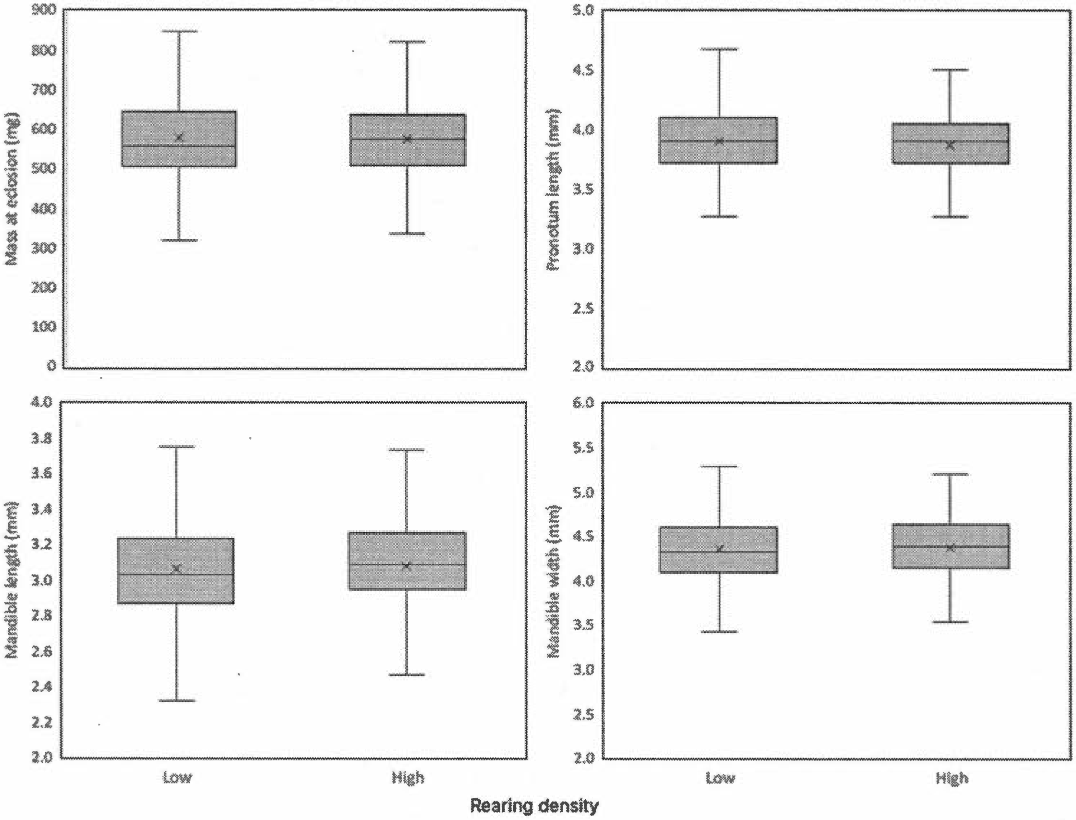

Figure 2.4 Boxplots showing body mass, pronotum length, and mandible width and length at eclosion for a male sand field crickets (*Gryllus firmus*) reared under low or high density. × represent means. (Low density: N = 390, High density: N = 442)

2.4.3 Effect of diet on body mass and fat load

Our diet treatments successfully manipulated body mass and fat load (but not SMI) as individuals in the NR treatment were significantly heavier than R males (GLM: t = -4.746, d.f. = 829, p < 0.0001) and had significantly more body fat (GLM: t = -20.45, d.f. = 828, p < 0.0001). The difference in body mass observed at the beginning of the contest between opponents of different treatments was due to the diet treatment and not the density treatment (Table 2.4). However, fat mass was influenced by an interaction between diet treatment and density treatment (Table 2.4): individuals reared at high density seemed to eat more than individuals reared at low density.

2.4.4 Effect of diet and rearing density on contest outcome

Contrary to our prediction, males reared at high density did not win significantly more fights than males reared at low density when controlling for adult diet (Table 2.5). Even though our diet treatment affected fat mass as predicted, males given an NR diet did not win significantly more fights than males provided an R diet when controlling for rearing density (Table 2.5).

Table 2.1 Summary statistics of RHP-related morphological traits in adult male sand field crickets (*Gryllus firmus*) reared under low and high densities.

	Low density		**High density**	
Trait	**Mean (±s.d.)**	**n**	**Mean (±s.d.)**	**n**
Mass at eclosion (mg)	578.00±5.15	442	574.49±4.96	390
Pronotum length (mm)	3.92±0.01	440	3.89±0.01	388
Mandible length (mm)	3.75±0.02	442	2.77±0.01	390
Mandible width (mm)	4.36±0.02	442	4.38±0.02	390

Table 2.2 Summary statistics of mass-related measures at the time of fighting for male adult sand field crickets (*Gryllus firmus*) reared in high and low population density, and given a restricted or non-restricted diet at adulthood.

	Low density				**High density**			
	Non-Restricted diet		**Restricted diet**		**Non-Restricted diet**		**Restricted diet**	
Trait	Mean (±s.d.)	n	Mean (±s.d.)	n	Mean (±s.d.)	n	Mean (±s.d.)	n
Mass (mg)	588.22±6.53	222	563.80±6.41	220	600.77±6.13	195	566.54±5.70	195
Fat mass (mg)	35.58±1.00	222	18.95±0.70	220	46.35±0.99	195	24.43±0.83	194
Scaled mass index	164.06±0.72	221	164.08±0.59	219	164.69±0.90	193	163.75±0.89	195

Table 2.3 Analysis of variance summary of the effect of density on various morphological trait at eclosion in male sand field crickets (*Gryllus firmus*).

