The effect of population density on mating dynamics and the interaction between pre- and post-copulatory selection in the New Zealand giraffe weevil

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Michaela Lambert

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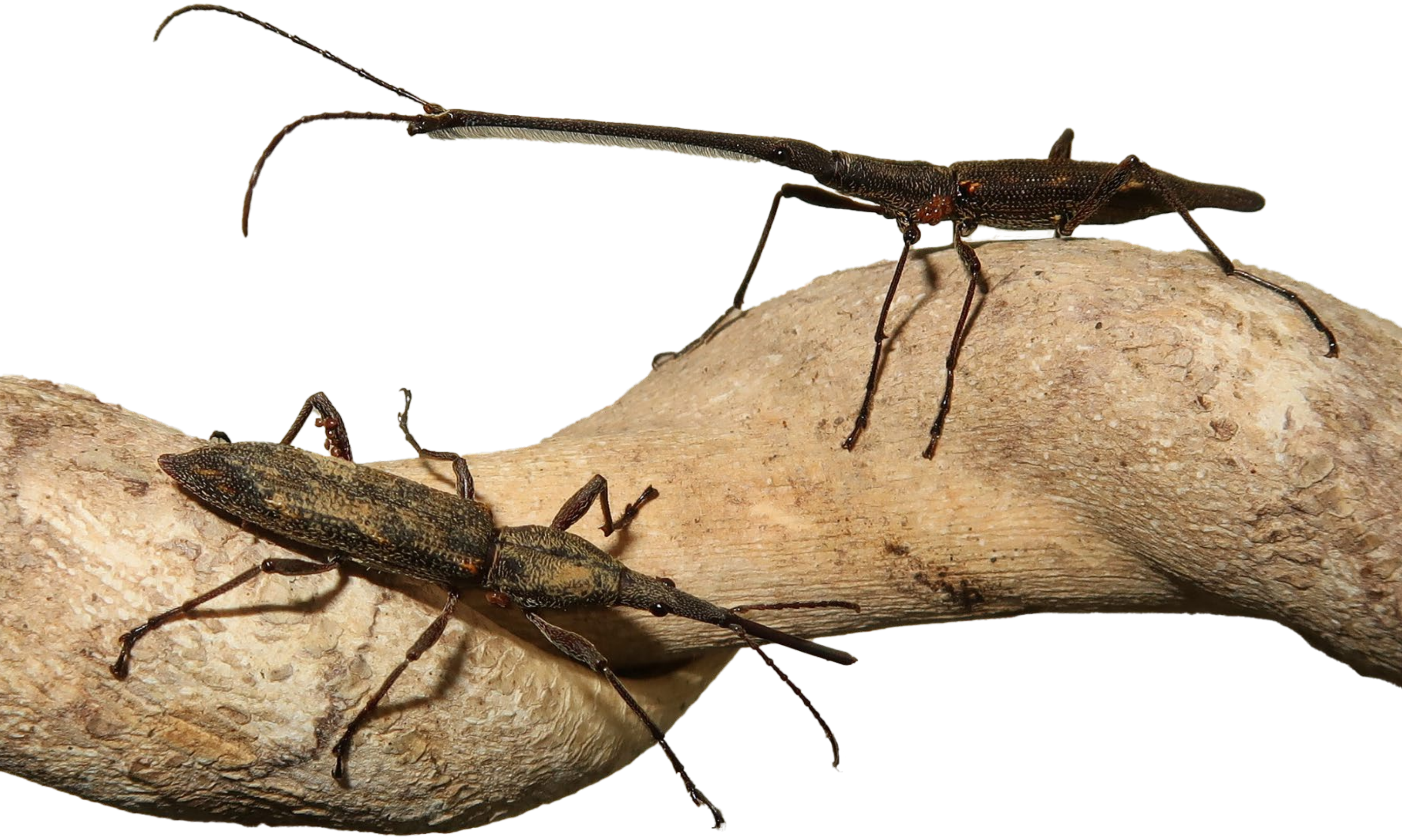

