UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LE MAINTIEN DE STRATÉGIES ALTERNATIVES CHEZ LE WELLINGTON TREE WETA (*HEMIDEINA CRASSIDENS*): TESTER LES PRÉDICTIONS DE L'HYPOTHÈSE DE POLYMORPHISME GÉNÉTIQUE

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THE MAINTENANCE OF ALTERNATIVE STRATEGIES IN WELLINGTON TREE WETA (*HEMIDEINA CRASSIDENS*): TESTING THE PREDICTIONS OF THE GENETIC POLYMORPHISM HYPOTHESIS

MÉMOIRE

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SARAH NASON

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AMS	Alternative mating strategy
IUCN	International Union for the Conservation of Nature
SSC	Species Survival Commission
H_o	Observed heterozygosity
H_e	Expected heterozygosity

RÉSUMÉ

Les stratégies alternatives reproductives (SARs) sont des ensembles de caractères comportementaux et morphologiques qui sont exprimés chez un sexe pour obtenir accès aux accouplements avec l'autre sexe. Il y a plusieurs idées dans la littérature pour tenter d'expliquer pourquoi les SARs se développent et comment elles se maintiennent, avec deux hypothèses principales : l'hypothèse de polymorphisme génétique, qui suggère que chaque stratégie ait une base génétique discrète, ou l'hypothèse condition dépendante, qui suggère que chaque stratégie est déterminée par la condition de l'individu (c.-à.-d., leur capacité d'acquérir et d'allouer les ressources). La première hypothèse prédit que la valeur sélective (« fitness ») de chaque stratégie sera égale, tandis que la deuxième ne fait pas cette prévision. Nous présentons une étude de terrain fait sur le *Wellington tree weta* (*Hemideina crassidens*), un insecte nocturne endémique à la Nouvelle-Zélande, pour investiguer ces deux hypothèses et pour générer des recommandations pour la conservation de l'espèce. *H. crassidens* est une espèce polygynandre qui forme des harems. Les mâles expriment un polymorphisme sélectionné sexuellement pour la taille de leur tête, qui pourrait représenter les SARs distincts. Nous avons testé la prévision de l'hypothèse de polymorphisme génétique que chaque stratégie devrait avoir un « fitness » égal, ainsi que deux autres prévisions concernant le maintien des SARs : (i) les morphes plus petites devront investir plus dans les traits post-copulatoires par rapport aux morphes plus grandes; et (ii) un facteur environnemental, la taille de l'entrée des cavités d'arbres, limite la distribution spatiale des morphes. De plus, nous avons testé si les individus des deux sexes gagnent des bénéfices en termes de la quantité de progéniture quand ils ont plus de partenaires, et aussi si les femelles gagnent des bénéfices en termes de la qualité de la progéniture.

Nous n'avons trouvé aucune différence en « fitness » relatif entre les morphes, ce qui suggère que les morphes sont principalement attribuables à un polymorphisme génétique. Nous avons trouvé aussi des évidences que les morphes plus petites investissent plus dans leur sperme parce qu'ils ont obtenus plus de paternité au sein des couvées par rapport aux morphes plus grandes. Il n'y avait pas de relation entre la taille de l'entrée de cavités d'arbres et la résidence des morphes à l'intérieur de la cavité. Il y avait un fort effet positif d'avoir plus de partenaires sur la quantité de progéniture pour chacun des sexes. Nous avons aussi trouvé des attestations des effets d'avoir plus de partenaires sur la qualité de progéniture chez les femelles : les femelles qui ont eu plus de partenaires ont pondu des œufs avec un taux d'éclosion plus haut. Nos résultats sont pertinents pour la gestion de la conservation, car les groupes de *H. crassidens* sont souvent transloqués pour des raisons de conservation mais, suites à des transferts dans le passé, certaines des morphes ont été perdues ou leur nombre a été réduits. Nos résultats indiquent que le polymorphisme sera conservé plus effectivement dans le futur si tous les trois morphes étaient transférés en fonction de leur fréquence dans la population source. À cause de la haute diversité génétique et la présence des trois morphes dans notre population (Isle Maud/*Te Pakeka*), nous recommandons aussi que les gestionnaires considèrent cette population comme source pour les translocations futures.

Mots clés : sélection sexuelle, morphe, évolution, réintroduction, restauration, invertébré, analyse de paternité, polyandrie

RÉSUMÉ

Alternative mating strategies (AMSs) are suites of behavioural and morphological characteristics expressed by one sex to gain access to matings. There exists considerable debate surrounding why AMSs arise and how they are maintained, with two hypotheses predominating: the genetic polymorphism hypothesis, positing that each strategy is determined by discrete genetic variation, and the condition-dependent hypothesis, positing that each strategy is determined by individual condition (ability to acquire and allocate resources). The former predicts equal relative fitnesses among strategies, while the latter does not. Here we present a field study conducted on the Wellington tree wētā (*Hemideina crassidens*), a nocturnal insect endemic to New Zealand, to investigate these hypotheses and generate recommendations for conservation. *H. crassidens* is harem polygynandrous and exhibits a sexually-selected polymorphism in head size in males, thought to represent distinct AMSs. We tested the prediction of equal fitnesses posited by the genetic polymorphism hypothesis along with two additional predictions regarding the maintenance of AMSs: (i) smaller male morphs invest relatively more in post-copulatory traits; and (ii) a limiting environmental factor, tree cavity entrance size, influences male morph spatial distribution. We also tested whether there were benefits to multiple mating in terms of offspring quantity in both sexes or offspring quality in females.

We found no significant differences in relative fitness among male morphs, suggesting that morphs are primarily determined by genetic polymorphism. We also found some evidence that smaller morphs invest more in sperm, as they achieved a greater proportion of paternity within broods sired by multiple morphs, and found no relationship between cavity entrance size and morph residency. There was a strong positive effect of multiple mating on offspring quantity in both sexes and some evidence for benefits to offspring quality in females, as females that mated multiply had greater hatching success. Our results have relevance to conservation management because groups of *H. crassidens* are often translocated for conservation purposes, but the polymorphism has been reduced in expression following previous translocations. This could negatively affect the fitness of translocated populations by reducing adaptive variation and disrupting selective processes associated with the species' mating system. Our results indicate that the polymorphism will be best conserved in future translocations if all three morphs are translocated in approximately the frequencies they occur in the source population. Based on the high genetic diversity and presence of all three morphs in our study population, we recommend managers consider our population (Te Pakeka/Maud Island) as a source for future translocations.

Key words: sexual selection, morph, evolution, reintroduction, restoration, invertebrate, paternity analysis, polyandry

INTRODUCTION

0.1. Sexual selection and mating strategies

Sexual selection is the competition between members of one sex for fertilization access to the gametes of the opposite sex. Sexual selection may be divided into two categories: intra-sexual selection, where individuals of one sex compete amongst themselves for access to mates of the opposite sex (e.g., rutting elk fighting each other for harems of females), or inter-sexual selection, where individuals of one sex select mates of the opposite sex (e.g., female tropical birds selecting mates based on the elaborate displays of males) (Darwin, 1871). As a result, intra-sexual selection generally promotes traits that improve an individual's ability to compete in contests against members of the same sex, such as body size and weaponry, while inter-sexual selection promotes traits that are preferred by the opposite sex, such as ornamentation and colour. Over evolutionary time these selective forces have generated striking phenotypic diversity in nature, including extreme phenotypic differences between the sexes, referred to as sexual dimorphism. For example, male Northern elephant seals (*Mirounga angustirostris*) may be more than twice the size of females because larger males have greater success when fighting other males for harems (Briggs & Victor Morejohn, 1975; Haley, Deutsch, & Le Boeuf, 1994).

Sexual selection is typically stronger for males than females because of the evolutionary constraints imposed by anisogamy, or the differential investment in gametes between the sexes (Bateman, 1948; Lehtonen, Parker, & Schärer, 2016).

Because the time and energy required to produce gametes and offspring is comparatively low for males, selection generally favours males that mate frequently and non-discriminately. By contrast, females are selected to be ‘choosy’ because each offspring is a relatively large investment and they cannot mate as frequently due to long periods of gestation. Females are therefore often a limiting resource for which males compete. When intra-sexual selection is intense, it can lead to the emergence of new phenotypes within the competing sex (Shuster & Wade, 2003). These alternative mating phenotypes use behaviours different from the conventional strategy to achieve mating success: for example, males of the giant cuttlefish (*Sepia apama*) typically guard females, but some smaller-bodied males adapt their body shape and colouration to mimic females, using this strategy to go undetected by guarding males and gain access to matings with their females (Norman, Finn, & Tregenza, 1999).

The forces of sexual selection can therefore produce phenotypic diversity not only between, but also within, the sexes. In this mémoire I will focus on the evolution of diversity within the sexes, specifically within males.

0.1.1. Alternative mating strategies (AMSs)

Individuals within a sex may express a variety of phenotypes related to mating, such as mate guarding, mimicking the opposite sex to gain access to matings, or ‘sneaking’ copulations when a mate guarder is unaware. When alternative mating phenotypes are plastic, they are referred to as mixed strategies (Gross, 1996); for example, in male field crickets (Orthoptera: Gryllidae), individuals can shift tactics between singing to attract mates and acting as ‘satellites’ that do not sing but settle near singing males to intercept the females attracted by the song (Cade, 1980). When individuals express fixed alternative mating phenotypes (i.e., the phenotype cannot change within an individual's lifetime), these are referred to as alternative mating strategies (AMSs; Gross, 1996; Shuster & Wade, 2003). For example, in the long-horned dung beetle *Onthophagus taurus,* males may be one of two morphs: 'majors' with large horns that fight other males for females that reside in tunnels, or 'minors' with short horns that evade fighting by ambushing females or bypassing majors using side tunnels (Knell & Simmons, 2010). In this mémoire I will refer to males that monopolize females as 'conventional morphs' and males that use other strategies to gain access to females (i.e., 'sneaking', mimicking females) as 'alternative morphs' (Taborsky, Oliveira, & Brockmann, 2008). These phenotypes are irreversible, and are thus likely to have a stronger genetic basis and undergo stronger sexual and natural selection. For this reason I will focus on the hypotheses for how AMSs, rather than mixed strategies, arise and are maintained.

AMSs are observed across animal taxa and are thought to be the product of intense sexual selection, usually on males. AMSs are most frequently observed in males of polygynous species wherein a single male may mate with multiple females. In the context of polygyny, a male's reproductive success is dictated by his ability to monopolize 'breeding resources': mates (i.e., female defense polygyny) or resources critical for mate attraction and/or successful reproduction (i.e., resource defense polygyny) (Emlen & Oring, 1977; Kelly, 2008c). The degree to which such breeding resources are 'clumped' in space and time determines the strength of sexual selection on males (Emlen & Oring, 1977). If breeding resources are dispersed they are more likely to be evenly distributed among male competitors, leading to weaker sexual selection (e.g., Forsgren, Kvarnemo, & Lindstr, 1996; McLain, 1986; Reichard et al., 2009; but see Muniz & Machado, 2015). When breeding resources are clumped, a single male can monopolize mating access to several females and rival males are excluded from mating, all else being equal. The group of non-mating (or 'zero class') males creates a 'mating niche' (Shuster & Wade, 2003), which, if sufficiently large, can permit an alternative strategy to invade.

0.1.2. Maintaining AMSs: a review of the hypotheses

Why do alternative mating strategies persist in nature, rather than populations becoming fixed for a single optimal strategy? Two leading hypotheses suggest that strategies are either condition-dependent or genetically polymorphic (*reviews in* Gross, 1996; Neff & Svensson, 2013; Shuster & Wade, 2003; Tomkins & Hazel, 2007). The condition-dependent hypothesis suggests that differences in mating strategy reflect an underlying continuum of 'condition', defined as the pool of resources accumulated within an individual that are then allocated towards fitness-related traits (Rowe & Houle, 1996). Under this hypothesis, males that have accumulated less resources use alternative behaviours in order to 'make the best' of their situation (Dawkins, 1980). The phenotypes of these males have traditionally been assumed to be determined solely by environmental factors (i.e., males are genetically monomorphic; Gross, 1996); however, more recent formulations of the condition-dependent hypothesis have included gene-by-environment interactions (Robinson & Beckerman, 2013; Tomkins & Hazel, 2007; discussed below). By contrast, the genetic polymorphism hypothesis posits that each strategy, and its associated morphology, is dictated by Mendelian inheritance. Accordingly, AMSs will only be maintained in a natural population if they achieve relative equal fitness to the dominant strategy; otherwise, selection should remove these phenotypes/genotypes from the population (Shuster & Wade, 2003). Historically there has been little consensus in the literature on this debate.

With regards to the condition-dependent hypothesis, differences in condition among individuals are hypothesized to arise due to differences in individual ability to acquire and allocate resources (a trait that may have a significant genetic component) as well as resource abundance in the environment (Tomkins, 1999; Tomkins & Hazel, 2007). Gross (1996) predicted that the frequency of condition-dependent strategies observed in a population depends on a 'switchpoint' at which the fitness of either strategy is equal (Gross, 1996; see Box 3). Individuals with a condition higher than the switchpoint should obtain greater fitness through the conventional phenotype/strategy, whereas individuals with condition lower than the switchpoint maximize fitness via the alternative phenotype/strategy. According to this hypothesis alternative morphs should be in poorer condition and have lower relative fitness, which could explain why they typically exist at lower frequencies in the population (Gross, 1996). Conditional polymorphisms are considered to be ubiquitous in nature (Rowland, Qualls, & Buzatto, 2017; Tomkins & Hazel, 2007), yet studies have historically relied on the assumption that all AMSs are genetically monomorphic, ignoring the necessity of underlying genetic variation to produce adaptation (Hazel, Smock, & Johnson, 1990; Shuster & Wade, 2003; Shuster, 2010).

In contrast to the condition-dependent hypothesis, Shuster & Wade (2003) argued that if AMSs were genetically monomorphic and yielded poor relative fitness returns, they would be eliminated from the population. They argue that the opportunity to gain fitness is what generates and maintains AMSs, and have demonstrated this principle in the marine isopod *Paracerceis sculpta* in which each of three male morphs have fitness that is equal to its frequency in a wild population (Shuster & Wade, 1991). The assumption of genetic monomorphism mandated by Gross' conception of the condition-dependent hypothesis is generally considered unrealistic, leading researchers to suggest that genetic polymorphism is more widespread than is currently assumed (Neff & Svensson, 2013; Shuster, 2010). In a genetically polymorphic AMS, discrete genes with Mendelian inheritance determine phenotype. Such AMSs may still exhibit a ‘switchpoint’, but its position would be encoded genetically. Genetic polymorphism has only been confirmed in a handful of species: a marine isopod (*P. sculpta;* Shuster & Sassaman, 1997; Shuster & Wade, 1991), ruffs (*Philomachus pugnax;* Küpper et al., 2015; Lank et al.,1995), side-blotched lizards (*Uta stansburiana;* Sinervo, 2001), white-throated sparrows (Tuttle, 2003), and pygmy swordfish (*Xiphophorus nigrensis;* Zimmerer & Kallman, 1989). There is not yet strong evidence for the existence of an AMS based on genetic polymorphism in an insect.

Support for the genetic polymorphism hypothesis is lacking because it requires information on non-mating males. This information is necessary for testing the key prediction that fitnesses are equal among strategies. These data are rarely available because mating males are typically more conspicuous than non-mating males, making collection of this data difficult in the field. Assessing fitness in the field is generally challenging because reproductive success is accumulated over a lifetime, but for many species it is not possible to continuously track individuals throughout their life. Males using alternative strategies may also be dismissed as non-mating males, resulting in estimates of success that are biased toward conventional strategies. However, the genetic polymorphism hypothesis merits investigation, as there are many ways that alternative morphs might acquire fitness. Alternative morphs may acquire mates in different locations or through different means than conventional morphs, facilitating equal fitnesses among strategies (Skrzynecka & Radwan, 2016; Slatkin, 1978). Furthermore, ignoring non-mating males tends to result in overestimates of average mating success and underestimates in the variance of mating success among males exhibiting the conventional strategy (Shuster, 2010; Wade & Shuster, 2004).