Trait	**F**	**d.f.**	**p**
Body mass at eclosion	0.177	1 & 825	0.674
Pronotum length	1.452	1 & 825	0.229
Mandible length	1.175	1 & 825	0.279
Mandible width	0.888	1 & 825	0.346

Table 2.4 Results from analysis of variance (ANOVA) examining the effect of rearing density and post-eclosion diet on three mass-related traits in male sand field crickets (*Gryllus firmus*).

Trait	**Treatment**	**F**	**d.f.**	**p**
Body mass at time of contest	Density	1.548	1 & 823	0.214
	Diet	**21.511**		**<0.001**
	Density*Diet	0.425		0.515
SMI	Density	0.029	1 & 823	0.864
	Diet	0.317		0.574
	Density*Diet	0.402		0.526
Fat mass	**Density**	**82.660**	1 & 823	**<0.0001**
	Diet	**466.730**		**<0.0001**
	Density*Diet	**8.387**		**0.004**

Significant components are in bold. Body mass and SMI were measured at the time of the contest whereas fat load was measured after the contest.

Table 2.5. Results from $\chi^{\square}$ tests showing fight outcomes in relation to phenotype of both opponents when controlling for the effect of density or the effect of diet in male sand field crickets (*Gryllus firmus*). HD=High density, LD=low density, NR=Non-restricted diet, and R=Restricted diet.

Focal male treatment	**Opponent treatment**	$\chi^{\square}$	**d.f.**	**p**
(a) Effect of rearing density when controlling for diet				
HD-NR	LD-NR	1.00	1	0.32
HD-R	LD-R	0.71	1	0.40
(b) Effect of diet when controlling for rearing density				
HD-NR	HD-R	0.00	1	1.00
LD-NR	LD-R	0.81	1	0.37

2.4.5 Effect of male RHP on contest outcome

Contrary to prediction, the probability of winning a contest was not related to any RHP-related trait (GLM: Body mass at time of fight: $z = 1.665$, $p = 0.096$; Pronotum length: $z = -0.904$, $p = 0.366$; Mandible length: $z = 0.791$, $p = 0.429$; Mandible width: $z = 0.710$, $p = 0.478$; Fat mass: $z = 0.770$, $p = 0.441$; SMI: $z = 0.837$, $p = 0.402$). We also found that males that initiated fights (i.e. males that moved towards the other male first) won a significantly greater proportion of fights (Chi-squared test: $\chi\square = 59.81$, d.f. = 1, $p < 0.0001$).

2.4.6 Effect of diet and rearing density on contest settlement

Contrary to expectation, contests involving males with similar RHP (i.e. control contests) were not consistently longer in duration than treatment contests (i.e. combatants differing in an aspect of their rearing or dietary history) (Table 2.6). The lone exception was the HD-NR control contest being significantly longer than the other two HD-NR treatment contests (i.e. HD-NR/HD-R and HD-NR/LD-NR; Table 2.6; Figure 2.5). No other treatment contests differed significantly from their control contests (Table 2.6; Figure X). Also contrary to prediction was the lack of significant treatment effects on intensity (Table 2.7; Figure 2.6).

Table 2.6 Results from general linear models (GLMs) examining effect of rearing density and diet treatments on mean (± SE) contest duration in sand field crickets (*Gryllus firmus*). HD=High density, LD=low density, NR=Non-restricted diet, and R=Restricted diet.

Control contest	N	Treatment contest	N	Estimate±SE	t	p
HD-NR vs HD-NR	36	HD-NR vs HD-R	35	**-0.311±0.129**	**-2.42**	**0.016**
HD-NR vs HD-NR	36	HD-NR vs LD-NR	35	**-0.358±0.129**	**-2.79**	**0.006**
HD-NR vs LD-NR	35	LD-NR vs LD-NR	33	-0.019±0.132	-0.14	0.888
LD-NR vs LD-NR	33	LD-NR vs LD-R	35	0.0349±0.132	0.265	0.791
LD-R vs LD-R	34	LD-NR vs LD-R	35	0.070±0.131	0.534	0.594
LD-R vs LD-R	34	HD-R vs LD-R	35	0.137±0.131	1.047	0.296
HD-R vs HD-R	37	HD-R vs LD-R	35	-0.208±0.128	-1.626	0.105
HD-R vs HD-R	37	HD-NR vs HD-R	35	-0.244±0.128	-1.906	0.058

Table 2.7 Results from general linear models (GLMs) showing effect of density and diet treatment combinations on mean contest intensity (±standard error) in sand field crickets (*Gryllus firmus*). HD=High density, LD=low density, NR=Non-restricted diet, and R=Restricted diet.