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Abstract

Sexual selection is thought to be the main driver for the evolution of pre-copulatory traits (e.g., weapons and ornaments) used to gain access to mates, and post-copulatory traits (e.g., sperm form and function) used to maximize fertilisation success. Individuals that have a greater ability to acquire resources from their environment may be able to invest in both types of traits equally, and therefore may have a competitive advantage during mate acquisition and sperm competition. For males that are competitively inferior, they may choose to invest more into one type of trait, resulting in a trade-off between the investment in one trait over another. The New Zealand giraffe weevil, *Lasiorhynchus barbicornis*, provides an ideal study system to explore size-dependent investment in sexual traits. Males show extreme size variability, leading to differences in mating success between males of different body sizes. Large males are better at acquiring mates and winning fights and as a result, small males have adopted a sneaking tactic to avoid pre-copulatory competition with larger rivals. Therefore, small males may choose to invest more into their post-copulatory traits. Further, giraffe weevils form large aggregations on host trees during the summer months, making them an ideal subject to explore how demographic factors can influence the mating success of males. In giraffe weevils, how body size and population density interact to influence mating success is not well understood. In this study, I used an experimental behavioural assay and morphometrics to investigate how males of different body sizes may be investing into their pre- and post-copulatory traits to engage or avoid sperm competition. I found that small males may be investing more into post-copulatory traits than large males. However, I found no evidence of a phenotypic trade-off between pre- and post-copulatory traits. I also used an observational dataset collected from a natural population to explore how population density, among other demographic factors, interact with body size to influence male mating success. I found that male giraffe weevils have density-dependent mating success, with small males doing best at high densities, and large males at low densities. My research will contribute to a growing body of work looking at how sexual selection can shape the life-history traits of insects.

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General Introduction

Sexual selection as a driver of phenotypic diversity in animals

Sexual selection has been the subject of debate and extensive research since it was first described by Darwin (1871). It is described as a form of selection that drives the adaptation and evolution of traits which increase an individual's reproductive success, and thus, fitness (Andersson, 1994). Sexual selection is a key driver of the incredible diversity of phenotypic traits in the animal kingdom such as weapons, used in contests, and ornaments, used in courtship displays (Bateman, 1948; Kodric-Brown & Brown, 1984; Zahavi, 1975). Common examples include the tail of peacocks, which are used in courtship displays (Loyau et al., 2005); or the eye stalks of male stalk-eyed flies (Diopsidae), used in male-male competition for access to females (Burkhardt & de la Motte, 1983; Panhuis & Wilkinson, 1999). One of the most well-studied exaggerated traits are the large horns borne by dung beetles (Scarabaeinae) and rhinoceros beetles (Dynastinae), used as weapons in male contests for access to females (Emlen et al., 2005, 2007; Emlen, 2008; Kawano, 1995; Ohde et al., 2018; Pomfret & Knell, 2008). Sexual selection typically acts more strongly on the more abundant sex, which is most commonly males (Kirkpatrick, 1987; Lande, 1980; Zahavi, 1975). Thus, diverse, and often bizarre traits are almost exclusively found in males, and as a result, sexual dimorphism is seen in many taxa (Kirkpatrick, 1982; Lande, 1980; Zahavi, 1975). For example, male fiddler crabs of the genus *Uca*, possess a small feeding claw, and an extremely enlarged claw that can comprise half their total body mass in some species, compared to females that have two normal claws used for feeding (Crane, 2015). Males use this enlarged claw in a visual courtship display, but also in aggressive contests with other males for access to females (Christy, 1983; Pope, 2000). Males with larger claws are more attractive to females, and thus they continue to persist in populations, despite being costly to maintain (Christy, 1983; Jennions & Blackwell, 1998; Pratt et al., 2003). The type of cost (e.g., locomotory, metabolic, developmental) and magnitude of cost is variable among taxa (McCullough et al., 2012; McCullough & Emlen, 2013; McCullough & Tobalske, 2013). That elaborate traits continue to persist despite the costs demonstrates how strongly sexual selection can act on a species.