Under the genetic polymorphism hypothesis, frequency-dependent selection could explain why alternative strategies tend to fluctuate but persist over time: as one strategy becomes sufficiently common, the strategy of its rare counterpart gains a fitness advantage (Ajuria Ibarra & Reader, 2013; Gross, 1996; Slatkin, 1979). This is the case for the three male morphs of side-blotched lizard, which are maintained by an evolutionary game of 'rock, paper, scissors': the frequencies of each morph oscillate over time but ultimately coexist (Bleay, Comendant, & Sinervo, 2007; Sinervo & Lively, 1996). Generally, however, alternative strategies are expected to reach an evolutionarily stable state within a population whereby the frequency of each strategy is relatively stable over time (Gross, 1996; Skrzynecka & Radwan, 2016; Slatkin, 1978). In contrast, no predictions based on evolutionary processes can be made for condition-dependent polymorphisms underlined by genetic monomorphism because there is no genetic variation among strategies for selection to act upon.

An issue with contrasting the condition-dependent and genetic polymorphism hypotheses is that each hypothesis represents an extreme assumption: phenotypes are either determined exclusively by environment (condition-dependent) or exclusively by genes (genetic polymorphism) (Neff & Svensson, 2013). In reality, genes, environmental factors, and the interaction between these influence the vast majority of phenotypic traits. In several systems it has been demonstrated that different morphs still arise even when environmental factors are held constant (Lagueux-Beloin & Kelly, in prep; Plaistow, Tsuchida, Tsubaki, & Setsuda, 2005; Tomkins, 1999), indicating that genetic factors likely underlie morph determination to some degree. In their review of alternative mating strategies, Neff & Svensson (2013) explored the flaws inherent in the assumptions of each hypothesis, concluding that investigations of AMSs should be framed around *how much* additive genetic variance contributes to a phenotype rather than whether genetics have any influence at all. In accordance with this conclusion, researchers have advanced models for the maintenance of condition-dependent polymorphisms by relaxing the assumption of genetic monomorphism and instead assuming that morphs are determined by polygenes (Engqvist & Taborsky, 2016; Hazel et al., 1990; Tomkins & Hazel, 2007). In these models, fluctuations in the frequency of each morph depend on variation in a heritable switchpoint as well as variation in an environmental variable that cues the switchpoint (Tomkins & Hazel, 2007). Such models effectively unify the theory underlying genetic polymorphisms and condition-dependent strategies into so-called ‘conditional alternative strategies’ by permitting a genetically polymorphic trait to be influenced by environmental conditions and epigenetics (Neff & Svensson, 2013; see Figure 2).

0.1.3. Insect life histories and their implications for AMSs

Insects are of particular interest to the study of AMSs because they are frequently polymorphic (e.g., Ajuria Ibarra & Reader, 2013; Kelly & Adams, 2010; Matsumoto & Knell, 2017; Rowland & Emlen, 2009) and their life history stages are partitioned by discrete growth stages (instars) that precede sexual maturity (i.e., molting and/or metamorphosis). Following from the condition-dependent hypothesis, these discrete growth stages could enable life history-dependent trajectories for polymorphic species: for example, an individual that has accrued few resources during its growth stages could potentially molt to sexual maturity early and adopt an alternative morph phenotype, with less energetic investment in pre-copulatory structures (e.g., weapons, ornaments), while an individual having accrued more resources might delay sexual maturity to become a conventional morph with energetic investment in elaborated structures. There is evidence that aquatic insects express plasticity in the timing of their final molt in response to drying conditions (Harper & Peckarsky, 2006;

Tronstad, Tronstad, & Benke, 2005), suggesting that terrestrial polymorphic species might also be able to time their molt in response to environmental cues.

Perhaps due to this hypothesized sensitivity to environmental cues, insects have featured prominently in investigations of condition-dependent mechanisms of morph determination. In particular insect species have been used to test the environmental threshold model, which posits that morph expression is triggered by individuals responding to an environmental cue (Hazel et al., 1990; Tomkins & Hazel, 2007). This model has been successfully applied to horned dung beetles, which shift their allocation of resources to horn development based on larval body size (Rowland & Emlen, 2009; Rowland et al., 2017). An important aspect of this model is that it also assumes individuals vary genetically in their sensitivity to a given environmental cue. This 'switchpoint' is heritable and allows a range of morphs to be maintained by selection; however, long-term selection acts upon the heritable switchpoint, not the morph/phenotype. While there has been limited investigation of how insects might change timing of sexually maturity in response to environmental cues, growth rate and delayed sexual maturity have been shown to affect morph determination in several species of fish (Gross & Charnov, 1980; Rios-Cardenas & Webster, 2008; Thorpe & Morgan, 1980).

For insects, overall instar number and the time spent in each instar can vary between individuals (Etilé & Despland, 2008), suggesting that there could be ample flexibility for individuals to adjust their growth trajectory. Species also differ in whether instar number is fixed or variable, which could affect the likelihood of a species evolving genetically fixed versus conditionally expressed morphs (Engqvist & Taborsky, 2016). In species with a fixed instar number, individuals with a low growth rate mature later and at a smaller body size (e.g., Tammaru, 1998), whereas species with a variable instar number can simply continue adding instars until they reach a larger body size (e.g., Kingsolver, 2007). Engqvist & Taborsky (2016) developed a model to predict whether species are more likely to evolve genetic or condition-dependent AMSs based on environmental and social conditions, finding that species with determinate growth were more likely to express genetic polymorphism. Because species with fixed instar number have less flexibility to change their growth trajectory in response to environmental cues, one can predict that such species are more likely to express AMSs that are strongly determined by genetic factors.

0.1.4. Evolutionary and conservation significance of AMSs

AMSs are a ubiquitous form of intraspecific diversity that may be an important substrate for speciation when associated with genetic polymorphism. Alternative male morphs could give rise to new species through a variety of mechanisms (*reviews in* Mclean & Stuart-Fox, 2014; Smith & Skulason, 1996): individuals might choose mates assortatively or breeding opportunities may become spatially/temporally segregated based on morph (prezygotic barriers); morph hybrids may have reduced fitness (postzygotic barriers); or certain morph(s) might be disfavoured in a new environment or under different selective pressures, resulting in morph loss and subsequent population differentiation (West-Eberhard, 1986). This final mechanism is responsible for the higher rate of speciation associated with colour morphs in non-passerine birds: the repeated progression of poly- to mono-morphism across taxa has resulted in a proliferation of new species (Hugall & Stuart-Fox, 2012). Corl et al. (2010) also found support for speciation via morph loss in a species with three alternative mating morphs, the side-blotched lizard (*Uta stansburiana*): here, geographic variation in the number and frequency of morphs is associated with evolutionarily distinct lineages, with subspecies more likely to be di- or mono-morphic. Sexual selection itself is strongly associated with species richness across the animal kingdom (Janicke, Ritchie, Morrow, & Marie-Orleach, 2018), indicating that processes driven by sexual selection are of overall importance to biodiversity conservation.

AMSs may thus be an important source of future biodiversity and are worthy of attention during conservation management, which ultimately aims to combat biodiversity loss (IUCN & SSC, 2013). It is unlikely that the genetic variation embodied by AMSs would be captured by conventional molecular measures of genetic diversity employed by conservation managers (e.g., expected heterozygosity), as these measures do not correlate strongly with quantitative traits (Reed & Frankham, 2001). Furthermore, there is a need to better integrate adaptive genetic variation alongside neutral genetic variation for the designation and management of conserved populations (De Guia & Saitoh, 2007). The possibility of morph loss and its potential outcomes should be considered when planning to transfer individuals to a new site for conservation purposes ('conservation translocations'; IUCN & SSC, 2013). Morph loss can occur as a result of natural selection and produce greater biodiversity, as observed in the above examples of non-passerine birds and side-blotched lizards. However, it has also been associated with reduced population size (Eckert & Barrett, 1992), and thus could negatively impact the demographic goals of a conservation translocation. Artificial morph loss due to the absence of a morph(s) in the translocated cohort is therefore a risk that can and should be mitigated by conservation managers. I address this concern by investigating the potential mechanisms maintaining AMSs in a frequently translocated insect, the Wellington tree weta (*Hemideina crassidens*).

0.2. Mechanisms underlying equal fitnesses among mating strategies

If fitnesses are equal among strategies, this begs the question of what mechanisms enable alternative morphs to perform equally to individuals that focus on monopolizing females (i.e., conventional morphs). There are many possibilities, the most prominent idea being that alternative morphs use distinct, but equally successful, behavioural strategies such as ambushing females when a guarding male is absent or unaware ('sneaking'; Neff & Svensson, 2013; Taborsky et al., 2008). This behavioural phenotype is hypothesized to allow alternative morphs to obtain an equal number of matings without the expense of guarding females (Parker, Lessells, & Simmons, 2012).

Here, I provide a brief overview of two proposed causal mechanisms underlying AMSs: 1) alternative morphs produce a greater quantity and/or quality of sperm per ejaculate, thus enabling them to outcompete conventional morphs for fertilization of a common mate's eggs (Parker, 1990; Simmons, Emlen, & Tomkins, 2007); or 2) morphs occupy different environmental niches ('resource polymorphism'), reducing their spatial overlap and thus also reducing male-male competition (Parker et al., 2012; Smith & Skulason, 1996).

0.2.1. Sperm competition

Sexually selected traits may be pre- or post-copulatory: pre-copulatory traits influence mate acquisition (e.g., body size, ornamentation, weaponry), while post-copulatory traits influence fertilization success (e.g., sperm number/quality). Post-copulatory traits are of particular importance when females are polyandrous and the sperm of two or more males can therefore simultaneously compete for fertilization of a given set of ova within the female reproductive tract ('sperm competition'; Parker, 1970).

Insects have been identified as a group where sperm competition is particularly strong owing to their propensity to re-mate as well as the capacity of females to store and maintain sperm from multiple ejaculates (Simmons, 2001). Evidence suggests that factors potentially influencing fertilization success include sperm morphology, sperm length, sperm viability, ejaculate size, and mating order ('sperm precedence') (Kelly & Jennions, 2016). Because the best sperm allocation strategy depends on interactions with competitors, models to predict how males ought to allocate energy towards sperm have been rooted in game theory (*reviewed in* Parker & Pizzari, 2010). The null model of sperm competition is referred to as the 'fair raffle': this model assumes all sperm have an equally good chance of fertilizing an egg and sperm storage space is unlimited, so the only determinant of fertilization success is the quantity of sperm provided compared to other male competitors (Parker, 1990). There are many reasons why sperm competition may not conform to these assumptions: some males may possess competitively superior sperm ('loaded raffle'), sperm may mix non-randomly in the female reproductive tract, sperm storage space is often limited, and females may play a role in the process by cryptically 'choosing' the sperm that fertilizes her eggs ('cryptic female choice'; *see* 0.3.1 Cryptic female choice) (Eberhard, 1996). Competing males might also occupy different 'roles' that influence fertilization success, which could be randomly assigned (e.g., being the first or second male to mate) or non-randomly assigned (e.g., possessing a particular phenotype).

The role that sperm competition plays in AMSs is a relatively recent topic of research (Lüpold et al., 2015). Conventional morphs that monopolize females are characterized by a comparatively high investment in pre-copulatory traits related to mate guarding or sexual signaling (Taborsky, 1997). By contrast, alternative morphs evade these costs by 'sneaking' (passing by a dominant male unnoticed, often via female mimicry) or 'streaking' (mating or depositing sperm rapidly) to acquire copulations with females that are usually associating with a dominant male (Taborsky et al., 2008). Models integrating AMSs generally treat each male phenotype as a non-randomly assigned 'role' that may express a fixed sperm allocation, determined by the average selection on that phenotype, or a facultative sperm allocation, determined based on available cues at ejaculation (Parker & Pizzari, 2010). When sperm allocation is fixed alternative morphs are predicted to allocate more sperm per mating than dominant strategists, owing to the fact that they can gain paternity through extra-pair copulations without the cost of monopolizing females (Ball & Parker, 2000; Parker, 1990). When sperm allocation is facultative, dominant males are predicted to vary sperm allocation depending on the detection of extra-pair matings (Parker, 1990).

Another factor predicted to affect a male's allocation of resources towards sperm is the probability that his ejaculate will encounter that of another male's, or the so-called 'risk' of sperm competition (Parker, Ball, Stockley, & Gage, 1996). Overall, alternative morphs are expected to invest more in sperm than dominant strategists; however, all males are predicted to invest more in sperm with an increasing number of alternative morphs in the population due to the increased risk of sperm competition (Parker, 1990; Simmons, Emlen, & Tomkins, 2007). Some examples of evidence for this concept include an analysis of 16 species of onthophagine beetles, where increasing frequency of the alternative strategy was associated with increased testis size across species (Simmons et al., 2007), and a study across 10 populations of myobatrachid frog *Crinia georgiana*, where testis size and number of sperm positively co-varied with density of breeding males (Dziminski, Roberts, Beveridge, & Simmons, 2010).

Given that each individual has a finite level of resources to invest in reproductive traits, it is likely that tradeoffs exist between investment in pre- and post- copulatory traits (Parker, Lessells, & Simmons, 2012). For example, in the horned beetle (*Onthophagus nigiventris*), experimental restriction of the development of horns (a pre-copulatory sexually selected trait) resulted in individuals growing larger testes, suggesting a tradeoff between the two structures (Simmons & Emlen, 2006). Such tradeoffs are pertinent to the study of AMSs as they provide a potential explanation for how alternative morphs might have fitnesses equal to conventional morphs: if alternative morphs consistently have greater fertilization success than conventional morphs, this could offset the consequences of having fewer total copulations (Lewis, Sasaki, & Miyatake, 2011; Parker et al., 2012). Differential investment between pre- and post- copulatory traits is also predicted to increase with increasing strength of male-male competition, indicating that this tradeoff should be particularly important when females are monopolizable (Lüpold et al., 2014).

0.2.2. Resource polymorphism and differential niche use

While AMSs are strikingly phenotypically distinct, research into how these diverse phenotypes may interact differently with the environment has been historically scarce. Environmental conditions could strongly influence the expression of AMSs as they might influence the ability of breeding resources to be clumped in space/time, thus influencing the ability of males to monopolize females (Emlen & Oring, 1977; McLain, 1986); they might alter the perceived strength of a sexual signal, particularly for colour polymorphisms (e.g., strength of sunlight changing female perception of male colour displays; Gamble, Lindholm, Endler, & Brooks, 2003; Reynolds, Gross, & Coombs, 1993); environmental heterogeneity could destabilize selection, leading to fluctuations in the number and frequency of AMSs (Corl, Davis, Kuchta, & Sinervo, 2010; Cornwallis & Uller, 2010; West-Eberhard, 1986); and AMSs might be stabilized by morphs using discrete niches in terms of their habitat or feeding behaviour ('resource polymorphism'; *reviewed in* Smith & Skulason, 1996). There is no reason to assume that such selective forces are not acting on the sexually selected polymorphisms represented by AMSs, yet environmental selection is often ignored for sexually selected traits (Cornwallis & Uller, 2010).