Pair 1	N	Pair 2	N	Estimate±SE	z	p
HD-NR vs HD-NR	36	HD-NR vs HD-R	35	-0.107±0.114	-0.942	0.346
HD-NR vs HD-NR	36	HD-NR vs LD-NR	35	-0.128±0.114	-1.120	0.263
HD-NR vs LD-NR	35	LD-NR vs LD-NR	33	0.146±0.116	1.261	0.207
LD-NR vs LD-NR	33	LD-NR vs LD-R	35	-0.052±0.113	-0.462	0.644
LD-R vs LD-R	34	LD-NR vs LD-R	35	0.037±0.115	0.324	0.746
LD-R vs LD-R	34	HD-R vs LD-R	35	0.056±0.115	0.491	0.623
HD-R vs HD-R	37	HD-R vs LD-R	35	0.012±0.111	0.113	0.910
HD-R vs HD-R	37	HD-NR vs HD-R	35	-0.080±0.114	-0.701	0.483

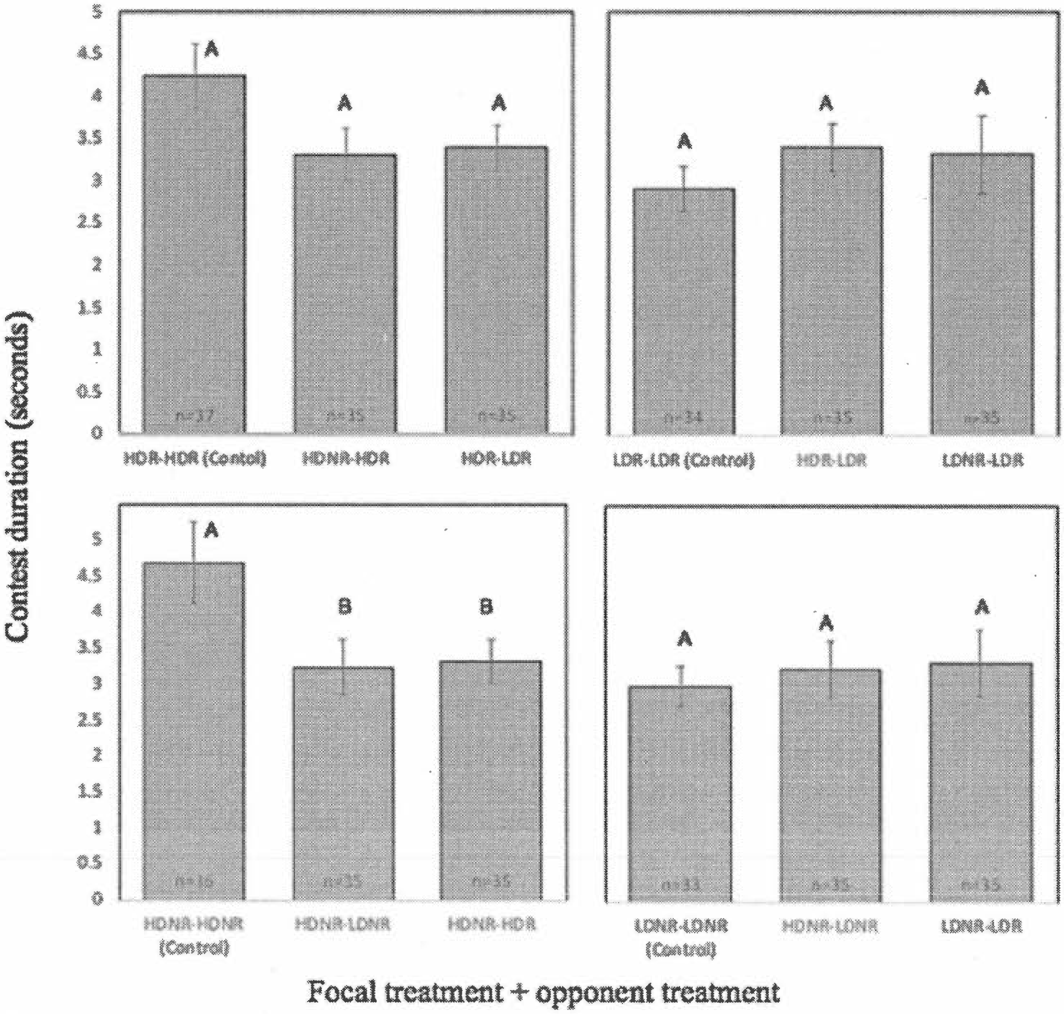

Figure 2.5 Bar plots showing the mean (± SE) duration (seconds) of contests featuring male sand field crickets (*Gryllus firmus*) in which combatants have the same treatment history (control contests) or differ in either their rearing density or diet (treatment contests). The duration of treatment contests was compared with control contest duration. Letters above bars indicate significant differences. HD=High density, LD=low density, NR=Non-restricted diet, and R=Restricted diet.