Exaggerated sexually selected traits are expected to be condition-dependent where the benefits of an exaggerated display or ornament in terms of mating success are thought to be balanced by the costs of producing and/or carrying the trait (Zahavi, 1975). Generally speaking it is thought that stronger sexual selection has led to higher condition-dependence in these traits (Bonduriansky & Rowe, 2005; Cotton et al., 2004; Johnstone et al., 2009). Males often invest a significant amount of resources into the exaggeration of weapons to gain access to females, resulting in variation in both shape and size within populations (Emlen et al., 2007). Indeed, many sexually selected traits are extremely large relative to the individual bearing them, exhibiting positive allometry (Eberhard et al., 2018). Positive allometry has been interpreted as evidence of sexual selection as it leads to variation in mating success between individuals (Andersson, 1994; Bonduriansky, 2007; Kodric-Brown et al., 2006), and larger weapons have been associated with increased mating success (Andersson, 1994). For example, male tree wētā (*Hemideima crassidens*) with larger mandibles, which are used as a weapon during contests, have increased success in male contests and access to female harems, therefore increasing their mating opportunities (Kelly, 2005, 2006). Furthermore, male snapping shrimp (Alpheidae) with the largest body and weapon are more likely to win contests for burrows used by females (Hughes, 1996). Thus, exaggerated traits are typically associated with increased mating success, and as a result, maintains exaggeration of the trait within the population.

Polyandry as a driver of pre- and post-copulatory selection

Polyandry is a common occurrence in the animal kingdom and is defined as multiple mating by females (Parker & Birkhead, 2013). Multiple mating by females therefore means that multiple ejaculates co-occur at the site of fertilisation. Because paternity is not guaranteed, males have evolved traits to give them a competitive advantage over their male counterparts, allowing them to increase their reproductive success (Andersson, 1994). Bateman (1948) argued that male reproductive success is not limited by sperm production, but rather the number of available females, whereas female reproductive success is limited by the number of eggs she can produce. Because multiple mating requires time and energy (Huchard et al., 2012), risks exposure to disease (Thrall et al., 2000), predation (Rowe, 1994) and injury (Arnqvist et al., 2005), females should only mate once or a few times to ensure fertilization (Simmons, 2005). However, polyandry is a ubiquitous phenomenon across many taxa (Arnqvist & Nilsson, 2000; Gowaty, 2013; Simmons, 2005). A review of Bateman's principle has since shown that his theory was controversial, and females actually benefit from mating multiple times (Parker & Birkhead, 2013; Simmons, 2005). For example, a direct benefit may be receiving nuptial gifts from multiple males, which can increase female egg production by up to 85%, despite an increased risk of disease (Knell & Webberley, 2004; Rowe, 1994). On the other hand, increasing offspring quality may be an indirect benefit of polyandry, allowing females to select the fittest males following copulation (McCullough et al., 2017). Therefore, both preand post-copulatory choice by females are expected to shape the indirect and direct benefits of polyandry, likely influencing its ubiquity in the animal kingdom (Simmons, Beveridge, et al., 2007).