Differential niche use, or resource polymorphism, might help to explain how AMSs could achieve equal relative fitness. This mechanism has been shown to reduce intraspecific competition and stabilize selection in other polymorphic species: for example, in the cichlid *Herichthys minckleyi* competition was reduced when cage experiments contained multiple morphs that use different microhabitats and feeding behaviours compared to only one morph at the same density (Swanson, Gibb, Marks, & Hendrickson, 2003). This mechanism could equally apply to AMSs if they acquired mates in different microhabitats, resulting in decreased male-male competition. For example, the conspicuous differences among AMSs with colour polymorphism could make them differentially suited to particular substrates/backgrounds to avoid predation (Hoekstra, Drumm, & Nachman, 2004; Sandoval, 1994), with natural selection reinforcing their segregation into these microhabitats. Evidence of genetic differentiation and assortative mating in resource polymorphic fishes is suggestive of mating behaviours being associated with resource polymorphism (Moore, Loewen, Harris, & Tallman, 2014; Smith & Skulason, 1996), yet these characteristics are rarely considered together as unified syndromes. Conversely, maintenance of AMSs are almost exclusively discussed in the context of sexual selection with little regard for the influence of natural selection or gene by environment interactions (Cornwallis & Uller, 2010; Neff & Svensson, 2013).

One issue with detecting resource polymorphism is that a similar distribution of morphs among microhabitats might be observed due to competitive exclusion:

conventional morphs may simply outcompete alternative morphs for high quality sites, resulting in a biased distribution of morphs among sites. It is therefore important to tease apart whether competitive interactions among morphs or microhabitat characteristics determine morph-specific spatial distribution. Polymorphism associated with differential niche use is predicted to decrease competitive interactions among morphs (Smith & Skulason, 1996). In the context of AMSs, this would also encompass reduced sperm competition as morphs would be less likely to compete for fertilization of the same females, resulting in reduced post-copulatory expenditure (Parker et al., 2012). Polyandry is also generally predicted to decrease the strength of sexual selection via sperm competition unless mating success and fertilization success co-vary positively (Shuster & Wade, 2003; Shuster, 2010). However, these factors would be unlikely to completely eliminate the effects of sperm competition in species where females mate with multiple morphs (i.e., incomplete or non- assortative mating). This dynamic has been demonstrated in many taxa with AMSs (Bleay et al., 2007; Shuster & Wade, 1991; Tuttle, 2003), suggesting that sperm competition is likely to remain a factor even when pre-copulatory male-male competition may be reduced by niche partitioning.

0.3. Female mate choice

My discussion to this point has mainly focused on competition between members of one sex (males) for access to mates, or intra-sexual selection; however, mate choice by the opposite sex (females), or inter-sexual selection, is an equally important component of sexual selection (Darwin, 1871). Female preference may not only drive sexual dimorphism by selecting for colourful or ornamented male phenotypes, but may also drive phenotypic diversity within males by influencing the relative success of alternative morphs (e.g., Morris, Rios-Cardenas, & Brewer, 2010). There are many characteristics apart from colour, ornamentation or morph that females may discriminate mates by: some include a male's immune status, physical performance, traits correlated with cognitive performance, and body size (Beltran-Bech & Richard, 2014; Boogert, Fawcett, & Lefebvre, 2011; Byers, Hebets, & Podos, 2010; Holveck, Geberzahn, & Riebel, 2011). Furthermore, a female may make choices based on her own condition: the costs of remaining unmated may influence females to be less selective in their mate choice early in life (Kokko & Mappes, 2005), whereas mated females may exert stronger 'choosiness' (Gabor & Halliday, 1997).

Such factors might influence more than just a female's choice to copulate: it is increasingly recognized that females mate multiply (Taylor, Price, & Wedell, 2014) and may therefore exert mate choice during and after copulation by manipulating sperm ('cryptic female choice'; Eberhard, 1996; Thornhill, 1983). Cryptic female choice can drastically change our estimates of male reproductive success because it can create situations where successful copulation does not necessarily guarantee fertilization. Prior to the discovery and understanding of these concepts, studies often relied on indirect measures to represent male reproductive success: for example, monopolization of females or assumed mating success (Shuster & Wade, 1991; Sinervo & Lively, 1996). I help to close this knowledge gap by directly quantifying the number of offspring sired by each male and using maternal identities to take into account polyandry and cryptic female choice in the Wellington tree weta (*Hemideina crassidens*).

0.3.1. Benefits of polyandry

While the benefits of multiple mating for males are undisputed and generally thought to arise due to anisogamy driving a higher potential mating rate and decreased investment in offspring in males (Bateman, 1948; Lehtonen et al., 2016), the benefits of multiple mating in females (polyandry) are less intuitively clear. The fitness benefits of polyandry can be divided into direct and indirect benefits (Slatyer, Mautz, Backwell, & Jennions, 2011). Direct benefits increase a female's direct fitness (i.e., lifetime fecundity); examples include nourishment provided with the ejaculate ('nuptial gifts'), chemicals in male ejaculates that improve egg production (Eberhard, 1996) or immune function (Worthington & Kelly, 2016b), assurance of fertilization (Hasson & Stone, 2009), increased parental care (Rubenstein, 2007), and decreased risk of infanticide (Klemme & Ylönen, 2010). Indirect benefits increase the mean fitness of a female's offspring, which can occur via increased genetic diversity, an increased ability for females to exert post-copulatory choice, or maternal half-sibling signaling stimulating increased female brood investment (Slatyer et al., 2011).

Research on direct benefits has been prolific, allowing for several meta-analytic studies. Arnqvist & Nilsson (2000) examined 122 experimental studies on the direct benefits of polyandry in insects, showing an overall positive effect on egg production and fertility. This study also demonstrated a negative effect on longevity for species without nuptial gifts, which was outweighed by the positive effects on fecundity only at intermediate mating rates. This analysis pooled together two types of multiple mating experiments: those in which females could re-mate the same partner (repeated mating), or those in which females could mate with multiple partners (polyandry). A meta-analysis of 84 studies on arthropod taxa by South & Lewis (2011) corroborated these findings, this time distinguishing between repeated and polyandrous matings. In both cases, larger ejaculate quantity increased lifetime fecundity more compared to monandrous females. It is therefore clear that multiple mating confers direct benefits whether via repeated or polyandrous matings; however, hypotheses to explain why females might acquire direct benefits from mating with multiple partners rather than re-mating the same partner are lacking (these benefits are presumed to be indirect; see below). One possibility is that polyandry is a natural consequence of certain mating systems: for example, insects rarely form long-term pair bonds, prohibiting them from re-mating the same partner (Choe & Crespi, 1997).

Indirect benefits of polyandry comprise genetic diversity benefits, post-copulatory choice benefits, and maternal half-sibling signaling benefits. Yasui (1998) identifies two situations in which increased genetic diversity within a brood could be beneficial: 1) males vary widely in genetic quality, so mating with multiple males ensures that a female's offspring are not entirely sired by a low quality male ('genetic bet-hedging'); and 2) the environment is unpredictable from one generation to another, so incorporating a variety of genotypes ensures that at least some offspring will survive ('genetic diversity bet-hedging'). Empirical tests of these hypotheses are rare because it is difficult to manipulate female mating strategy (i.e., monandry versus polyandry) across generations in an experimental design; however, an empirical study on the purple sea urchin *Heliocidaris erythrogramma armigera* did overcome these limitations and showed support for the genetic bet-hedging hypothesis but not the genetic diversity bet-hedging hypothesis (Garcia-Gonzalez, Yasui, & Evans, 2015).

The second proposed indirect benefit of polyandry is that females may use cryptic choice to bias paternity towards sires that would increase their offspring's fitness. Mechanisms of cryptic female choice and its evolutionary implications are discussed in the section below (0.3.2. Cryptic female choice). Briefly, a female might realize benefits by selectively using sperm from higher quality males ('good sperm' hypothesis; Yasui, 1997) or from males less related to herself ('genetic compatibility' hypothesis; Zeh & Zeh, 1997). The third proposed indirect benefit of polyandry is a maternal half-sibling signaling effect: some males may produce ejaculates or offspring that are better at producing signals to induce the female to increase her investment in a brood, resulting in a shared beneficial maternal effect among all offspring (Simmons, 2005). This indirect benefit has rarely been demonstrated and is only known in the cricket *Teleogryllus oceanicus* (García-González & Simmons, 2007) and the pseudoscorpion *Cordylochernes scorpioides* (Zeh & Zeh, 2006).

Apart from the fitness benefits discussed above, there are several logistical reasons that might explain why females mate multiply. Historically it has been assumed that females easily acquire the necessary quantity of sperm to fertilize their eggs in a single mating (Kokko & Mappes, 2005); however, recent work shows that viable sperm may be a limiting resource, with multiple mating helping to ensure that females receive an adequate supply (Hasson & Stone, 2009; South & Lewis, 2011; Worthington & Kelly, 2016a). The costs of expressing 'choosiness' to the degree of accepting only a single, best mate may also be too high, as this could critically extend the amount of time a female remains unmated (Kokko & Mappes, 2005, 2013). Furthermore, organisms with limited cognitive and sensory abilities such as insects may not be capable of simultaneously comparing many potential mates, making it an easier decision rule to accept most or all available mates (Kokko & Mappes, 2013). Finally, females might also mate multiply to avoid physical injury via male harassment ('convenience polyandry'; Slatyer, Mautz, Backwell, & Jennions, 2011).

0.3.2. Cryptic female choice

Cryptic female choice refers to inter-sexual selection taking place during and after copulation, which can occur when females receive ejaculates from multiple mates and bias fertilization towards sperm from particular mates (Eberhard, 1996; Thornhill, 1983; *reviewed in* Firman, Gasparini, Manier, & Pizzari, 2017). This phenomenon can be difficult to demonstrate because mechanisms of sperm competition could produce similar results to those expected from cryptic female choice (Eberhard, 2015; Evans, Rosengrave, Gasparini, & Gemmell, 2013). For example, females might bias paternity towards 'sneaker' males if they are currently rare in the population, because this would produce sons with a negative frequency-dependent fitness advantage; however, the same bias could be observed due to 'sneakers' producing a comparatively larger ejaculate than the conventional morph and winning out in sperm competition. Lab-based experiments are therefore essential to the study of cryptic female choice to allow for control of ejaculate traits as well as other confounding factors such as plastic male response (Firman et al., 2017).

Several mechanisms of cryptic female choice are hypothesized across the stages of copulation, sperm storage, and fertilization. During copulation, females may influence the quantity of sperm received by controlling the duration of copulation or terminating it prematurely (Herberstein et al., 2011; Pilastro, Mandelli, Gasparini, Dadda, & Bisazza, 2007). Immediately following copulation, females may eject sperm (Dean, Nakagawa, & Pizzari, 2011; Peretti & Eberhard, 2010; Pizzari & Birkhead, 2000), use contractions of their reproductive tract to preferentially uptake sperm (Friesen, Uhrig, Mason, & Brennan, 2016; Troisi & Carosi, 1998), or destroy sperm via immune response or spermicidal chemicals (Holman & Snook, 2008). At the sperm storage stage, the morphology of the female reproductive tract may allow for control of this process (i.e., in species where females possess multiple sperm storage organs) or sperm may be subsequently lost from the sperm storage organ via 'sperm dumping' (Snook & Hosken, 2004). Finally, at the fertilization stage sperm swimming performance may be mediated by female reproductive fluids, which has been shown to bias paternity towards particular male phenotypes (Alonzo, Stiver, & Marsh-Rollo, 2016) and unrelated males (Gasparini & Pilastro, 2011). Sperm may also be differentially activated by secretions of the female reproductive tract (Herberstein, Schneider, Uhl, & Michalik, 2011), and there is some evidence that sperm-egg signaling could be a mechanism of cryptic female choice through surface binding protein interactions (Ghaderi et al., 2011; Stapper, Beerli, & Levitan, 2015).

The fitness benefits of cryptic female choice are likely to be indirect benefits that increase the fitness of a female's offspring (*see* 0.3.1. Benefits of polyandry). All females might prefer a particular male genotype, producing directional selection, or females may differ in the genotypes they prefer, producing non-directional selection. There are two prominent hypotheses to explain directional selection: the 'good sperm' hypothesis (Yasui, 1997), where females select for ejaculate traits associated with male genetic quality, and the 'sexy sperm' hypothesis (Curtsinger, 1991), where males with the most competitive sperm produce sons with superior ejaculate traits. Non-directional selection is predicted to occur if females preferentially use sperm from males that are less similar to her in genotype ('genetic compatibility' hypothesis; Trivers, 1972; Zeh & Zeh, 1997), which could increase her offspring's fitness via hybrid vigour and avoidance of inbreeding (Firman et al., 2017; Tregenza & Wedell, 2002).

What consequences does cryptic female choice have for the evolution of male alternative mating strategies? Either form of selection might act as a mechanism to maintain male AMSs: non-directional selection is expected to maintain genetic variance and polymorphism in males, while directional selection could provide an opportunity for some males to maximize fitness through post-copulatory selection and others to specialize in traits favoured by pre-copulatory selection (Firman et al., 2017). The hypothesis that alternative morphs should invest more in sperm than dominant strategists (*see* 0.2.1. Sperm competition) assumes that it is principally competitive characteristics of sperm/ejaculates that determine fertilization success and that the influence of cryptic female choice is negligible (i.e., 'sexy sperm' hypothesis). This assumption may be justified at least within insects due to the prevalence of last-male sperm precedence (Simmons & Siva-Jothy, 1998), which suggests that females have limited control of the fertilization of their eggs after copulation. However, there are several studies providing evidence for cryptic female choice in insects (Peretti & Aisenberg, 2015), and cryptic female choice may modulate the effects of sperm precedence (Mack, Priest, & Promislow, 2003). The effects of sperm competition and cryptic female choice should therefore be evaluated separately on a species by species basis if possible.

0.4. Study species and field site

Wētā are a group of roughly 70 insect species belonging to the order Orthoptera that are endemic to New Zealand. Wētā are a major prey item for native insectivorous predators (Gibbs, 1998; Haw, Clout, & Powlesland, 2001) and herbivorous/frugivorous wētā species likely play a prominent role in seed dispersal and nutrient cycling in natural forest ecosystems (Duthie, Gibbs, & Burns, 2006; Griffin, Morgan-Richards, & Trewick, 2011; Weisser & Siemann, 2008). In New Zealand invasive species such as rats (*Rattus* spp.) and stoats (*Mustela erminea*) threaten native wildlife that evolved in the absence of mammalian predators (Armstrong & McLean, 1995). To ensure the continued survival of wētā and preserve their roles in New Zealand forest ecosystems, wētā are frequently translocated among offshore island sanctuaries and mainland reserves by the federal Department of Conservation (Watts et al., 2008).

The Wellington tree wētā (*Hemideina crassidens*) is a large, flightless, nocturnal species that aggregates in tree cavities, known as galleries, for diurnal refuge and mating (Gibbs, 2001). Male *H. crassidens* express three morphs (trimorphism), with individuals maturing at one of three instars (8th, 9th, or 10th; Kelly, 2005; Kelly & Adams, 2010; Spencer, 1995) to produce three distinct classes of head size while females mature at the 10th instar only. After eclosion to adulthood, individuals cease moulting and remain the same size for the duration of their ca. 1-year reproductive lifespan (Kelly, 2006c). While adults mate and reproduce throughout this time period, peak mating and oviposition occur over the austral summer and autumn months (approximately December – May; Rufaut & Gibbs, 2003). Females may lay eggs every few days to weeks during this time, making lifetime reproductive success likely to be strongly dependent on lifespan (Kelly, 2011).

A rearing experiment suggests that male trimorphism has a significant genetic basis, as males still matured at different instars even when environmental conditions were held constant (Lagueux-Beloin & Kelly, in prep.). Trimorphism in head size is driven by sexual selection (Kelly, 2005): males engage in aggressive contests whereby the individual with larger mandibles consistently controls access to harems of females (Kelly, 2006b; Kelly & Adams, 2010). Strong pre-copulatory sexual selection on male weapon size has thus apparently opened a mating niche for smaller alternative morphs in *H. crassidens*, although it is unknown what behavioural strategies they employ (Kelly, 2008a; Spencer, 1995). Males with smaller weaponry produce larger ejaculates than males with larger weaponry, indicating that smaller morphs may be alternative strategists that invest more in post-copulatory traits (Kelly, 2008b). Some morphs of Wellington tree weta have been reduced or lost following previous conservation translocations (Watts et al., 2008), indicating there is a need to improve our knowledge of how the polymorphism in males is generated and maintained in order to prevent losses of intraspecific diversity during future translocations.