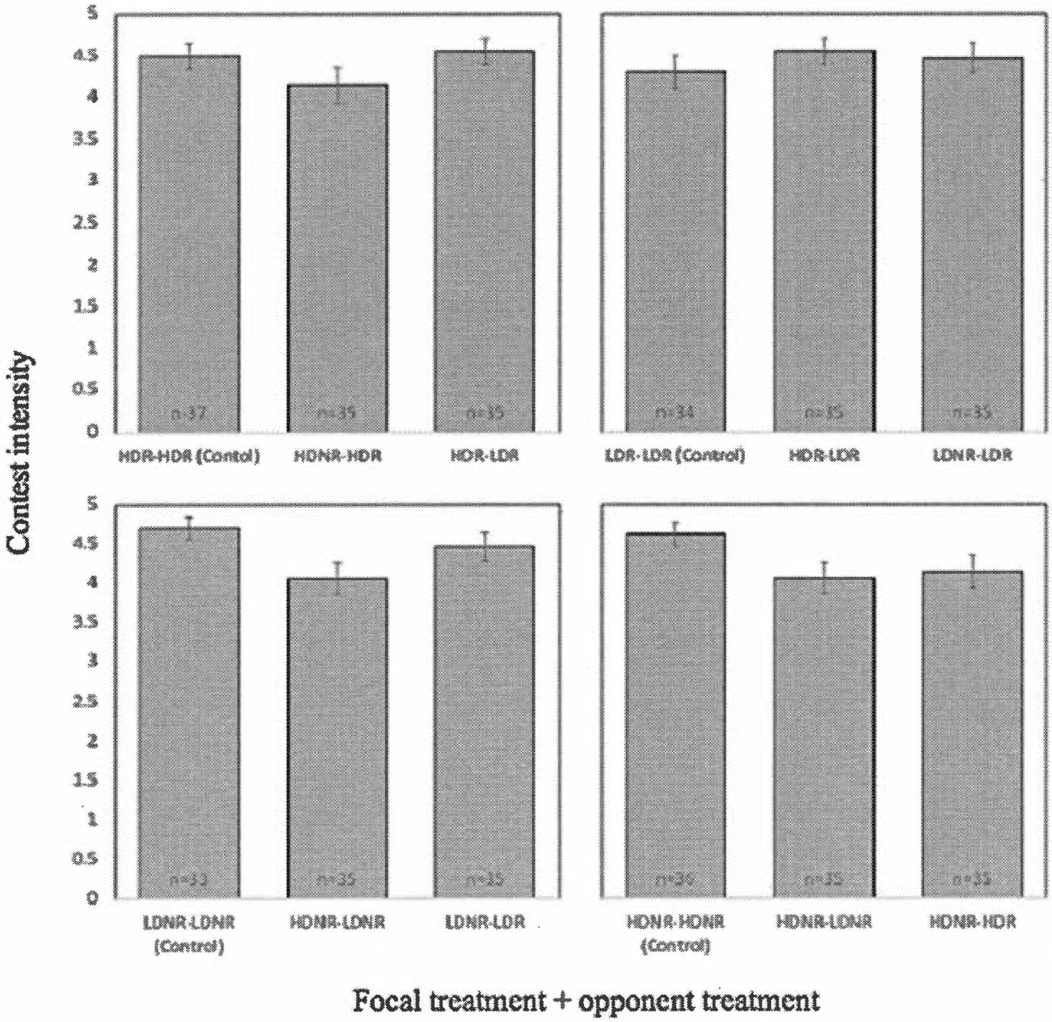

Figure 2.6 Bar plots showing the effect of treatment on contest intensity when comparing to a control and modifying only diet or density of one opponent in male sand field crickets (*Gryllus firmus*). There was no significant difference for any treatment. HD=High density, LD=low density, NR=Non-restricted diet, and R=Restricted diet. (± standard error)

2.4.7 Effect of male RHP on contest settlement

Contrary to prediction, contest duration was significantly positively correlated with the heaviest rival mass, longest rival pronotum, and longest rival mandible (Table 2.8). In other words, contest duration was generally dictated by the largest combatant of a pair; however, duration was also positively related to the body mass of the lightest combatant (Table 2.8). Longer durations with larger combatant (and not smaller) is predicted by the CAM but not the SAM model. Contest were longer and more intense when both opponents were bigger than when they were smaller. These results are consistent with CAM, but not with mutual assessment models such as SAM (Arnott & Elwood, 2008).

Contest intensity was significantly positively related to the heavier rival mass and widest rival mandible (Table 2.9). However, in agreement with our prediction, intensity was also significantly positively related to the lightest rival body mass, shortest rival pronotum, narrowest and longest rival mandibles (Table 2.9). These results suggest that contest intensity was generally dictated by the smallest rival of a pair. Results of analysis of RHP-related traits on contest duration and intensity are shown in Table 2.8 and 2.9.

2.4.8 Offensive and defensive trait allometry and contest duration

Palaoro and Briffa (2016) predict non-linear (quadratic) relationships between body size (averaged for the two combatants) and contest duration when offensive traits (i.e. mandible length) scale allometrically with body size (assuming defensive traits are fixed at 1) but a linear relationship when defensive traits (i.e. stamina in the form of fat content) scale allometrically (and offensive traits are fixed at 1). As assumed by Palaoro and Briffa (2016), we found that mandible length scaled allometrically with body mass (slope = 1.54, 95% CI = 1.489 and 1.59).

Table 2.8 Results of generalized linear models (GLMs) examining the effect of the smallest and largest values of different RHP-related traits in contest pairs on contest duration and intensity in male sand field crickets (*Gryllus firmus*). Models of contest duration assumed a gaussian error distribution whereas models of contest intensity assumed a Poisson error distribution.

Trait		**Contest response variable**	**Estimate**	**d.f.**	**t**	**p**	**Pearson r**
Mass at time of contest	Smallest mass	**Duration**	0.001	**278**	**2.17**	**0.03**	0.105
		Intensity	0.002	**279**	**2.62**	**0.01**	0.090
	Largest mass	**Duration**	0.000	**278**	**1.99**	**0.05**	0.100
		Intensity	0.002	**278**	**2.74**	**0.01**	0.090
Pronotum length	Smallest pronotum length	**Duration**	0.293	**275**	**2.30**	**0.02**	0.093
		Intensity	0.598	**275**	**2.50**	**0.01**	0.039
	Largest pronotum length	**Duration**	0.271	**275**	**2.10**	**0.04**	0.090
		Intensity	0.445	275	1.83	0.07	0.039
Mandible width	Smallest mandible width	Duration	0.171	278	1.78	0.08	0.116
		Intensity	0.428	**278**	**2.36**	**0.02**	0.113
	Largest mandible width	Duration	0.164	278	1.81	0.07	0.115
		Intensity	0.435	**278**	**2.55**	**0.01**	0.113
Mandible length	Smallest mandible length	Duration	0.196	278	1.76	0.08	0.117
		Intensity	0.482	**279**	**2.30**	**0.02**	0.117