Pre- and post-copulatory selection in the context of sperm competition

While polyandry has been demonstrated to offer benefits to females, it also creates the opportunity for sexual selection on males to occur both before and after mating. Like many of the classical traits involved in male contests, it has become apparent that post-copulatory traits such as sperm can also be greatly influenced by sexual selection. For example, some *Drosophila* species have evolved giant sperm that are now thought to be one of the most extreme ornaments, even when compared to antlers or peacock feathers (Lüpold et al., 2016). Post-copulatory sexual selection operates after mating and is driven by the process of sperm competition. Sperm competition occurs when the sperm of two or more males co-occur at the site of fertilisation, resulting in competition between sperm for fertilisation of the female ova (Simmons, 2005). In contrast, pre-copulatory selection operates before mating through male-male competition for access to mates. Males may avoid sperm competition through male-male competition, and often the males that are endowed with the most exaggerated of weapons and ornaments experience a disproportionately high mating success when compared to males who bear smaller traits (Andersson, 1994; Emberts et al., 2018; Parker, 1974b). Further, to avoid sperm competition through post-copulatory selection, males may evolve genitalia that impose harm on females to prevent them from remating (Hotzy & Arnqvist, 2009; Kyogoku & Sota, 2015), or evolve structures on their genitalia for the removal of rival sperm (Córdoba-Aguilar et al., 2003; Waage, 2008). For example, damselflies (Odonata) have evolved genital structures that allow them to remove rival sperm before transferring their own (Waage, 1979). Therefore, sperm competition has resulted in the evolution of diverse traits and strategies that allow males to avoid post-copulatory competition.

Males can also engage in sperm competition through post-copulatory selection on traits such as testes or sperm length, quality, or number. Sperm competition favours traits that maximize competitive fertilisation success. A common evolutionary response to an increase in sperm competition is an increase in testes size (Hosken, 1997; Hosken & Ward, 2001; Moller, 1991; Stockley et al., 1997), which has shown to be correlated with sperm production (Amann, 1970; De Reviers & Williams, 1984; Møller, 1988). Theory predicts that with increasing sperm competition, males should allocate more resources to sperm production (Parker, 1970), and there is growing evidence for this in the literature

(Kelly & Jennions, 2011; Simmons & Emlen, 2006; Wedell et al., 2002). For example, in a comparative study using the Australian Maluridae, sperm concentration was positively correlated with sperm competition level, suggesting males increase sperm production in response to sperm competition risk (Rowe & Pruett-Jones, 2011). Furthermore, in the dung beetle *Onthophagus taurus,* males from polygamous lines had larger testes than those from monogamous lines (Simmons & García-González, 2008).

Sperm competition models predict that males have a limited resource pool and therefore may experience a trade-off between mate acquisition (e.g., mate searching, male-male competition), and ejaculate expenditure (e.g., sperm number, testes size) (Lüpold et al., 2014; Parker & Pizzari, 2010; Simmons, 2001). Where males compete in contests for direct access to females, these models predict that expenditure on weapons should increase and expenditure on ejaculates should decrease (Parker & Pizzari, 2010; Simmons & Buzatto, 2014). This has been shown in the dung beetle *Onthophagus taurus,* where under high levels of competition, large horned (major) males allocated more resources to body size (Simmons & Buzatto, 2014). Because pre-copulatory traits (e.g., antlers) influence a male's access to mates, and post-copulatory traits (e.g, sperm size) can affect fertilisation success, overall reproductive success is determined by how much a male invests into both pre- and post-copulatory traits (Andersson & Simmons, 2006; Kvarnemo & Simmons, 2013). Similar to pre-copulatory traits, there is evidence to suggest that post-copulatory traits are condition-dependent (Bonduriansky & Rowe, 2005; Perry & Rowe, 2010; Rowe & Houle, 1996; Wigby et al., 2016). For example, Wylde et al. (2019) found that condition-dependence and male-male competition interact to influence ejaculate investment in the neriid fly, *Telostylinus angusticollis.* High condition males had lower latency to mate and increased ejaculate expenditure but only when they were second to mate, suggesting strategic allocation of ejaculates (Wylde et al., 2019). Despite this, how male condition, and therefore body size influences expenditure on pre- and post-copulatory traits is poorly understood.