Gallery size is likely a strong factor affecting the clumping of females in space (harem size) and could therefore be an important determinant of the intensity of sexual selection in this species (Field & Sandlant, 2001; Kelly, 2008a). Field observations suggest that male head size in *H. crassidens* is correlated with gallery size: males inhabiting forest patches with small galleries (capacity of < 3 adults) had significantly smaller heads on average than those inhabiting patches with large galleries (capacity of > 3 adults; Kelly, 2008a). This could be an example of resource polymorphism associated with an AMS wherein smaller galleries favour smaller morphs, resulting in morphs being distributed according to appropriate microhabitat; alternatively, larger morphs may competitively exclude smaller morphs from high quality sites (i.e., large galleries where larger harems may form). The size of available galleries does appear to have implications for expression of the trimorphism: the Somes Island population of *H. crassidens* was seeded from Mana Island, where males express a wide range of head sizes including large morphotypes (>21 mm head length), but is now restricted to smaller head sizes in association with the smaller average gallery size on Somes Island (Kelly, 2008a). This observed shift in the trimorphism could indicate a reduced fitness for larger morphs introduced onto the island, perhaps because they were unable to exploit the small cavities afforded by their new habitat.

Our study population of *H. crassidens* is located at Te Pākeka/Maud Island, New Zealand (41°02'S, 173°53'E). Te Pākeka is a 309-ha scientific reserve that is free of invasive predators commonly present in New Zealand (i.e., rodents [*Mus* and *Rattus* spp.] and stoats [*Mustela erminea*]). The endemic ruru owl (*Ninox novaeseelandiae*) is the only known predator of *H. crassidens* present on the island (Kelly, 2006b). The forest is mainly comprised of broad-leaved trees (e.g., kohekohe, *Dysoxylum spectabile*) as well as an understory of scrub (e.g., manuka *Leptospermum* spp. and mamaku/tree fern *Cyathea medullaris*). Males of each morph can be found at this site, but there are typically more 8th instars than 9th or 10th (Kelly & Adams, 2010). A 7-year survey showed that the frequency of each morph is constant over time at this site, indicating that the effects of frequency-dependent selection are likely stabilized in the current environment (Kelly & Adams, 2010). A head size distribution for the three male morphs has been established for the population at this site and is used to interpolate the morphs of males used in the present study based on head size measurement (Kelly & Adams, 2010).

CHAPTER I

SUPPORT FOR MAINTENANCE OF ALTERNATIVE MATING STRATEGIES VIA GENETIC POLYMORPHISM IN THE WELLINGTON TREE WETA

Sarah E. Nason and Clint D. Kelly

1.1. Abstract

Alternative mating strategies (AMSs) are suites of behavioural and morphological characteristics expressed by one sex to gain access to matings. AMSs are well documented across many taxa, yet it is not often clear how they evolve and are maintained. We conducted a field study on the Wellington tree wētā (*Hemideina crassidens*) to investigate the maintenance of AMSs. *H. crassidens* is polygynandrous, forms harem groups, and exhibits a sexually-selected polymorphism in head size in males thought to represent distinct AMSs. We tested the hypothesis that the polymorphism is genetic and thus maintained by each morph achieving equal relative fitness. We measured and compared the number of offspring produced by each morph in a cross-sectional study of a natural population of *H. crassidens* on Te Pākeka/Maud Island, Aotearoa/New Zealand using a molecular paternity analysis. The results show no differences in relative fitness among morphs and that each morph produced a quantity of offspring proportional to that morph's historic frequency in the wild population, suggesting that the polymorphism is primarily determined by genetics. We further analyzed the data to examine rates of multiple mating and potential fitness benefits in each sex. We found evidence of direct fitness benefits in both sexes, as they produced more offspring when they had more mates, and indirect fitness benefits in females, as their offspring had greater hatching success when they had more mates. Lastly, we examined whether there was directional post-copulatory selection for any particular male morph, finding that smaller morphs sired more offspring within broods. This fertilization bias could be explained by sperm competition or cryptic female choice and should be investigated in future studies. As this species is frequently translocated for conservation purposes, we offer recommendations to ensure the polymorphism is maintained at translocation sites: cohorts to be translocated should include males of each morph in the proportions observed in the source population or be of a propagule size to adequately capture genetic variation (at least 20-50 breeding individuals).

Keywords: alternative mating strategy, sexual selection, fitness, evolution, intraspecific diversity

Running title: Support for maintenance of alternative mating strategies via genetic polymorphism in the Wellington tree weta (*Hemideina crassidens*)

1.2. Introduction

Individuals of one sex (usually males) often have the potential to express one of several behavioural phenotypes to gain access to mates (Gross, 1996; Taborsky et al., 2008). When these phenotypes are fixed over the lifetime of an individual, they are referred to as alternative mating strategies (AMSs) (Gross, 1996). Systems with AMSs are typically characterized by the presence of one strategy that invests in mate acquisition by monopolizing females, and one or more other strategies that evade this cost (Taborsky et al., 2008). For example, male bluegill sunfish (*Lepomis macrochirus*) may either be large, dominant 'guarders' that display parental care or one of two small-bodied alternatives: 'sneakers' and female mimics (Gross & Charnov, 1980). For the purposes of this paper, we refer to males that monopolize females as 'conventional morphs' and males that use other strategies (e.g., 'sneaking', female mimicry) as 'alternative morphs'. Why AMSs arise and how they are maintained from an evolutionary perspective is still a subject of much debate (see discussions in Engqvist & Taborsky, 2016; Neff & Svensson, 2013; Shuster & Wade, 2003; Tomkins & Hazel, 2007), with two competing hypotheses historically predominating: the condition-dependent hypothesis and the genetic polymorphism hypothesis (Shuster & Wade, 2003; Tomkins & Hazel, 2007).

The condition-dependent hypothesis has traditionally assumed that all individuals are genetically monomorphic and alternative phenotypes arise from inter-individual differences in 'condition,' defined as the pool of resources accumulated within an individual that are then allocated towards fitness-related traits (Gross, 1996; Rowe & Houle, 1996). In this case, individuals that are in 'poor condition' (i.e., having accumulated relatively few resources) are assumed to adopt alternative mating behaviours in order to maximize their fitness (Dawkins, 1980; Gross, 1996). By contrast, the genetic polymorphism hypothesis suggests that each AMS is determined by a discrete genotype with Mendelian inheritance. This hypothesis is based on the reasoning that selection should disfavour genotypes related to poor relative fitness returns and so they should not be maintained in the population (Shuster & Wade, 2003). Both hypotheses have been supported in the literature, with condition-dependence being found most notably in several families of beetles (Knell & Simmons, 2010; Perry & Rowe, 2010; Rowland & Emlen, 2009) and genetic polymorphism in the marine isopod *Paracerceis sculpta* (Shuster & Sassaman, 1997; Shuster & Wade, 1991), the lekking ruff *Philomachus pugnax* (Küpper, Stocks, Risse, Dos Remedios, et al., 2015; Lank et al., 1995), and the side-blotched lizard *Uta stansburiana* (Sinervo & Lively, 1996; Sinervo, 2001).

Directly contrasting the condition-dependent and genetic polymorphism hypotheses is unlikely to be a useful exercise, because each hypothesis relies on an extreme assumption: either phenotypes are determined solely by the environment (condition-dependence) or solely by genes (genetic polymorphism) (Neff & Svensson, 2013). In light of the fact that the majority of phenotypic traits are determined by both of these factors, as well as the interaction between them, we instead assume that all AMSs encompass some degree of genetic polymorphism. This assumption is supported by theoretical work that has produced a unified theory for the evolution and expression of AMSs, wherein all AMSs are genetically polymorphic but may also be influenced by biotic/abiotic environmental factors and epistasis ('conditional alternative strategies'; Neff & Svensson, 2013). We therefore focused our study on the relative influence of genes versus the environment in determining the expression of AMSs, rather than whether one or the other is exclusively responsible.

According to Shuster & Wade (2003), the existence of AMSs that are primarily determined by genetics can be explained by the opportunity to gain fitness: when sexual selection is strong and some dominant males are able to monopolize females, other dominant males are unable to mate and this results in an empty 'mating niche'. This niche can only be filled by an AMS that is at least as good as the conventional strategy in terms of fitness returns (Shuster & Wade, 2003). In order for the AMS to then be maintained by selection, it must also be heritable. Therefore, one of the key predictions for an AMS that is strongly determined by genetic polymorphism is that each morph should have equal relative fitness. Importantly, this prediction only holds when the population is at an evolutionarily stable state: the relative rarity of a strategy may confer it a fitness advantage due to negative frequency-dependent selection (Ajuria Ibarra & Reader, 2013; Maynard Smith, 1982), a mechanism that has been shown to explain cycles in the frequencies of three male morphs of side-blotched lizards (Sinervo & Lively, 1996). However, strategies are ultimately predicted to reach stable frequencies at the point where their fitness functions intersect (i.e., strategies have equal fitness) (Gross, 1996; Maynard Smith, 1982). The detection of equal fitnesses can be difficult because it requires confirmation of a null hypothesis, and can be confounded if a population is not at an evolutionarily stable state (Neff & Svensson, 2013). As a result, AMSs underlined by genetic polymorphism have only been identified in a handful of species, despite genetic polymorphism likely being widespread (Shuster, 2010).

Alternatively, an AMS might be driven more strongly by the environment. While such an AMS is still likely to be determined by some degree of underlying genetic variation, allowing it to be maintained in the long-term by selection, the expression of these genes can be regulated by epigenes and/or environmental conditions. The environmental threshold model (Hazel et al., 1990; Tomkins & Hazel, 2007) assumes that individuals genetically vary in their sensitivity to an environmental cue which triggers the expression of an alternative morph; for example, ear wigs *Forficula auricularia* produce long-forcep morphs more commonly when their diet is high in protein (Tomkins, 1999). The strength of the environmental cue may also vary, meaning that a sudden shift in the environmental cue could trigger a short-term difference in the relative frequency of a particular morph unrelated to long-term selection (Tomkins & Hazel, 2007). Because it is the degree of sensitivity to an environmental cue ('switchpoint') that is heritable in this type of system, and not the morph, according to the same principles of negative frequency-dependent selection as above we would predict that fitnesses would be equal among switchpoints at equilibrium, but not necessarily among morphs.

We investigated the relative influence of genes versus the environment in determining polymorphism using an insect species with three male morphs, the Wellington tree wētā (*Hemideina crassidens*). As part of efforts to restore native ecosystems on offshore islands in Aotearoa/New Zealand, *H. crassidens* are frequently translocated. Expression of some morphs have been reduced following previous translocations (Watts et al., 2008), indicating a need to improve our knowledge of how the polymorphism in males is generated and maintained. We aimed to answer the question: is the polymorphism primarily driven by genetic factors or by environmental factors? Put another way, is the polymorphism driven by the inheritance of alleles of large effect (e.g., Küpper, Stocks, Risse, Dos Remedios, et al., 2015) or by facultative thresholds (e.g., Rowland & Emlen, 2009)? To address this question, we tested two key predictions of the genetic polymorphism hypothesis: i) that morphs should achieve equal relative fitness, and ii) that each morph should produce offspring in proportion to its equilibrium frequency in the population. If our predictions were supported, we would conclude that the polymorphism is driven by alleles of large effect (i.e., presents like a genetic polymorphism). If our predictions were not supported, we would conclude that our alternative hypothesis was more likely: AMSs in this species are primarily determined by environmental factors, likely via an environmental threshold mechanism.

In order to test these predictions without the assumption of equal fitnesses being violated, we studied a population that expressed the three morphs at stable proportions annually over a recent 7-year survey period, suggestive of an evolutionarily stable population (Kelly & Adams, 2010). We estimated number of offspring sired using a genetic paternity analysis based on one novel microsatellite marker developed for *H. crassidens* as well as four other markers cross-amplified from other *Hemideina* (Hale, Alabergère, & Hale, 2010; King, Hanotte, Burke, & Wallis, 1997). While we acknowledge that fitness is accumulated over a lifetime, we were unable to continuously track individual reproductive success in this species due to time constraints (*H. crassidens* have a ca. 1-year reproductive lifespan; Kelly, 2006c); we therefore sampled near the peak of the mating and oviposition season (March/April; Rufaut & Gibbs, 2003) to attempt to best approximate fitness for each indvidual. Based on 95% confidence intervals for the average frequency of each morph at our site (data from Kelly & Adams, 2010), we predicted that the smallest morph would produce 45% (±3.7%) of offspring, the intermediate would produce 29% (±3.9%), and the largest would produce 26% (±3.4%).

Testing the predictions of the genetic polymorphism hypothesis can help us understand how morphs are determined, but cannot explain what strategies alternative morphs might use to achieve equal fitness. We therefore additionally tested the hypothesis that alternative morphs gain fitness by seeking matings in different environmental niches than conventional morphs ('resource polymorphism'; Smith & Skulason, 1996). In the context of the *H. crassidens* system, we hypothesized that tree cavities with small entrances would prohibit entry of larger morphs and therefore be preferentially occupied by small morphs, providing a space where smaller morphs could monopolize smaller groups of females without competition from larger morphs (Kelly, 2008a). We therefore predicted that larger males would be excluded from cavities with smaller entrances.

The data collected allowed us to test additional hypotheses surrounding AMSs and polygamous mating systems. We examined whether there were fitness benefits of multiple mating in terms of offspring quantity (direct benefits) for both sexes, and in terms of offspring quality (indirect benefits) for females (Evans & Magurran, 2000; Slatyer et al., 2011; Worthington & Kelly, 2016). A positive relationship between number of mates and fecundity has long been predicted for males (Arnold & Duvall, 1994; Bateman, 1948; Jones, 2009) and more recently for females (Arnqvist & Nilsson, 2000; South & Lewis, 2011); we therefore predicted that both males and females would experience direct benefits, producing more offspring when they had more mates.

Females are predicted to additionally accrue indirect benefits through post-copulatory selection (*reviewed in* Slatyer et al., 2011): this might occur via directional selection for high quality males ('good sperm' or 'sexy sperm' hypotheses; Curtsinger, 1991; Yasui, 1997) or via non-directional selection for less related males ('genetic compatibility' hypothesis; Trivers, 1972; Zeh & Zeh, 1997). Based on the hypothesis that there is directional post-copulatory selection for high quality males, we predicted that females with a higher rate of multiple mating would produce higher quality offspring. We further investigated if there was evidence for directional selection by examining whether there was fertilization bias towards any one morph when multiple morphs shared paternity of a brood. Here we predicted that smaller morphs would sire a greater proportion of offspring within broods since they produce a larger ejaculate (Kelly, 2008b). We also predicted that this would translate into a direct benefit, with females laying more eggs when mated by smaller morphs due to a higher likelihood of fertilization (South & Lewis, 2011). Finally, we hypothesized that a fertilization bias towards any particular morph would be associated with indirect benefits, predicting that morphs that sired more offspring within broods would also produce offspring of higher quality.

1.3. Methods

1.3.1. Collection of harems and offspring in the field

A cross-sectional survey of tree cavities was performed at our field site on Maud Island from March 22-April 4, 2017 (details of the study species and field site are given in section 0.4). Cross-sectional studies have been implemented previously to study fitnesses among alternative mating strategies (e.g., Cogliati, Balshine, & Neff, 2014; Shuster & Wade, 1991). We used three different sampling methods: 1) opportunistic gathering of fallen branches from the forest floor; 2) destructive sampling by chain-sawing branches off live trees; and 3) inspection of nest boxes and artificial cavities (initially installed for other projects and now commonly inhabited by *H. crassidens*). To include apparently unsuccessful males, males that were found without harems were also collected and included in our analyses. We also assessed whether cavity entrance size was a limiting factor for males of different morphs (i.e., different head sizes) to gain access to cavities. Before splitting open branches to survey the individuals inside, we took a high resolution photograph of each cavity entrance with a scale (15cm ruler) and then measure the width and area of the entrance using ImageJ (Schindelin, Rueden, Hiner, & Eliceiri, 2015).