	Largest mandible length	**Duration**	0.206	**278**	**1.95**	**0.05**	0.119
		Intensity	0.510	**278**	**2.57**	**0.01**	0.117
Fat mass	Smallest fat mass	Duration	0.005	277	1.89	0.06	0.062
		Intensity	0.001	277	1.59	0.11	0.066
	Largest fat mass	Duration	0.003	277	1.71	0.09	0.096
		Intensity	0.006	277	1.51	0.13	0.066
SMI	Smallest SMI	Duration	-0.004	275	-1.09	0.28	-0.049
		Intensity	-0.009	275	-1.30	0.20	0.016
	Largest SMI	Duration	0.001	275	0.23	0.82	0.026
		Intensity	-0.001	275	-0.21	0.84	0.016

Significant components are in bold.

Table 2.9 Results of generalized linear models (GLMs) for RHP-related morphological traits of loser and winner on contest duration and intensity in sand field crickets (*Gryllus firmus*).

Trait		**Contest variable**	**Estimate**	**d.f.**	**t**	**p**	**Pearson r**
Mass at time of contest	Loser mass	**Duration**	0.001	**276**	**2.08**	**0.04**	0.104
		Intensity	0.002	**277**	**2.57**	**0.01**	0.082
	Winner mass	**Duration**	0.001	**278**	**2.20**	**0.03**	0.108
		Intensity	0.002	**279**	**2.57**	**0.01**	0.079
	Mass difference	Duration	-0.003	278	-0.64	0.52	-0.028
		Intensity	0.000	278	0.05	0.96	-0.020
Pronotum length	Loser's pronotum length	**Duration**	0.278	**275**	**2.26**	**0.03**	0.089
		Intensity	0.407	276	1.75	0.08	0.024
	Winner's pronotum length	**Duration**	0.244	**276**	**1.98**	**0.05**	0.084
		Intensity	0.560	**277**	**2.40**	**0.02**	0.050
	Pronotum length difference	Duration	-0.158	275	-0.53	0.60	-0.010
		Intensity	-0.892	275	-1.59	0.11	-0.002
Mandible width	Loser's mandible width	Duration	0.149	276	1.61	0.11	0.102
		Intensity	0.378	**277**	**2.16**	**0.03**	0.097
	Winner's mandible width	Duration	0.170	278	1.90	0.06	0.125
		Intensity	0.423	**279**	**2.52**	**0.01**	0.129
	Mandible width difference	Duration	0.093	278	0.36	0.72	0.010
		Intensity	0.433	278	0.88	0.38	0.002
Mandible length	Loser's mandible length	Duration	0.185	276	1.70	0.09	0.104
		Intensity	0.427	**277**	**2.09**	**0.04**	0.093
	Winner's mandible length	Duration	0.198	278	1.92	0.06	0.129
		Intensity	0.492	**279**	**2.53**	**0.01**	0.136

	Mandible length difference	Duration	0.235	278	0.77	0.44	0.018
		Intensity	0.607	279	1.06	0.23	0.009
Fat mass	Loser's fat mass	Duration	0.003	276	1.52	0.13	0.060
		Intensity	0.004	277	1.13	0.26	0.016
	Winner's fat mass	Duration	0.003	277	1.64	0.10	0.091
		Intensity	0.006	278	1.64	0.10	0.057
	Fat mass difference	Duration	0.001	277	0.18	0.86	-0.016
		Intensity	0.001	277	0.25	0.80	-0.033
SMI	Loser's SMI	Duration	-0.001	275	-0.39	0.69	-0.038
		Intensity	-0.004	276	-0.71	0.48	-0.078
	Winner's SMI	Duration	-0.001	276	-0.45	0.65	0.019
		Intensity	-0.003	277	-0.55	0.59	0.043
	SMI difference	Duration	0.004	275	1.29	0.2	-0.039
		Intensity	0.007	275	1.04	0.30	-0.084

Significant components are in bold.

Fat mass also scaled allometrically with body mass (density treatments pooled) in the R (slope = 3.69, 95% CI = 3.23 to 4.23) and NR (slope = 2.53, 95% CI = 2.21 to 2.89) diet treatments; however, the slope was steeper in R than in NR crickets (R slope = 3.69, NR slope = 2.53). Despite this difference we pooled crickets from both diets because both showed positive allometry. Unfortunately, Palaoro and Briffa's (2016) model does not predict contest duration when *both* offensive and defensive traits scale allometrically. We found that the relationship between contest duration and body mass fitted with a quadratic (hump-shape) model (GLM: t = 2.11, d.f. = 279, p = 0.04) was not significantly better (Δ AIC =0.17) than a linear model (GLM: t = 2.16, d.f. = 279, p = 0.03). These findings do not support the Palaoro & Briffa prediction that non-linear scaling between offensive RHP traits and body size results in a non-linear effect on contest duration. Our results, however, do support the prediction of a linear relationship between combatant body size and contest duration when defensive traits scale allometrically (with the caveat that RHP traits are assumed by Palaoro & Briffa to scale linearly and not allometrically in this scenario).