How sperm competition shapes the use of alternative reproductive tactics

Polyandry, and therefore the risk and intensity of sperm competition, can influence the mating behaviour of males of different body sizes. Large males typically have higher mating success due to competitively excluding smaller males in pre-copulatory competition. This disparity in mating success between small and large individuals has led to the evolution of alternative reproductive tactics (ARTs) (Shuster & Wade, 2003). Alternative reproductive tactics allow smaller males to engage in copulations without a pre-copulatory fight (Taborsky et al., 2008). Most commonly two or three morphs of males are selected for; a large, dominant male, which possesses an exaggerated trait, and one or two smaller, subordinate male morphs that sneak, mimic females, or act as satellites (Emlen, 1997; Okada et al., 2008; Rowland & Emlen, 2009; Shuster, 1987). For example, the yellow dung fly (*Scathophaga stercoraria)* uses size-specific ARTs (Parker, 1970; Pitnick et al., 2009; Tomkins & Simmons, 2002). Large males compete in aggressive contests for gravid females on dung, but smaller males search for females at foraging sites and as a result, have higher mating success (Gress et al., 2014; Pitnick et al., 2009). Therefore, although sexual selection typically acts on large males with exaggerated traits, if individuals can gain reproductive opportunities using alternative phenotypes, these phenotypes can continue to persist within the population (Gross, 1996).

Differential allocation of resources into pre- and post-copulatory traits are likely to be more common in species with alternative mating tactics (Simmons & Emlen, 2006). Within a population, fighter and sneaker male morphs are exposed to varying levels of both sperm competition risk and intensity, ultimately influencing their relative fitness. Sneaker males typically face higher levels of sperm competition than fighter males as they are more likely to mate after the dominant males (Parker, 1990b). Therefore, theory predicts that sneaker males should invest more into testes size or sperm characteristics that allow them to overcome the competitive disparity and increase their reproductive success (Parker, 1990b; Vahed & Parker, 2012). This is seen in the dung beetle genus *Onthophagus,* where minor (sneaker) males have greater testes size than major (fighter) males (Simmons et al., 2007). However, a review of 29 species across five taxonomic classes by Kustra and Alonzo (2020) found no significant differences in ejaculate traits between sneaker and fighter males. Thus, there is contrasting evidence for this theory and the exact mechanisms explaining why there is a difference in resource allocation are poorly understood. Understanding how males using different alternative mating tactics invest resources into their pre- and post-copulatory traits can provide a deeper understanding of the interaction between developmental trade-offs of sexually selected traits.

The effect of population density and sex ratio on mating dynamics

Pre- and post-copulatory selection will be subject to variation based on the number of individuals in a population and the ratio of sexually receptive males to females (operational sex ratio [OSR]). However, little is known about the mechanisms underlying how population density and the OSR may affect sexual selection and the mating success of an individual. Increasing the number of competing males and the number of females available to mate will increase competition for access to mates, and may also increase the intensity of sperm competition. For example, when population density increases, males are likely to have more mating opportunities as their encounter rate with female increases (Kokko & Rankin, 2006). However, with a greater number of males competing for access to females, the efficiency of competition and monopolisation of females reduces as encounter rates with other males have increased (Cai et al., 2019; Knell, 2009). Similarly, the OSR can have a strong influence on the intensity of competition for mates which is typically thought to correlate with the strength of intrasexual selection (Weir et al., 2011). It is predicted that as the OSR becomes increasingly more male-biased, male aggression and competition increases and females becomes more choosy (Emlen & Oring, 1977). On the other hand, as the OSR becomes more female-biased, male aggression and competition is expected to decrease, and females are expected to be less choosy (Emlen & Oring, 1977). The effects of population density and OSR are closely intertwined and therefore it can be difficult to resolve the effects of these individual parameters on sexual selection (Head et al., 2008).

Population density is predicted to influence mating behaviours such as mating frequency and duration. For example, male water striders (*Aquarius remigis*) invest more effort into mate acquisition (Lauer et al., 1996), but have lower mating success at high densities (Sih & Krupa, 1995). Furthermore, in the seed bug *Nysius huttoni,* males increase mating duration in response to high population density (Wang et al., 2008). The exact functions behind increasing mating duration in response to population density is currently unknown, although it is predicted that males expend more resources on ejaculate in response to sperm competition (Simmons, 2001). Indeed, this is what sperm competition theory predicts, that males should maximize post-copulatory expenditure to secure as many fertilisations as possible when facing sperm competition from other males (Parker et al., 2013a). However, there is some empirical evidence that brings the generality of this theoretical prediction into question. For example, in the Australian polydesmidan millipede (*Gigantowales chisholmi*), males decrease mating duration in response to increasing density, suggesting mate acquisition is more important than securing fertilisations and ensuring paternity in this species (Holwell et al., 2016). However, this may be due to the mating system this species employs, showing an adaptive response to population density by changing mating tactics.