For all individuals sampled from cavities, we determined sex, removed their middle left leg and placed it in a 1.5 ml microtube with 80% ethanol for later DNA analysis, and took measurements of pronotum width (a plate-like structure covering the thorax used as a proxy for body size; Kelly, 2011), length of the left and right hind femurs, and head length (from top of head to tip of left mandible; males only). Males were assigned to morph according to the trimorphic distribution identified by Kelly & Adams (2010). Males were then released into the forest while females were held captive for 1-2 weeks to lay eggs. Females were housed in 5L buckets with moist vermiculite as oviposition substrate and a wooden artificial refuge. Females were fed a piece of apple that was replaced every 2-3 days, also acting as a source of water. The isolation of females during this period guaranteed knowledge of maternal identities. To avoid any adverse effects of DNA sampling on female condition during oviposition, we sampled tissue from females at the end of their oviposition period.

We removed females from their buckets after laying 15 or more eggs or 14 days after isolation, whichever occurred first. Females were then preserved by freezing at -20° C for later dissection for any remaining eggs to determine total fecundity. If a female did not lay fertilized eggs during the oviposition period, she was euthanized by decapitation, dissected while alive, and her spermatheca (sperm storage organ) removed to determine her fertilization status. Following Kelly (2008b), we placed spermathecae in 0.5mL of tap water, pierced them with a needle and mixed with a pipette to suspend sperm in the water. 20 μL of this solution was then transferred to a microscope slide and air-dried for later examination to determine the presence or absence of sperm. Eggs oviposited by females were counted and stored in 1.5mL microtubes with a drop of water to avoid desiccation. We transported these eggs to the lab at the Université du Québec à Montréal where they were placed in moist vermiculite and incubated in a Percival 141VL incubator (Percival Scientific Inc., Perry, IA) synchronized to the local temperature in Wellington, New Zealand. When eggs hatched, we measured the nymph's pronotum width and body mass before euthanizing the animal in 95% ethanol. We stored nymphs and eggs that did not hatch in 95% ethanol at room temperature to await genotyping.

1.3.2. Paternity assignment of wild-caught males

We determined male reproductive success by assigning paternity to the offspring produced by the females collected. The number of offspring sired by each male was determined by matching the microsatellite genotype of offspring to father, and this was used as a proxy of fitness for that male. Development of a set of novel microsatellite primers was conducted prior to fieldwork by Dr. D. Coltman's lab (University of Alberta, Edmonton, Canada) using archived tissue samples from adult weta (n = 6) previously collected from Maud Island. Microsatellite-enriched libraries were built and microsatellite sequences isolated following previously established protocols (see details in Glenn & Schable, 2005; Hamilton, Pincus, Di Fiore, & Fleischer, 1999). Briefly, DNA was extracted from each tissue sample using a standard acetate-alcohol precipitation protocol (Sambrook, Fritsch, & Maniatis, 1989) or using DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands). Restriction enzymes RsaI and BstUI (New England Biolabs, Ipswich, MA) were used to create 200-500bp fragments that were enriched for microsatellites, amplified via polymerase chain reaction, and transformed into Escherichia coli DH5α. Colonies were sequenced bi-directionally to identify microsatellite-bearing regions and primers were designed for the flanking regions. Polymorphism of these loci were assessed using an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) and GENEMAPPER 3.5 software (Applied Biosystems).

This labwork resulted in a set of 12 novel primers. To supplement this set, we also tested for cross-amplification of 16 primers previously developed for other species of *Hemideina* (Hale et al., 2010; King et al., 1997). Loci that reliably amplified in > 50% of individuals and which expressed > 2 alleles were retained for use as genetic markers for paternity analysis (n = 5 loci; see Table 1.1 for details). One locus, HR14, was later excluded due to a significant homozygote excess in the sample, suggesting a high incidence of null alleles that would confound assignment (H_o = 0.10; null allele frequency estimate = 0.35).

Table 1.1. Details of 5 microsatellite loci used for paternity assignment (n = 393 individuals genotyped). The combined non-exclusion probability for a second parent using the 4 final loci (HR14 excluded*) was 0.0011.

Locus	**Core sequence**	**H_o (N)**	**H_e**	**No. of alleles**	**Primer sequences**	**Source**
Hma04	[TC]24	0.706 (309)	0.809	13	**F:**CACGAAACTAGACAGAGTTACA **R:**CCAACCTTCAGGTTATACAC	King et al., 1997
HR3	[CT]10	0.877 (390)	0.898	25	**F:**TGACGGTGTGCTTCGATAAG **R:**CACGAGGGCGATAGATGTTT	Hale et al., 2010
HR14*	[GAT]8	0.105 (381)	0.215	3	**F:**TTTTGACTCTGTTCAGAATGACC **R:**TACAGAGCCTGGGGAAGAAA	Hale et al., 2010
HR35	[CTT]7	0.839 (386)	0.920	26	**F:**CAACTGGGGATCAATTCCTG **R:**GGAGGGAAATGGAAGAGTCC	Hale et al., 2010
HC1	[TC]25	0.808 (364)	0.955	39	**F:**GCGGACCAATTTCGAGACTA **R:**AGAAATAATGGGCGTTGGTG	Present study

H_o (N): observed heterozygosity (number of individuals typed)
H_e: expected heterozygosity
**later excluded due to homozygote excess*

Because female insects are known to store sperm for long periods of time (Parker, 1970), it is possible that offspring in our sample were sired by males with whom the focal female was no longer residing (e.g., a previous harem owner or a "sneak" mating). To examine how often offspring could be attributed to the current harem owner versus other males in the population, we considered three scenarios: 1) population-level: paternity matches with all males sampled; 2) branch-level: paternity matches with all males from the same branch as the mother; and 3) harem-level: paternity matches only with those males that were found residing with the harem containing the mother. Paternity was assigned at 95% confidence using CERVUS 2.0 (Marshall, Slate, Kruuk, & Pemberton, 1998).

1.3.3. Statistical analyses

All statistical analyses were performed in R v3.1.3 (R Core Team, 2015) and all statistical tests were deemed significant at $\alpha = 0.05$. For each of our linear models we ran an ANOVA on the model object to extract Wald X^2-values and p-values based on type III sums of squares. We first calculated the harem success of each male, which we defined as the number of females found residing with a male. We occasionally found multiple males residing with the same harem, making assignment of harem ownership to a single male ambiguous; we therefore defined harem success using three methods: i) full success: males were assumed equally likely to be full owners, so all females in a cavity were assigned to each male; ii) shared success: males were assumed to equally share females, so harem success was calculated as the number of females divided by the number of males in a cavity; and iii) large morph dominance: the largest male was assumed to be the harem owner (sensu Kelly, 2006a), so all females were credited to the male with the largest mandibular weaponry. Values of harem success were transformed to relative measures by dividing each individual's harem success by the population mean. To determine whether harem success varied by morph, we used generalized linear models with male morph as a fixed effect and harem success as a response variable. Because the data were overdispersed, with many males in our sample having no harem, we used a negative binomial error distribution.

We compared morph-specific reproductive success by using two estimates of fitness derived from our paternity analysis: the number of offspring sired and the number of females mated. These values were standardized as relative fitness by dividing the values for each male by the population mean. We determined the effect of morph on fitness using generalized linear models with a negative binomial distribution. The proportion of total offspring sired by each morph was then compared to the historic distribution of morphs in the population from Kelly & Adams (2010) using a X^2 goodness-of-fit test. We acknowledged that these estimates could be biased based on which level males were sampled at: for example, if only males that appeared successful (i.e., were found residing with a harem) were considered as potential fathers. We therefore examined the number of offspring in the sample sired by each morph at each level of analysis (population, branch, and harem) using a X^2 test of independence and a post-hoc test using adjusted residuals. The goal of this analysis was to determine if our estimation of a morphs' relative success changed depending on which male competitors were considered to be present.

We next tested hypotheses related to the potential benefits of multiple mating, particularly in females. We first asked whether there were direct fitness benefits of multiple mating in either sex: we used generalized linear models with a Poisson family of errors to examine the number of offspring produced for each sex as a function of number of mates. Because females laid many eggs that could not be genotyped, we ran this model only for the subset of individuals that had offspring assigned a father or that only laid 1 egg, for which we assumed there was only 1 sire (n= 39). For each female we calculated a proxy of body size as the mean of her hind femur measurements (significantly correlated with body weight: Kelly, 2005) and found this to be significantly correlated with fecundity, so included this factor as a fixed effect. To investigate indirect benefits for females, we used a series of models to examine the effect of number of mates on hatching success and proxies of offspring quality (mass, body size, and number of days to hatch; summarized in Table 1.2).

Indirect benefits might arise as a result of directional post-copulatory selection. To explore evidence of directional selection, we used three methods: first, we used the paternity data to determine the morphotypes that contributed to each female's brood and examined whether there were within-brood differences in paternity among morphs. We used a generalized linear mixed model with a binomial distribution, proportion of brood sired as a response variable, morph as a fixed effect, and female ID as a random effect. We calculated proportion of brood sired as the number of offspring sired divided by the total number of offspring that could be assigned a father within each brood. Second, we examined whether the morphotypes each female mated with affected the number of eggs laid using a generalized linear model with a Poisson distribution. Finally, we examined whether a sire's morph influenced proxies of offspring quality: linear mixed models were used with sire morph as a fixed effect, female ID as a random effect, and proportion of eggs hatched (hatching success), offspring mass, pronotum width (log-transformed) or the number of days between egg lay date and hatch date (incubation period) as responding variables. For incubation period, we used a Poisson family of errors and for hatching success we used a binomial family of errors. We additionally used a cox proportional hazard analysis to examine likelihood of hatching earlier given an offspring's father's morph.

1.4. Results

We found weta in 68 of 192 galleries surveyed (n = 7 artificial), resulting in the collection of 188 adults (n=103 females and n=85 males). Based on the head size distribution determined by Kelly & Adams (2010), 61% (n= 51) of males were categorized as 8th instar, 28% (n= 23) as 9th instar, and 11% (n= 9) as 10th instar. Only one male had a head measurement falling within the small overlap in ranges between 8th and 9th instar; this male was assigned 8th instar status because the measurement was closer to the 8th instar mean than the 9th instar mean. Multiple males were found residing in the same cavity on nine occasions; in five instances males were of the same morph (8th with 8th: n = 4; 9th with 9th: n = 1) and in four instances 8th instar males were with a larger morph (8th with 10th: n = 3; 8th with 9th: n = 1).

1.4.1. Harem success

As predicted, larger morphs tended to hold larger harems: full success was greater for 10th instars compared to 8th instars (GLM: $\beta = 1.19$, SE = 0.42, $p = 0.0047$), and larger morphs had larger harems compared to any smaller morph in the shared success model (GLM 8th-9th, 8th-10th, 9th-10th: $\beta = 0.737$, SE = 0.35, $p = 0.033$; $\beta = 1.75$, SE = 0.38, $p = 3.41 \times 10^{-6}$; $\beta = 1.015$, SE = 0.39, $p = 0.0094$) and the large male dominance model (GLM 8th-9th, 8th-10th, 9th-10th: $\beta = 0.876$, SE = 0.38, $p = 0.021$; $\beta = 1.83$, SE = 0.40, $p = 5.52 \times 10^{-6}$; $\beta = 0.949$, SE = 0.403, $p = 0.018$). We predicted that this pattern might be explained by larger morphs being excluded from tree cavities with smaller entrances. This prediction was not supported, as even the smallest cavity entrance could accommodate the largest male head width (Appendix A).

1.4.2. Paternity assignment

Of the 103 females collected, 45 females laid eggs in captivity (n = 393 eggs; mean ±SE per female: 8.73±1.59) and 22 females had eggs hatch, totaling 255 offspring (mean±SE per female: 5.67±1.29; n = 138 unhatched). Of the females that did not lay eggs, 82% had mature eggs in their ovaries and sperm stored in their spermatheca at dissection. We attempted to genotype all nymphs and eggs; however, we were successful in extracting DNA from only n=30 eggs. Due to the high diversity of our microsatellites (n = 4 loci; mean number of alleles per locus±SE = 25.8±5.31; mean polymorphic information content±SE = 0.886±0.0345), the probability of failing to exclude an incorrect parent was not much changed when any one locus was removed (combined non-exclusion probability for second parent±SE = 0.006737±0.001837 across simulations excluding each locus one at a time). We therefore included individuals in the paternity analysis if they were successfully typed at 3 or more loci. In our first scenario where all males sampled could be fathers, 275 offspring were evaluated against 83 candidate fathers, resulting in 184 offspring being matched to 51 fathers (67% assignment). In our second scenario where only males from the same branch as the mother were considered as candidate fathers, 92 offspring were matched with 95% confidence to 17 out of 44 fathers (38% assignment). Finally, our third scenario considered only males within the same harem as the mother as candidate fathers, resulting in 50 offspring being assigned with 95% confidence to 6 out of 20 fathers (23% assignment; see Appendix C for details of differences in assignment).

1.4.3. Male reproductive success and relative fitness

To assess reproductive success among morphs, we used the population-level paternity analysis results which considered all males sampled as potential fathers. As predicted according to the genetic polymorphism hypothesis, morphs appeared to have equal relative success: male morph did not significantly influence the relative number of offspring sired (GLM: $X^2 = 3.66$, $df = 2$, $p = 0.16$; Figure 1.1). Our second measure of relative fitness, the total number of females each male mated, was also not significantly affected by morph (GLM: $X^2 = 1.60$, $df = 2$, $p = 0.45$; Figure 1.1). We then tested a second prediction of the genetic polymorphism hypothesis: that each morph should sire offspring in proportion to its equilibrium frequency in the population. In our sample 8th instar males fathered 51% of the offspring, 9th instars 28%, and 10th instars 20%. We used a X^2 goodness-of-fit test to compare this distribution to the historic frequency distribution of morphs obtained from Kelly & Adams (2010): 45% 8^{th} instar, 29% 9^{th} instar, and 26% 10^{th} instar. Our results confirm the prediction of the genetic polymorphism hypothesis, as we found that the proportion of offspring sired among morphs was not equal and instead mirrored the historic frequency of morphs in the population ($X^2 = 3.57$, $df = 2$, $n = 184$, $p = 0.17$).

We used a second X^2 test of independence to examine if the proportion of offspring assigned to each morph varied based on the level of analysis (population-level, branch-level, harem-level), which was significant ($X^2 = 52.6$, $df = 4$, $n = 326$, $p = 1.02 \times 10^{-10}$). A post-hoc test using adjusted residuals revealed that assignment was lower for 8th instars in the harem and branch-level and for 10th instars in the population-level analysis, while assignment was higher for 10th instars at the harem level, 9th instars at the branch level, and 8th instars at the population level (Appendix B). To briefly summarize the range of difference observed, we note that the majority of offspring (60%) were assigned to 10th instars in the harem-level analysis while this number dropped to 20% in the population-level analysis. Finally, we investigated correlations between harem success derived from field observations and estimates of reproductive success derived from the paternity analysis. We found all measures of harem success were positively correlated with the number of offspring sired (full: $r = 0.22, p = 0.046$, n = 82; shared: $r = 0.40, p = 0.0002$, n = 82; large male dominance: $r = 0.37, p = 0.002$, n = 68) but not with the number of females mated (full: $r = 0.05, p = 0.64$, n = 82; shared: $r = 0.21, p = 0.056$, n = 82; large male dominance: $r = 0.18, p = 0.15$, n = 68).