Table 2.10 Predictions of three assessment models of conflict resolution and the results obtained in this study.

	Sequential assessment model (SAM)	Cumulative assessment model (CAM)	This study
Decision based on:	Mutual assessment	Sum of opponent's actions	
Number of phases	Multiple	One	One
Escalation	Interphase with change in behaviour	Increase in intensity	Increase in intensity
Contest duration vs absolute RHP asymmetry	Negative	Negative	Non-significant
Contest intensity vs absolute RHP asymmetry	Negative	Negative	Non-significant
Contest duration vs contest intensity	Positive	Positive	Positive
Contest duration vs loser RHP	Positive	Positive	Positive
Contest duration vs winner RHP	Negative	Negative	Positive
Contest intensity vs loser RHP	Positive	Positive	Non-significant
Contest intensity vs winner RHP	Negative	Negative	Non-significant

RHP: resource holding potential. We used body mass at time of fight as a measure of RHP.

*Modified from Table 2 in Payne (1998) and Table 1 in Kelly (2006)

2.5 Discussion

In this study, we tested the hypothesis that rearing density would influence investment in RHP, with males reared in high rearing density investing more in RHP in response to the higher competition for mates. We also tested how conflict are resolved between size-matched opponents in *Gryllus firmus*.

Density treatments did not affect RHP traits like mandible size the way we predicted. Males in high density treatments did not have significantly larger mandibles than males in low population density. The prediction was that mandible size was a sexually selected trait, and juvenile males should invest more in their mandibles to help them in male-male competition when they perceive a higher competition risk in the future for mates. Although we confirmed in another study that males had significantly larger mandibles than females (Chapter 1), population density did not affect investment in mandible size.

These surprising results might be caused by density treatments not being different enough. Males reared under high population density should develop faster and be smaller on average than low-density males, which is the pattern we would have expected from previous studies in crickets (McFarlane, 1962; Niemelä *et al.*, 2012; Wey, Réale & Kelly, 2019) and other insects (Applebaum & Heifetz, 1999). We did not find any significant difference in body mass between the two density treatments (Table 2.2), which leads us to believe the high-density treatment was not dense enough. It is possible that male crickets invest more in their sexual traits at intermediate density and our density treatments were on both sides of that peak in investment. Yet, we used the same density treatments for Chapter 1 and found the expected results for body mass and development time in that experiment. In addition, our sample size was greater in this second experiment.

Another possible explanation for the lack of effect of population density on morphological traits in this experiment and more specifically RHP-related traits, would be that males respond more specifically to the sex ratio in the population than the overall population density. Increasing the number of competitors (males) has a greater impact on sexual traits and mating behavior than increasing overall population density, which could also increase the number of potential mates (Alonso-Pimentel & Papaj, 1996; Grant & Foam, 2002; Head *et al.*, 2007; Kvarnemo & Ahnesjö, 1996; Pomfret & Knell, 2008).

We also hypothesized that manipulation of adult diet would influence energy reserves which, in turn, would influence stamina and contest settlement. We predicted well-fed males to have more energetic reserves in the form of body fat, thus more stamina. Starved males were expected to lose more often and give up the fight more quickly than well-fed males. We found a significant difference between diet treatments on body mass and energetic reserves. Males that were starved after eclosion were significantly lighter and had significantly less body fat than males who were fed more food. We can conclude that we manipulated successfully energetic reserve (stamina) of the individuals, an important factor of conflict resolution (Palaoro & Briffa, 2016).

We did not find an effect of density and diet treatment on fight outcome: winning probability was also not influenced by rearing density. Our initial prediction was that rearing density would affect individual traits which will in turn influence contest outcome. Since morphological traits were not influenced by population density, it is not surprising that contest outcome did not differ between treatments, individuals being size-matched. However, it also confirms that there was no difference in motivation between the two density treatments. Also disagreeing with our predictions, we found no significant effect of diet on contest outcome. Males fed a NR diet did not win more often. We are therefore still unsure about the environmental factors influencing winning probability, since neither rearing density or diet seems to influence the outcome. However, we found that males who initiated the fight also won significantly more often. It is possible that males who initiate the contest are bolder and more aggressive, which intimidates their opponent. This would confirm Brown *et al.* (2007) study showing males who initiate fights to often be more aggressive than their opponent.

Contrary to our predictions, we found no significant effect of diet on contest duration. Males fed a NR diet did not last longer in the contest than males fed a R diet. We also tested the effect of fat mass more directly on contest duration and found no effect of loser's (or winner's) fat mass on contest duration (Table 2.5). Fat mass might not be a good indicator of stamina, or having more stamina did not help individuals pursue the contest longer (Peixoto & Benson, 2011). Another possibility is that energy reserves are not important in determining contest resolution because fights last only a few seconds and energy reserve would likely be more important in fights that last minutes.

Population density did affect positively contest duration. Males reared under high densities fought significantly longer than males reared under low densities. It is possible that males reared under crowded conditions valued the territory of the arena more greatly that those reared under solitary conditions. This would confirm findings by Warner and Hoffman (1980) where coral reef fish were more territorial when population densities were high.