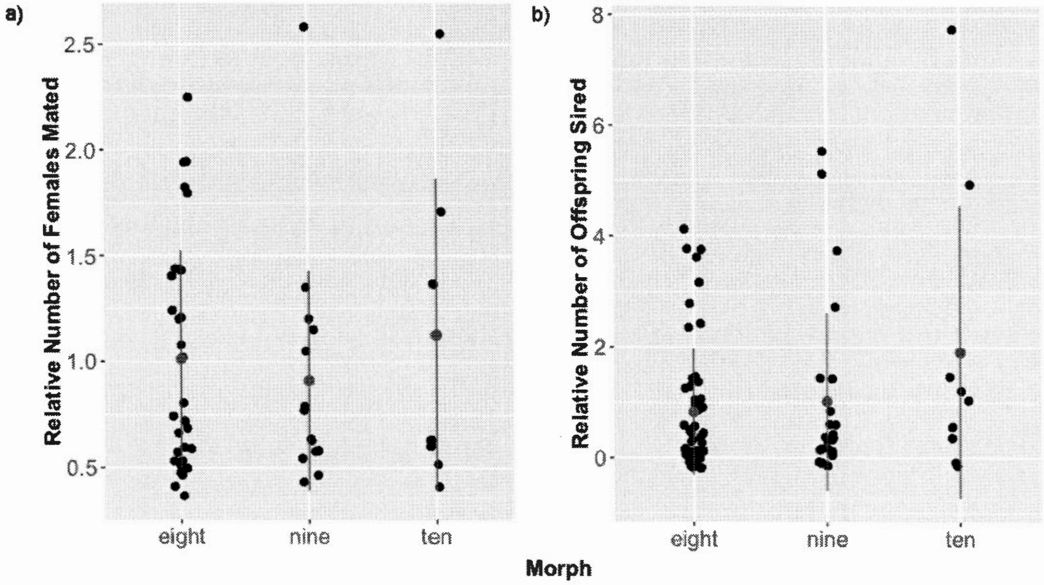

Figure 1.1. Relative reproductive success for each male morph ($n_{eight} = 51$; $n_{nine} = 23$; $n_{ten} = 9$) represented as **a)** the number of females each male mated with divided by the population mean; **b)** the number of offspring assigned to each male divided by the population mean. Morph did not significantly explain either value of relative fitness (GLM females mated, offspring sired: $X^2 = 1.60$, $df = 2$, $p = 0.45$; $X^2 = 3.66$, $df = 2$, $p = 0.16$).

1.4.4. Multiple mating and benefits of polyandry

As predicted, both sexes mated multiply with females mating 4.00±0.55 (n = 21; max = 9) males and males mating 1.65±0.13 (n = 51; max = 4) females. Also in line with our predictions, there was a positive relationship between mating success and reproductive success in males (Figure 1.2a) and females (Figure 1.2b). For females, the true number of males mated is likely underestimated by this study because 105 offspring could not be assigned a father from our sample, indicating females used sperm from male(s) unknown to us.

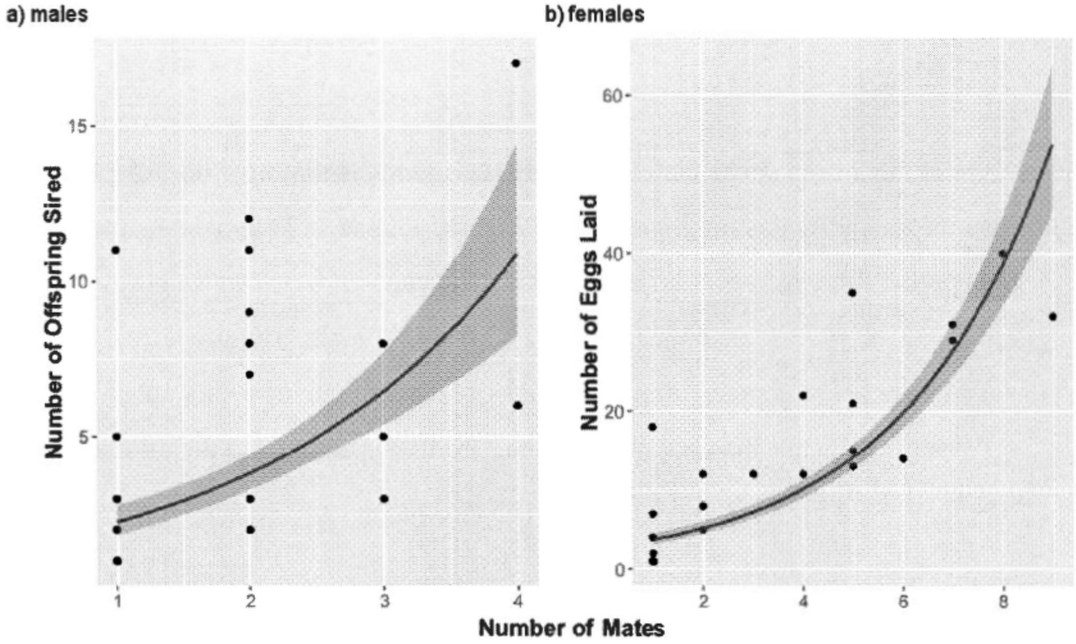

Figure 1.2. Reproductive success for each sex as a function of number of mates. **a)** Males (n = 83): reproductive success represented as the number of offspring sired (GLM: $\beta = 0.757$, SE = 0.05, $p < 0.0001$); **b)** Females (n = 39): reproductive success represented as the number of eggs laid (GLM: $\beta = 0.336$, SE = 0.02, $p < 0.0001$). The number of mates for each sex and the number of offspring produced by each male were determined using a genetic paternity analysis implemented in CERVUS 2.0.

Of the 44 females collected, 4 died in captivity and were excluded from the analyses. Because female fecundity was significantly correlated with female body size ($r = 0.30$, $p = 0.044$, n = 45), we included female body size as a fixed effect in our fecundity models (Table 1.2). As predicted, the number of sires per brood had a significant effect on fecundity (GLM: $\beta = 0.336$, SE = 0.02, $p < 0.0001$; Figure 1.2b). The types of morphs a female mated also had a significant effect on her fecundity (GLM: $X^2 = 100.57$, $df = 3$, $p < 0.0001$; Figure 1.3). Female body size was no longer significant when included as a covariate ($p > 0.05$), indicating the other variables were more important in explaining fecundity (Table 1.2). As predicted by the 'good sperm' and 'sexy sperm' hypotheses, there was an indirect benefit of multiple mating in females: hatching success was greater when more sires contributed to a brood (GLMM: $\beta = 0.432$, SE = 0.12, $p = 1.68 \times 10^{-4}$). There was no relationship between the number of sires and female body size, and we detected no other indirect benefits of multiple mating in terms of offspring mass (LMM: $X^2 = 0.407$, $df = 1$, $p = 0.52$), offspring pronotum size (LMM: $X^2 = 1.55$, $df = 1$, $p = 0.21$), or the number of days eggs incubated for (GLMM: $X^2 = 2.64 \times 10^{-1}$, $df = 1$, $p = 0.61$) (Table 1.2).

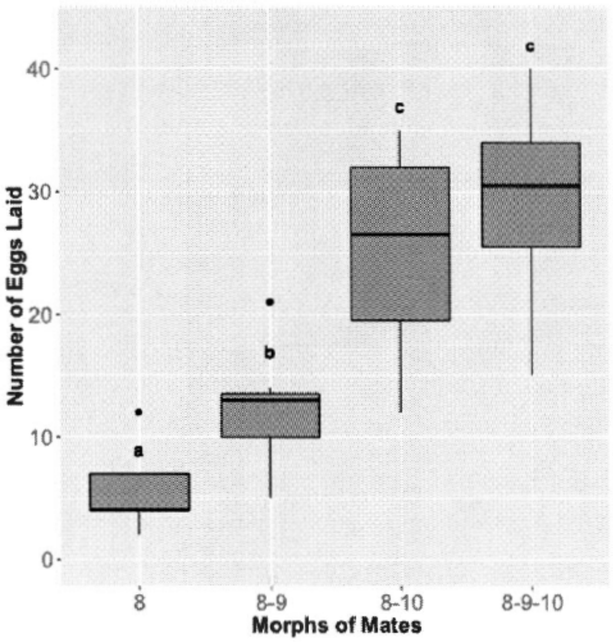

Figure 1.3. Female reproductive success as a function of the morphs of males she mated, determined using a genetic paternity analysis implemented in CERVUS 2.0 ($n_8 = 5$, $n_{8\text{-}9} = 7$, $n_{8\text{-}10} = 4$, $n_{8\text{-}9\text{-}10} = 4$). Data were analyzed using a generalized linear model (GLM: $X^2 = 100.57$, $df = 3$, $p < 0.0001$) and post-hoc Tukey test ($p < 0.05$ for all pair-wise comparisons). No females in the sample mated with only a 9th instar or with the combination of a 9th and 10th instar; only 1 female mated with a 10th instar only, and was thus excluded from analysis.

Using the mates identified by the paternity analysis for females that had genotypable offspring ($n = 21$), we found that females mated with a variety of morphs (Figure 1.3). We analyzed the relationship between the morph(s) mated and number of offspring using a generalized linear model and post-hoc Tukey test (Table 1.2). The results showed that mating with an 8th instar only was associated with a significantly lower number of eggs laid ($p < 0.05$ for all pairwise comparisons). Females that mated with 8th and 10th instars or with all three morphs also had greater fecundity than females that mated with 8th and 9th instars only (Tukey 8-9 versus 8-10, 8-9-10 versus 8-9: $\beta = -0.704$, SE = 0.15, $p = 6.89 \times 10^{-6}$; $\beta = 0.777$, SE = 0.16, $p = 6.89 \times 10^{-6}$).

Table 1.2. Summary of models used to analyze effects of female body size and rate of polyandry on reproductive success and proxies of offspring quality. Generalized linear models were used to model fecundity and female body size; generalized linear mixed models with female ID as a random effect were used to model the proportion of eggs hatched and incubation period; and linear mixed models with female ID as a random effect were used to model offspring mass and offspring pronotum width. A Poisson family of errors was used for response variables that were count data and a binomial family of errors for the proportion of eggs hatched.

Fixed effect(s)	**Response variable**	**Test statistic**	**p-value**
Number of sires*	Fecundity (number of eggs laid)	$z = 16.113$	$p < 0.0001$
Female body size		$z = -0.235$	$p = 0.814$
Types of morphs mated*	Fecundity (number of eggs laid)		
8th		$z = -0.325$	$p = 0.745$
8th and 10th		$z = 6.998$	$p = 2.59 \times 10^{-12}$
8th and 9th		$z = 3.623$	$p = 2.91 \times 10^{-4}$
8th, 9th and 10th		$z = 7.389$	$p = 1.48 \times 10^{-13}$
Female body size		$z = 1.003$	$p = 0.316$
Female body size	Number of sires	$t = 1.336$	$p = 0.190$
Number of sires*	Proportion of eggs hatched	$z = 3.763$	$p = 1.68 \times 10^{-4}$
Number of sires	Incubation period	$z = 0.500$	$p = 0.607$
Number of sires	Offspring mass	$t = 0.608$	-
Number of sires	Offspring pronotum width	$t = 1.198$	-

* = significant

Finally, we investigated evidence for directional post-copulatory selection towards any particular male morphotype. Our binomial GLMM confirmed our prediction that smaller morphs should sire a greater proportion of offspring within broods: the proportion of the brood sired was significantly skewed towards 8th and 9th instars compared to 10th instars (GLMM 10^{th}-8^{th}, 10^{th}-9^{th}: $\beta = -1.170$, SE = 0.28, $p = 2.23 \times 10^{-5}$; $\beta = -0.822$, SE = 0.32, $p = 0.0095$; Figure 1.4). In terms of effects on offspring quality, we found no effect of sire morph on hatching success (GLMM: $X^2 = 2.33$, *df* = 2, $p = 0.31$), offspring mass (LMM: $X^2 = 2.16$, $df = 2$, $p = 0.34$) or body size at hatch (LMM: $X^2 = 2.19$, $df = 2$, $p = 0.34$), but did find a significant effect of the sire being a 9^{th} instar on offspring incubation period (Cox: $\beta = 0.731$, SE = 0.18, $p = 0.0047$). The cox proportional hazard analysis showed that offspring with 9^{th} instar fathers hatched significantly earlier than offspring sired by 8^{th} or 10^{th} instar males (log-rank p value = 8.46×10^{-5}; Figure 1.5).

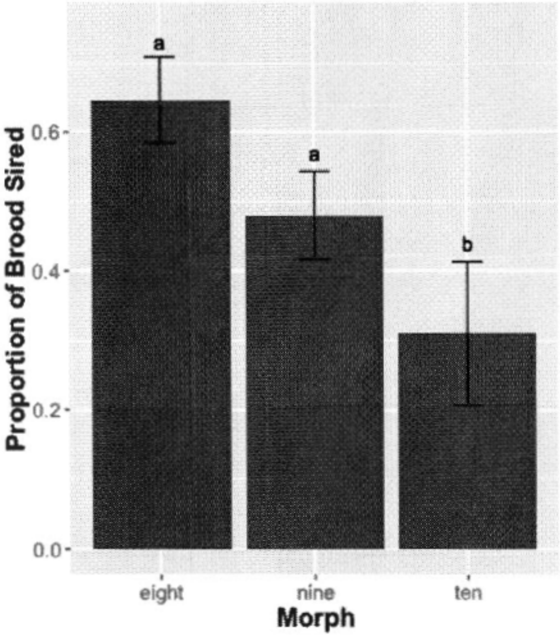

Figure 1.4. Mean within-brood paternity by morph ($n_{eight} = 20$, $n_{nine} = 11$, $n_{ten} = 9$), calculated as the number of offspring sired divided by the total number of offspring
]

assigned a father. A binomial generalized linear mixed model showed that 8th and 9th instars had greater within-brood paternity than 10th instars (GLMM 10^{th}-8^{th}, 10^{th}-9^{th}: β = -1.17, SE = 0.28, $p = 2.23 \times 10^{-5}$; β = -0.822, SE = 0.32, p = 0.0095). Bars indicate standard error.

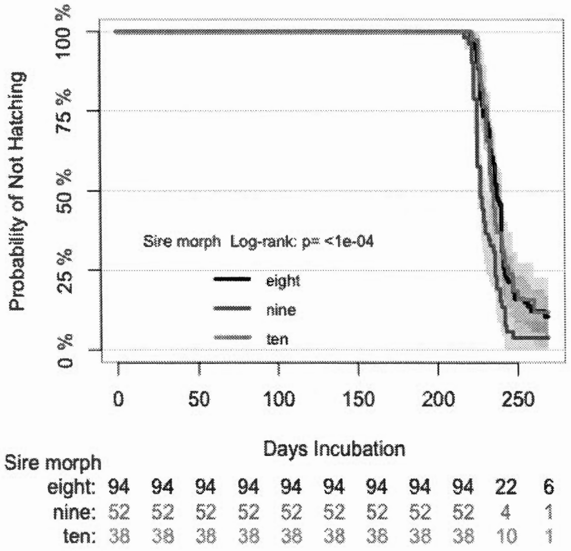

Figure 1.5. Cox proportional hazard analysis curves showing egg hatch rate for offspring as a function of the sire's morph. The offspring of 9th instar males hatched significantly earlier than offspring sired by 8th or 10th instar males (log-rank p value = 8.46×10^{-5}; hazard ratio = 2.08).