We predicted that contests between males of similar background and/or similar RHP would last longer and be more intense than contests between opponents that are more different. We found that fight duration was positively correlated with fight intensity, so longer fights also tended to be more intense, but we found no significant difference in level of aggression (intensity) between the different density treatments in our study. This result contradicts Iba *et al.* (1995) study who found that male crickets reared individually were more aggressive than males reared under high density. Another explanation could be that high rearing density does not increase mean energy investment, but increases variance, with only some males have enough energy to invest in aggression. This hypothesis might explain the result found by Radwan (1993) were male acarid mites (*Caloglyphus berlese*) developed a less aggressive morph at high rearing density. Furthermore, we considered the idea that our low-density treatment was in fact an asocial treatment and this lack of social interaction could negatively affect the individual's development. However, it was shown that individuals reared individually were very aggressive when initially transferred to a social environment (Yoerg, 2001). In our case, density treatment did not affect the individual one way or another. There was also no effect of diet on contest intensity in our study.

Other studies have found that male aggression is highest at intermediate density (Jirotkul, 1999). Indeed, at low population density, individuals should invest in structures for mate searching rather than male-male competition, and at high population density, the cost of fighting might be greater than the benefits, considering the number of rivals (Knell, 2009). At high population density, it might be more valuable to invest in traits that help in sperm competition, rather than pre-copulatory traits (Knell, 2009; Pomfret & Knell, 2008). It would be interesting to see if this applies to *Gryllus firmus* by adding an intermediate density to our experimental design.

Because we observed little effect of density and diet treatments on contest outcome and settlement we analyzed our contests based on the individual phenotypes of the combatants without regard to their rearing and diet treatments. We predicted that males with larger RHP would win more often in male-male competition. Surprisingly, we found that bigger mandibles did not increase winning probability. The male with the biggest mandibles of the two opponents was not always the one winning the contest, contrary to findings of Judge and Bonanno (2008). In fact, none of the morphological traits measured seem to influence contest outcome since we found no connection between probability of winning and morphological traits of the combatants. The combatants were size-matched, and thus had very similar body masses, so it is not surprising that body mass did not influence contest outcome. However, weaponry scales allometrically with body size so even a 5% difference in body mass should result in a large difference in weaponry.

Although we found no effect of treatment or RHP on contest outcome (i.e. probability of winning), we found significant correlations between RHP-related traits and contest duration and intensity. The SAM and CAM predict that contest duration is inversely related to the size difference between combatants. We found no effect of size difference on contest duration or intensity, which is not surprising given that pairs were size-matched. Both models predict a positive correlation between contest duration and contest intensity, which is congruent with our findings. The two models also predict a positive correlation between contest duration or intensity and loser's RHP. When using body mass as a measure of RHP, we found the same positive correlation as the models. We also found a positive relationship between winner's RHP and contest duration. These results are in accordance with pure self-assessment model where both opponent's RHP is positively related to contest duration, contrary to mutual assessment model (SAM) or CAM where the winner's RHP is negatively related to contest duration (Gammell & Hardy, 2003, Arnott & Elwood, 2008). Briffa *et al.* (2013) mentioned that the relationship between winner's RHP and contest duration is the main way to distinguish between pure-self assessment versus mutual assessment. All other significant results we had agree with both CAM and SAM.

Rillich *et al.* (2007) and Stevenson & Schildberger (2013) found that crickets settle fights according to the CAM. It does not imply that the loser can assess its opponent's RHP, but simply withdraws when he reaches a threshold he can no longer tolerate. Judge and Bonanno (2008) state that none of the settlement models match their results since they did not find a relationship between mandible size and contest duration.

Similarly, we cannot find a model that fits fully our results. However, we believe CAM is the model that describes best (even though not perfectly) what we observed in our cricket fights. We opted for this model because CAM is for fights with only one phase that increase in intensity (as seen in crickets) whereas SAM is for fights with multiple phases with interphase with change in behavior.

Taylor and Elwood (2003) explain that other factors could play a role in settlement. They predict positive correlations between smaller rival size and contest duration, or larger rival size and contest duration, meaning that contest should last longer when both opponents are bigger. They also predict a negative correlation between absolute size difference between the rivals and contest duration. We found a positive relationship between smallest opponent (and largest opponent body mass) and contest duration. When both opponents were larger, contests lasted longer. According to the models, this indicates that bigger males can tolerate spending energy for longer or they assess themselves as having a better chance of winning than smaller males. The other positive relationship we found was between contest duration and loser's mass (and winner's mass). The other correlations tested were non-significant (with contest intensity). Even though all models of contest settlement predict a linear relationship between weapon size and contest duration, our results cannot support this prediction, which was also not found by Judge and Bonnano (2008). Males with bigger mandibles did not fight for longer than males with smaller mandibles.

A recent simulation showed that violation of an assumption can affect model predictions (Palaoro & Briffa, 2016). Specifically, Palaoro & Briffa argue that RHP (e.g. weaponry) scales allometrically with body size and that incorporating this non-linear relationship in the analysis would results in a non-linear (hump-shaped) relationship between combatant body size (a unified measure of both rivals) and contest duration. In line with their predictions we found mandible length (the most important RHP trait found in other cricket studies) scales allometrically with body size.

Therefore, duration versus mass should be hump-shaped. Contrary to their predictions we found duration in our trials to be linear related to average body mass of fighters. Even though we might have to revisit the assumed relationship between mandible length and body size, it does not seem to affect contest settlement the way Palaoro & Briffa would have predicted.

Our findings reinforce the idea that models of contest settlement are theoretical models and as good as the theory can be, it does not always perfectly match what we observe in nature. Nature is always more complicated than we first think and still has a lot of secrets to unveil.

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CONCLUSION

Notre première expérience cherchait à approfondir les connaissances sur l'effet de l'environnement sur l'investissement dans les traits sexuels et l'immunité. La densité de population lors du développement est un facteur environnemental qui influence le développement des individus (Whitehouse & Lewis, 1973). Celle-ci peut apporter des indices de l'intensité de la compétition future pour la fécondation des femelles (Emlen & Oring, 1977; He & Tsubaki, 1991; Knell, 2009; Kokko & Rankin, 2006). Pour ces raisons, une haute densité de population peut amener les mâles à augmenter leur investissement dans leurs traits sexuels (Kasumovic *et al.*, 2012). Entres autres, un mâle peut développer de plus grandes armes en fonction du niveau de compétition qu'il connaît lors de son développement (Pomfret & Knell, 2008; Radwan *et al.*, 2002). Aussi, la densité de population étant un indice indirect du niveau de compétition spermatique dans le futur, elle influence également la taille des testicules (Hosken & Ward, 2001).

Bien que nous ayons trouvé un dimorphisme sexuel pour la taille des mandibules, celle-ci ne semble pas influencée par la densité de population, tout comme la taille des testicules. Il faut cependant considérer la possibilité que les grillons répondent plus directement au ratio sexuel plutôt qu'à la densité de population de manière plus générale (Alonso-Pimentel & Papaj, 1996; Grant & Foam, 2002; Head, Lindholm, & Brooks, 2007; Pomfret & Knell, 2008). Malheureusement, il nous était impossible de déterminer le sexe des grillons aux premiers stades juvéniles.

Le seul trait sexuel influencé par la densité de population lors du développement chez les mâles grillons est la taille des harpes. Les mâles élevés en isolation avaient de plus grandes ailes, ce qui les aiderait à s'éloigner sur de plus grandes distances pour trouver des femelles afin de se reproduire. D'un autre côté, les femelles semblent ajuster leur investissement dans leurs œufs en fonction de la densité de population. Elles ont de plus grands œufs lorsque le stress environnemental est moins grand, c'est-à-dire lorsqu'elles sont élevées en isolation.

Dans le but d'approfondir le sujet de l'investissement dans les traits pré-copulatoire et de tester des théories de règlement de combats, nous avons organisé des combats entre grillons. Tout comme notre étude précédente, nous n'avons trouvé aucun effet significatif de la densité de population sur la taille des mandibules. De plus, celles-ci ne semblaient pas influencer le résultat du combat lorsque les individus étaient de même poids. Bien que la diète influençât la masse de gras, celle-ci ne changeait pas la durée du combat, son intensité ou bien le résultat. Le seul facteur qui avait un impact sur la durée du combat était le traitement de densité dans lequel les individus avaient grandi. En effet, les mâles élevés en haute densité de population se battaient plus longtemps que ceux élevés en basse densité de population. Il est possible que cela s'explique par la valeur perçue du territoire. Les mâles élevés sous des conditions de surpeuplement accordaient peut-être plus de valeur au territoire de l'aréna en comparaison aux mâles élevés solitairement. En considérant le fait que l'intensité du combat augmentait parallèlement à la durée du combat, en addition aux autres résultats trouvés, il est difficile de déterminer lequel des modèles est le modèle le plus approprié afin d'établir les règlements de combats chez les grillons. Il semblerait que le modèle qui correspond le plus à nos résultats serait le CAM, mais ce modèle n'explique pas en entier ce que nous avons observé chez les grillons. Le modèle CAM est un modèle théorique et comme bien souvent, la théorie n'arrive pas à expliquer en entier ce qui est observé en nature. Autrement, nous avons testé les allégations de Palaoro et Briffa (2016) et nous pouvons confirmer que, bien que les armes et la taille corporelle soient allométriques, cette relation n'influence pas la durée du combat ou le règlement du combat.

Sur une autre note, nous avons testé l'effet de l'environnement social d'un individu sur ses capacités immunitaires. Nous avons tenté de démontrer qu'une prophylaxie densité-dépendante existait chez les grillons. Contrairement à nos hypothèses initiales, nous n'avons trouvés aucun effet de la densité de population sur l'investissement en immunité. Il semblerait que les grillons se basent sur d'autres facteurs que la densité de population pour modifier leur investissement. Il est aussi possible que les grillons investissent de manière plus marquée dans leur immunité seulement dans des cas de densité de population extrêmes et donc que la densité de population que nous avons choisie n'était pas assez élevée pour remarquer un effet significatif.

Afin d'avoir une vision plus globale et puisque les traits sexuels et ceux liés aux compétences immunitaires peuvent aussi être influencés par la génétique, nous avons considéré l'héritabilité de ses traits. Les traits sexuels masculins ne montraient que très peu d'héritabilité. Par contre, les compétences immunitaires semblaient plus fortement héritables, ce qui pourrait expliquer l'absence de différence entre les différents traitements de densité de population.

Comme des génotypes différents peuvent réagir de différentes manières à une variation dans l'environnement, c'est-à-dire, certains traits de divers génotypes ne performent pas de la même manière dans différents environnements (Bowman, 1972), nous avons testé l'effet d'une possible interaction entre l'environnement et le génotype sur les traits sexuels et l'immunité. Nous n'avons trouvé aucune interaction entre la densité de population, le génotype et les traits sexuels ou les compétences immunitaires. Par contre, il ne faut pas négliger que la densité de population n'est qu'un seul facteur environnemental et qu'il est possible qu'une interaction GxE existe avec d'autres facteurs environnementaux.

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