1.5. Discussion

1.5.1. Morph-specific fitness and the genetic polymorphism hypothesis

Our results confirm two key predictions of the genetic polymorphism hypothesis: first, that morphs should have equal relative fitness, and second, that they should produce offspring in proportion with their equilibrium frequency in the population. We found no significant difference in the relative number of offspring sired based on the father's morph (Figure 1.1), and the proportion of total offspring attributable to each morph did not depart significantly from that morph's frequency in the population. Taken together, these results show that the trimorphism expressed by male *H. crassidens* presents as a genetic polymorphism characterized by equal relative fitnesses among morphs; put differently, our results show that this trimorphism is mainly driven by alleles of large effect and not environmental factors. This study represents one of only a few successful attempts to quantify directly the relative fitnesses of male AMSs, and the first strong evidence for a genetically polymorphic AMS in an insect. Evidence for genetic polymorphisms has been demonstrated previously through controlled breeding experiments (Lank et al., 1995; Zimmerer & Kallman, 1989) and using indirect measures of fitness such as mating success (Shuster & Wade, 1991) or assumed paternity based on monopolization of females (Sinervo & Lively, 1996); however, reproductive success among male morphs has rarely been quantified in a wild population using a direct measure of fitness such as genetically confirmed paternity (e.g., Bleay, Comendant, & Sinervo, 2007; Neff, 2001).

A key result of our study is that no morph experienced a significant fitness disadvantage, which is often predicted for condition-dependent polymorphisms because individuals are assumed to adopt an alternative strategy based on being in poor condition relative to other male competitors ('best of a bad job'; Dawkins, 1980). In our case, alternative strategies are not associated with lower fitness and thus are unlikely to reflect ‘poor condition’, suggesting that a different mechanism underlies the determination of morphs in this species. Another study showed that all three morphs of *H. crassidens* develop in captivity even when individual condition and environmental cues are held constant (Lagueux-Beloin & Kelly, in prep.), indicating that these factors do not determine morph as predicted by the condition-dependent hypothesis. This finding, in combination with our own, suggests that the polymorphism is determined more strongly by genetic factors than by environmental factors. To provide conclusive evidence, future research should strive to clarify the genetic basis for morph determination in *H. crassidens* by means of a controlled breeding experiment.

The prediction of equal fitnesses among AMSs has previously been tested and confirmed in only a few other systems: the marine isopod *Paracerceis sculpta* (Shuster & Wade, 1991), the swordtail fish *Xiphophorus nigrensis* (Ryan, Pease, & Morris, 1992), and the pumpkinseed sunfish *Lepomis gibbosus* (Rios-Cardenas & Webster, 2008). Studies comparing fitness among morphs have helped to not only confirm the prevalence of genetic polymorphisms, but to reveal the potential presence of conditional polymorphisms: a study on the plainfin midshipman *Porichthys notatus* (Cogliati et al., 2014) and another on the bluegill sunfish *Lepomis macrochirus* (Neff & Lister, 2007) showed unequal fitnesses among morphs. It is therefore increasingly clear that the evolutionary mechanisms underpinning AMSs vary by species and that both genetic polymorphism and conditional polymorphism can be valid explanations (Neff & Svensson, 2013). A recent modeling study has embraced this idea and shows that differences in environmental and social conditions can influence whether species evolve strategies based on genetic polymorphism or condition-dependence (Engqvist & Taborsky, 2016). Our study aligns with the predictions of this model, as *H. crassidens* exhibits determinate growth and strong competitive selection on body size, both of which were predicted to favour genetically determined AMSs over condition-dependent ones (Engqvist & Taborsky, 2016).

AMSs determined by genetic polymorphism are predicted to be maintained by negative frequency-dependent selection (Shuster & Wade, 2003; Sinervo & Lively, 1996). Under negative frequency-dependent selection morphs fluctuate in frequency depending on their fitness relative to other morphs, as is predicted by the 'rock-paper-scissors' model based on a genetically determined male trimorphism in side-blotched lizards (Barreto, Marquitti, & de Aguiar, 2017; Sinervo & Lively, 1996). However, the frequency of Wellington tree weta morphs on Maud Island are stable over time (Kelly & Adams, 2010); given that the male morphs of *H. crassidens* appear to be determined by a similar mechanism, why would we not observe fluctuations in our population as well? Maynard Smith (1982) predicted that at a certain frequency of each strategy, an evolutionarily stable state exists where their fitness functions intersect (i.e., the average fitness of each phenotype is equal) (*reviewed in* Gross, 1996). Viewed through this framework, our result that the morphs all appear to have equal fitness may help to explain why morphs do not fluctuate over time: this population appears to be at an evolutionarily stable state characterized by stable frequencies of each strategy at which they achieve equal fitness. To confirm that negative frequency-dependence is the mechanism driving this apparent equilibrium, future work should strive to demonstrate that morph fitnesses change when morph frequencies are changed.

Interestingly, a different study on *H. crassidens* is suggestive of conditional polymorphism: when transferred to a new site, individuals from a donor population containing only 9th and 10th instar males apparently produced progeny including 8th instar morphs, a phenomenon that was attributed to environmental conditions at the release site (Watts et al., 2008). While these findings contradict our results, we are not aware of any thorough study on the size distribution of morphs present at either the source or recipient site in the case described in Watts et al. (2008), making comparison with the present study difficult. A possible explanation for the differences between our findings could be that the size range of the polymorphism varies by environment, which could prevent morphs from being determined and compared using a universal scale. Polymorphisms often vary geographically in their expression, which may occur due to differences in local selection pressures (Mclean & Stuart-Fox, 2014). More detailed research conducted alongside future translocations could aid in our understanding of potential size range variation in the polymorphism depending on differences in environmental conditions between sites.

Two main challenges limited our ability to assess male reproductive success: first, thoroughly sampling all candidate fathers in a large, open population; and second, successfully collecting and genotyping all offspring. Many offspring could not be assigned a father and many females did not produce offspring despite having mature eggs and sperm, indicating that sampling was incomplete at both these levels. These sampling issues may have resulted in inaccurate fitness estimates if males and offspring were excluded from the sample with bias towards particular morph(s), which could occur if our pool of candidate fathers was not representative of the population. Our sample contained ~15% more 8th instars and ~15% fewer 10th instars than the mean proportions previously estimated for this population (Kelly & Adams, 2010). Despite these differences, we still did not detect any significant differences in individual fitness across morphs; however, this may have biased overall assignment of offspring towards 8th instars, resulting in an overestimate of their contribution at the population level. The context of male competitors present also appears to be an important factor, as the morph with the highest relative success varied depending on whether candidate fathers were considered within a harem, within a branch, or as any male sampled (Appendix B).

Due to the time constraints of our study and the relatively long lifespan of *H. crassidens* (from eclosion, individuals take ~18 months to become sexually mature), along with the fact that morph can only be visually confirmed at sexual maturity, we were unable to determine the morphs of male offpsring in our study. The question of whether offspring express the same morph as their father is therefore unanswered and should be prioritized in future studies. We submit two recommendations for future research: i) repeating the study to see if year-to-year differences in sampling affect the results; and ii) using a controlled breeding experiment to confirm the genetic mechanism underlying the polymorphism and determine its heritability, preferably in different environments to test for gene-by-environment interactions (Neff & Svensson, 2013). The question of how environmental factors may modulate expression of this polymorphism is still largely open for investigation, and it would be particularly informative to determine how diet/nutrition at early nymphal stages might influence the trajectory of morph determination. Because instar number is so tightly linked to morph expression in *H. crassidens*, this could also represent an excellent opportunity to investigate whether time to sexual maturity is also influenced by environmental cues.

1.5.2. Harem success among morphs

While reproductive success was equal among morphs, harem success was not: as has been demonstrated in other studies on this species, there was a strong positive relationship between morph and harem success, with larger morphs tending to hold larger harems (Kelly, 2005, 2006d). This observation may provide a hint as to what behaviours characterize each mating strategy in our species: as has been proposed in other works, smaller morphs might target smaller harems or acquire matings outside of harems via a 'wandering' strategy (Kelly, 2006b; Spencer, 1995). Alternatively, larger morphs might be unable to access smaller harems because they occur in smaller tree cavities with entrance holes that are too small for large morphs to enter (Kelly, 2008a). We attempted to address this final explanation in the present study, predicting that cavities with small entrances would exclude larger morphs. Our results do not support this prediction, as even the smallest cavity entrances accommodated all sizes of male (Appendix A). However, the forest on Maud Island has been regenerating for >45 years (Department Of Conservation, n.d.), which is not the case for many other island sanctuaries in New Zealand (i.e., Matiu/Somes Island, Mana Island; Miskelly, 1998; Nester, Sawyer, & Sutton, 2000). In younger forests cavities might be less developed and have smaller entrances, which could reduce the ability of 10th instar males to use these cavities. By expanding research to other sites with younger forest, we could address whether cavity entrance size plays a role in determining the harem success of alternative strategists.

Because harem success is often taken to represent mating success (e.g., Shuster & Wade, 1991; Sinervo & Lively, 1996) we expected that there would be a positive relationship between these two measures, but found no significant correlation between any measure of harem success and mating success (number of females mated). However, it is unlikely that selection would maintain an energetically costly mating strategy such as defending a harem if it resulted in consistently low mating success (Shuster & Wade, 2003), and many other empirical studies have shown a positive relationship between harem size and mating success (e.g., Apollonio, Festa-Bianchet, & Mari, 1989; Balmford, Albon, & Blakeman, 1992; Heckel & Von Helversen, 2003).

There are several potential reasons why our measures of harem success and mating success might appear unrelated, despite a relationship existing: for one, a male's current harem size is not necessarily representative of his previous harems. A male that we found residing alone or with a small harem might have had high harem success during previous associations with the females in our sample, leading to our detection of high mating success in the genetic analyses. This issue is compounded by the fact that female *H. crassidens* store sperm (Kelly, 2008d), which potentially enables a female to be inseminated in one harem context and then to fertilize eggs using this sperm after residing in one or more other harems. Secondly, the timing of our sampling may have occurred before males succeeded in mating the female(s) in his harem. Male *H. crassidens* will remain in the same cavity for several nights, and this time period is positively related to harem size, suggesting males stay in a cavity until they have mated all the females in their harem (Kelly, 2006c). We sampled cavities with no knowledge of how long individuals had been residing there previously; it is therefore likely that we separated males from their harems before they had mated all the females, and this may have caused them to appear to have low mating success within their current harems. Our findings suggest that researchers should be wary of assuming that field observations of a male's current associations with females are representative of his true fertilization success.

1.5.3. Rates of multiple mating and fitness benefits

The high incidence of polygamy in both sexes detected by this study was expected, as polyandry is increasingly detected across animal taxa (Taylor, Price, & Wedell, 2014) and Kelly (2008b) previously detected sperm storage in females in this species. We hypothesized that multiple mating would result in greater reproductive success in both sexes: for males because the time and energy invested in gametes and offspring are relatively low, so fitness may be maximized by mating frequently (Bateman, 1948; Lehtonen et al., 2016; Trivers, 1972); and for females because mating multiply can help to guarantee fertilization and ensure access to high quality genes (Hasson & Stone, 2009; Slatyer et al., 2011). Our results support our hypothesis: we showed that there is a strong positive effect of the number of mates on the number of offspring sired for males (Figure 1.2a) and the number of eggs produced for females (Figure 1.2b).

While the idea that males should accrue fitness benefits from multiple mating has long been accepted, the fitness benefits of multiple mating in females (polyandry) are not immediately clear and more strongly debated. For this reason, we will focus on discussing the fitness benefits of polyandry, which are divided into direct benefits (increased number of offspring) and indirect benefits (increased mean fitness of offspring) (Slatyer et al., 2011). Our study detected direct benefits because females produced more eggs when they mated multiply. Some possible explanations for the direct benefits that we observed in our study could be that male ejaculates contain stimulants or nutrients for egg production (Arnqvist & Nilsson, 2000; Stanley-Samuelsson & Peloquin, 1986) or that viable sperm is a limiting resource (i.e., females need to replenish ejaculates to ensure fertilization) (Hasson & Stone, 2009; Worthington & Kelly, 2016a). Multiple mating might also benefit a female because acquiescing to mating attempts might reduce physical damage a female incurs due to male harassment ('convenience polyandry'; Slatyer et al., 2011); this could contribute to explaining polyandry in *H. crassidens* as males often physically injure females during mating attempts (Kelly, 2006d; S. E. Nason personal observation).

We also hypothesized that females would accrue direct benefits from mating with smaller morphs because they produce a larger ejaculate and more sperms per ejaculate (Kelly, 2008b), which could increase the likelihood of fertilization. Here, we predicted that females would produce a greater number of offspring when they had mated with a smaller morph. Our results do not support this prediction, as females that mated with 9th and 10th instar males laid more eggs than those that mated only with 8th instars (Figure 1.3). However, because no female mated with only 9th instars and only one mated with only a 10th instar, it is not possible to distinguish our results from what might be the effect of mating multiple morphs. Such an effect might arise if females employ post-copulatory 'bet-hedging': if the genetic quality of her potential mates or future environmental conditions are unpredictable, a female might 'hedge her bets' by mating with males of a variety of genotypes (i.e., morphs) (Yasui, 1998). Under this hypothesis, females that have only succeeded in mating one morph might lay fewer or no eggs until they have procured sperm representing more genotypes. Support for the bet-hedging hypothesis has recently been demonstrated in the sea urchin *Heliocidaris erythrogramma armigera* (Garcia-Gonzalez et al., 2015) and could be an intriguing avenue for future research to help explain the patterns we observed in this study.

Another possible benefit of polyandry is an indirect benefit to the female's offspring, as multiple mating could lead to more genetically diverse offspring, higher quality offspring, or provide the option for a female to exert post-copulatory choice (i.e., only use sperm from males that would elevate her offspring's fitness) (Slatyer et al., 2011; Yasui, 1998). We hypothesized that females would experience indirect benefits of multiple mating, with the prediction that females with more mates would produce higher quality offspring. Our prediction was partially supported because there was a positive effect of multiple mating on hatching success for females, indicating that a greater rate of multiple mating translated to more viable offspring. However, there was no effect of multiple mating on the other proxies of offspring quality (body size, time to hatch) that we used. Our study generally corroborates the findings of two meta-analyses on the benefits of polyandry (Arnqvist & Nilsson, 2000; Slatyer et al., 2011) that detected the presence of direct, but not indirect, benefits. However, our measures of offspring quality were coarse and it is presently unknown if they correlate with fitness in terms of future survival and reproductive success. Future studies should strive for a more comprehensive estimation of offspring fitness.

We further investigated the potential for indirect benefits by assessing whether there was evidence for directional post-copulatory selection towards any particular morph. Based on the 'sexy sperm' hypothesis (Curtsinger, 1991), we predicted that smaller morphs should sire a greater proportion of offspring within broods because they produce a larger ejaculate (Kelly, 2008b). Our results support this prediction, showing that within-brood paternity was significantly greater for 8th and 9th instars (Figure 1.4). This pattern might arise because the ejaculates of 8th and 9th instars are competitively superior ('sexy sperm' hypothesis; Curtsinger, 1991), but could equally be observed due to cryptic female choice favouring the sperm of these morphs ('good sperm' hypothesis; Yasui, 1997). Anecdotal evidence suggests females were choosy in using sperm in our study as the majority of females that we collected did not lay eggs despite having mature eggs and sperm at their disposal. Mating order might also contribute to explaining morph-specific biases in paternity; many insect taxa exhibit 'last-male precedence', where mating second results in greater fertilization success and paternity (Simmons & Siva-Jothy, 1998). The mechanism underlying the observed differences in within-brood paternity among morphs is therefore presently unclear and should be investigated in future studies. If such studies are able to confirm the 'sexy sperm' hypothesis, this could provide a mechanistic explanation for how 8th and 9th instar morphs are able to achieve equal fitness to 10th instars.

Finally, we predicted that any morph-specific bias in fertilization within broods would be associated with indirect benefits as well. We found partial support for this prediction as we only detected indirect benefits as a result of mating with 9th instars, even though within-brood paternity was biased towards both 8th and 9th instars. The offspring of 9th instar males hatched significantly earlier than the offspring of other morphs (Figure 1.5), indicating that females might increase the fitness of their offspring by preferentially using the sperm of 9th instars. This result supports the 'good sperm' hypothesis that females should bias paternity towards males of high genetic quality (Yasui, 1997) and helps us to start disentangling the relative influence of sperm competition versus cryptic female choice in determining morph-specific fertilization bias. However, we emphasize once more that our measures of offspring quality do not necessarily correlate with fitness and that this should be investigated to confirm whether this result represents a true indirect fitness benefit.

1.5.4. Implications of genetic findings

We gained a few novel insights from the genotyping and genetic analyses: firstly, we observed a high number of alleles and high heterozygosity at each locus (Table 1.1). No assessment of neutral genetic variation had previously been carried out for this population, nor any other population of *H. crassidens* to our knowledge. Given that our population is a historic one and not the result of a translocation, as many other island weta populations are (Watts et al., 2008), the level of genetic diversity observed in this study could serve as a reference state to compare translocated populations to. Furthermore, these results suggest that Maud Island could be a good choice of source population for future translocations in terms of maximizing genetic diversity, which can aid in reducing inbreeding (Fernández, Toro, & Caballero, 2004) and increasing translocation success (Forsman, 2014).

Secondly, we observed that the relative success of each morph was dependent on the level of paternity analysis used (i.e., the set of candidate fathers considered): when only males residing with a harem were considered as potential fathers, the paternity analysis assigned the majority of offspring (60%) to 10th instars. This number dropped to 20% when all males sampled were considered as potential fathers, indicating that males external to the female's current harem group contribute considerably to her offspring. Such males could be previous harem owners or males using alternative strategies. The morph assigned the most offspring was different at each level: at the harem level, 10th instars contributed disproportionately; at the branch level, 9th instars contributed disproportionately; and at the population level, 8th instars contributed disproportionately (Appendix B). These data are by no means conclusive, but could be suggestive of strategies wherein 10th instars may maximize their fitness within harems, while 8th and 9th instars may successfully 'sneak' matings by camping out in cavities near harems or by employing a 'wandering' strategy (Kelly, 2006b; Spencer, 1995). Regardless, it is clear that the reproductive success of 8th instars was underestimated when candidate fathers were restricted to only those males found within harems or their associated tree branch. This suggests that 8th instars gain reproductive success by means other than harem ownership and reinforces the importance of considering apparently unsuccessful males when assessing fitness (Shuster & Wade, 2003).

1.5.5. Speciation and conservation implications

In a conservation context, the intraspecific variation represented by genetic polymorphisms is important to consider because increased intraspecific diversity results in improved establishment of organisms when they are moved and released for conservation purposes ('conservation translocations'; Forsman, 2014; IUCN & SSC, 2013; Love Stowell, Pinzone, & Martin, 2017; Weeks et al., 2011). Furthermore, polymorphic species have been identified as a source of future biodiversity through the process of 'morphic speciation' (e.g., Corl et al., 2010; Hugall & Stuart-Fox, 2012b): new species can arise from polymorphic ones due to assortative mating, reduced morph hybrid fitness, or different selective pressures disfavouring certain morph(s) in a new environment, resulting in morph loss and subsequent population differentiation (West-Eberhard, 1986). This final mechanism might explain why there is widespread geographic variation in polymorphism (Mclean & Stuart-Fox, 2014).

Despite the speciation potential of polymorphisms and the demonstrated impact of intraspecific genetic diversity on population viability and establishment (Forsman, 2014; Spielman et al., 2004), genetic factors are often absent in conservation planning (Brichieri-Colombi & Moehrenschlager, 2016; Pierson et al., 2016). Insects in particular are under-studied with respect to conservation (Cardoso et al., 2011; Dunn, 2005; Kim, 1993; Seddon, Soorae, & Launay, 2005). The results of the present study can help to close these gaps and aid future translocation efforts for *H. crassidens.* We provide strong evidence that the polymorphism is genetically based, and is therefore unlikely to be fully expressed at a new site unless all three morphs are transferred. Considering that previous translocations have resulted in morph reduction/loss (Watts et al., 2008), we conservatively recommend that conservation managers take one of two approaches to translocating *H. crassidens* in the future: i) select individuals for translocation such that male morphs are obtained at approximately their frequency in the source population; or ii) transfer at least 20-50 breeding individuals to capture > 95% of the genetic variation from the source population (genetic capture; Weeks et al., 2011). If possible, we would recommend increasing this number to 60 individuals to account for post-translocation mortality and to guard against the loss of rare alleles (Tracy, Wallis, Efford, & Jamieson, 2011). Either of these approaches should ensure that the full range of variation in male genotypes is captured and will be available for selection to act upon at the receiving site.

1.6. Conclusion

We detected no significant fitness advantage for any male morph of *H. crassidens* (Figure 1.1), supporting the hypothesis that the morphs are relatively equally fit and determined by genetic polymorphism. This study complements additional evidence for genetic polymorphism in *H. crassidens* based on a rearing experiment in controlled conditions (Lagueux-Beloin & Kelly, in prep.). Future research should seek to confirm the genetic basis of this polymorphism through controlled breeding experiments and explore further the role of the environment in shaping polymorphism expression among sites. These pieces of information will help to better inform future conservation management to ensure the polymorphism is maintained at new translocation sites, which will likely increase translocation success (Forsman, 2014; Takahashi, Kagawa, Svensson, & Kawata, 2014) and preserve the potential for future speciation (Corl et al., 2010; Mclean & Stuart-Fox, 2014).

1.7. Acknowledgements

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CONCLUSION

Biologists have long observed the presence of alternative mating behaviours and morphologies, referred to as alternative mating strategies (AMSs) (Gross, 1996; Shuster & Wade, 2003). Researchers have posited two hypotheses to explain why AMSs are maintained: the genetic polymorphism hypothesis, suggesting each morph is a discrete genetic variant and morphs experience equal relative fitness, and the condition-dependent hypothesis, suggesting that morphs are genetically monomorphic and instead determined by their 'condition' (Rowe & Houle, 1996), resulting in unequal relative fitnesses (Gross, 1996; Shuster & Wade, 2003; Tomkins & Hazel, 2007). Because AMSs determined by genetic polymorphism are rare in the literature despite being predicted to be widespread (Shuster, 2010), we investigated this hypothesis by testing the key prediction that relative fitnesses should be equal among morphs. We additionally sought to apply our findings to conservation management by making clear recommendations for maintaining the polymorphism during future translocations of *H. crassidens*.

Our results support the hypothesis that the male morphs expressed by *H. crassidens* are determined by genetic polymorphism, contributing to mounting evidence that genetically-determined AMSs are more common than previously thought (Küpper, Stocks, Risse, Dos Remedios, et al., 2015; Shuster & Wade, 1991; Sinervo, 2001; Tuttle, 2003). Our study also provides information that contributes to characterizing the alternative strategies employed by males of *H. crassidens*: 8th instars possessed smaller harems than 10th instars and had low reproductive success when only harem owners were considered as potential fathers, suggesting they might sire offspring through means other than harem ownership. In the rare instances where we found males of different morphs with the same harem we most commonly found 8th and 10th instars together and females often mated with multiple morphs, showing there is potential for smaller morphs to use a 'sneaker' strategy. This is especially intriguing because male *H. crassidens* cannot distinguish sex based on chemical cues (Wehi, Monks, & Morgan-Richards, 2017), suggesting they might be unable to detect the presence of different morphs. 8th and 9th instars also sired a greater proportion of offspring within broods than 10th instars, backing up previous evidence that smaller morphs invest more in sperm (Kelly, 2008b) and providing a potential mechanism by which fitness could be equalized among morphs.

Based on our findings, we offered two recommendations to promote the maintenance of the polymorphism following future translocations: translocate all three morphs in the approximate proportions they occur in the source population, or translocate an adequate number of individuals to capture > 95% of genetic variation in the source population (Weeks et al., 2011). Conservation of all three morphs merits consideration in translocation planning, as a greater degree of polymorphism has been demonstrated to increase population fitness (Forsman, 2016; Takahashi, Kagawa, Svensson, & Kawata, 2014) and to act as a source for new species (Corl et al., 2010; Hugall & Stuart-Fox, 2012; Mclean & Stuart-Fox, 2014). Maintaining the full potential for this species' mating system, including the potential for sperm competition among morphs and polyandry, might also contribute to translocation success because rate of polyandry is correlated with indicators of population fitness (Taylor, Price, & Wedell, 2014). The first recommendation, if followed, could present an excellent opportunity to study shifts in the polymorphism's expression in a new environment given knowledge of the relative abundance of each morph in the translocated cohort.

There are many intriguing avenues for future research both within the *H. crassidens* study system and concerning genetically determined AMSs more broadly. To our knowledge, the functional genomic basis for morph determination has only been identified in two species: the white-throated sparrow, where colour morphs are determined by a pericentric chromosomal inversion (Tuttle, 2003), and the lekking ruff, where alternative male phenotypes are determined by a supergene (Küpper, Stocks, Risse, Dos Remedios, et al., 2015). Given that these two examples involve different mechanisms, there are likely more yet to be uncovered. This might represent an opportunity for a novel discovery in *H. crassidens*, but should not overshadow the need to also test predictions of the condition-dependent hypothesis in this species. Furthermore, there are only a few studies about how the environment shapes selection on AMSs (e.g., Corl et al., 2010; Muniz & Machado, 2015), and there has not yet been much consideration of how resource polymorphism might factor into AMSs. *H. crassidens* could serve as an excellent study organism for these topics: while we only investigated the effect of cavity entrance size at a superficial level at one site, this species occurs at a variety of sites with differing vegetation and anecdotal observations suggest that the polymorphism shifts depending on available cavity size (Kelly, 2008a). The opportunity therefore exists to examine whether there is selective pressure against large morphs where cavities are small or if morphs preferentially use specific sizes of cavity when all sizes are available. Finally, life history strategies might also contribute to the maintenance of AMSs in this species as smaller morphs mature faster in the lab (Lagueux-Beloin & Kelly, unpubl. data), meriting further investigation of trade-offs between growth rate and longevity in the field.

Outside of the *H. crassidens* study system and the few other species that have been studied in the context of genetically determined AMSs, we recognize that most alternative mating behaviours are considered to be plastic or condition-dependent (Gross, 1996; Neff & Svensson, 2013) and that some question the need for a distinction between 'strategies' and 'tactics' (Taborsky et al., 2008). This study, along with others demonstrating a discrete genetic basis for AMSs, suggest that Shuster (2010) may have been correct in his intuition that genetic polymorphism is more widespread than we think. We urge other researchers to explicitly test this hypothesis before discounting it. Neff & Svensson (2013) pointed out that genetic polymorphism and condition-dependence have historically been framed as mutually exclusive, ignoring the potential for gene by environment interactions. Recent theoretical models by Engqvist & Taborsky (2016) also show that both types of strategy can evolve simultaneously in certain environmental and social conditions. Both hypotheses should be considered and the role of the environment should not be forgotten as future research goes forward.

In the face of our current biodiversity crisis, valid strategies to combat biodiversity loss include preventing the loss of extant species as well as preserving the potential for future species (Moritz, 2002; Soulé, 1985). The latter strategy has historically received less attention, perhaps due to our limited ability to identify sources of future species. The present study contributes to this endeavor by investigating the origins of intraspecific diversity that might act as a substrate for speciation. Based on our finding that the AMSs in *H. crassidens* are likely genetically determined, we infer that selection could act upon this genetic variation to produce morphic speciation patterns based on geographic variation in the polymorphism or assortative mating (Corl et al., 2010; Gray & McKinnon, 2007; Mclean & Stuart-Fox, 2014). These mechanisms should be addressed in future research and point to the value of considering species that are not presently in decline as conservation targets.

Beyond the context of male AMSs, our study also contributed some new perspectives on polyandry and cryptic female choice in our species. We validated a long-standing assumption that females are polyandrous (Kelly, 2006c) and uncovered tangible benefits to polyandry, including greater fecundity and greater hatching success. We also showed that the morph(s) mated by a female affected both the number of eggs she laid and the time they took to hatch. These results indicate that females experience benefits not only based on the number of mates, but perhaps also from the number and type(s) of morphs mated. Furthermore, we showed that 8th and 9th instar males sired a greater proportion of a female's brood when multiple morphs competed for fertilization. These findings raise the question of whether it is the competitive ability of the sperm, the preference of the female, or a combination of these factors that lead to a morph-specific fertilization advantage. It is also unknown whether sperm precedence is a factor in this system, which should be investigated alongside other variables to determine the cause for the pattern we observed.

In summary, this study provides support for the rarely tested genetic polymorphism hypothesis, identifies a system that may be a source for future biodiversity via morphic speciation, and offers evidence-based recommendations directly applicable to conservation translocation management. This study also opens many doors to future research not only regarding AMSs but also cryptic female choice and polyandry. More research is needed to confirm our findings and answer the many questions that follow, and will contribute critical information to ensure that the evolutionary potential of this species is not lost in the future.

APPENDIX A

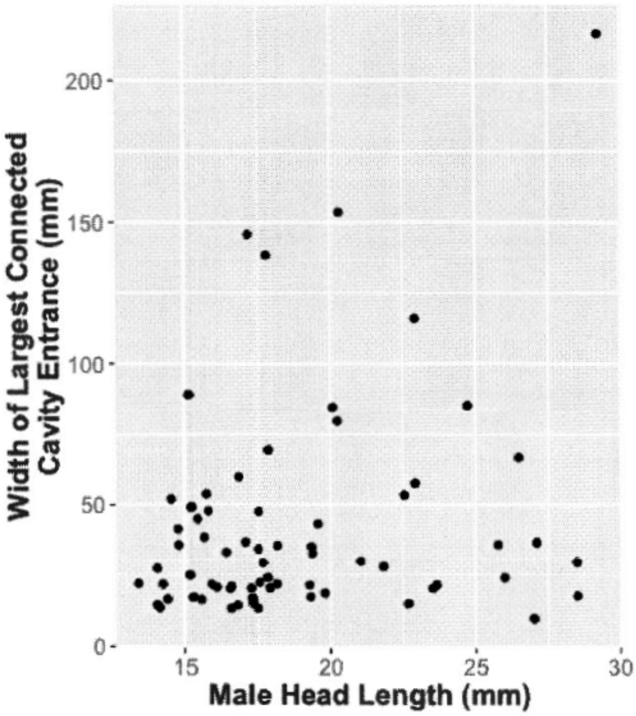

Figure A.1. Male head length and the width of largest connected entrance to the gallery he was found residing in. The full range of male head lengths occurred inside cavities of the smallest entrance size (< 25mm across at widest point), indicating this variable did not affect morph-specific residency at our site.

APPENDIX B

Table B.1. Table of standardized residuals obtained from a X^2 test of independence used to examine the proportion of offspring assigned to each morph at each of three levels of analysis: harem-level, branch-level, and population-level (all males sampled). An adjusted significance value of 0.0056 was used to obtain a critical cut-off value of -2.773.

		Morph		
		8	9	10
Level of Analysis	harem	**-3.592**	-1.164	**4.952**
	branch	**-3.983**	**3.273**	0.8192
	population	**6.223**	-2.126	**-4.343**

APPENDIX C

Table C.1. Rate of paternity assignment and differences in assignment at three different levels of analysis considering different sets of candidate fathers. Paternity was assigned in CERVUS 2.0 at 95% confidence.

Level of Analysis	**Fathers considered**	**Fathers assigned**	**Rate of assignment**	**Difference in assignment (harem)**	**Difference in assignment (branch)**
Population	83	51	70%	12.5%	10.2%
Branch	44	17	38%	2.17%	-
Harem	20	6	23%	-	-

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