Similarly, the OSR is another aspect of the population known to influence mating success. When the OSR becomes more male-biased the intensification of male competition for mating opportunities is expected to increase as well as the opportunities for females to exercise mate choice (Emlen & Oring, 1977; Smith, 2007). Therefore, male mating success is likely to vary with a male-biased OSR because some males (e.g., large, fighter males) will be able to exclude others (e.g., small, sneaker males) from securing fertilisations (Smith, 2007). For example, in the European bitterling, *Rhodeus sericeus*, a male-biased OSR changed the mating tactic of large males, and they competed against small males to release sperm when females spawned. Large males managed to competitively exclude smaller males, obtaining 66% of sperm releases compared to 6% in smaller males (Mills & Reynolds, 2003). This is expected under sexual selection theory, particularly Bateman's principles which suggest that reproductive success in males is primarily based on access to mates (Bateman, 1948). Others, however, have suggested more nuanced relationships between the OSR, Batemans gradient, and sexual selection (Kokko et al., 2012). Furthermore, the OSR can influence mating duration and frequency. In the walnut fly (*Rhagoletis juglandis*), a male-biased sex ratio led to males having low mating frequencies with short mating durations (Alonso-Pimentel & Papaj, 1996). However, the opposite was seen in the beetle *Menochilus sexmaculatus*, where mating duration increased under extremely male-biased sex ratios (Saxena et al., 2020). Increasing mating duration in response to a male-biased OSR is consistent with the theory of Parker et al. (2013a), which suggests that males should maximize post-copulatory expenditure to secure as many fertilisations as possible when facing sperm competition from other males. There is no denying that the OSR influences male mating success. However, how it interacts with population density to influence net male fitness is poorly understood.

Studies on sexual selection in the wild on non-model species

Insects are not often studied in the field as they can be difficult to observe and track in their wild populations (LeGrice et al., 2019; Rodriguez-Munoz et al., 2010). Conducting behavioural ecology research in the wild is advantageous because it allows individuals to interact without manipulation and therefore produces a complex and dynamic understanding of variation in natural populations that would otherwise not be seen (Fisher et al., 2015; Le Grice, 2015). However, a wild environment can introduce uncontrolled variation to the dataset and this should be taken into consideration when trying to decipher relationships between variables (Kawasaki et al., 2008; Lande & Arnold, 1983; Roulston & Goodell, 2011). Furthermore, it is often difficult to monitor the behavioural variation of an individual or population due to the labour required to accurately track and monitor the species in their natural habitat (Fisher et al., 2015; Herborn et al., 2010). In contrast, conducting research on insects in the laboratory is a more typical approach as insects are often easy to rear and observe in captive populations. However, laboratory environments can confound behavioural measurements (Archard & Braithwaite, 2010; Bonduriansky & Brassil, 2005; Bretman & Tregenza, 2005; Kawasaki et al., 2008). There has been some support for behavioural traits seen both in the wild and laboratory environments (Herborn et al., 2010; Šlipogor et al., 2021; Yuen et al., 2016). However, repeated measures of the same behavioural trait are needed to provide an accurate depiction of the effect of both wild and laboratory environments (Fisher et al., 2015). Recently, a study by Osborn and Briffa (2017) measured the behaviour of beadlet sea anemones (*Actinia equina*) in the wild and the laboratory. Despite repeatability of behavioural measurements between the wild and laboratory environments, startle responses differed between environments. Anemones in the wild had shorter startle responses (i.e., less risk averse) compared to those in the laboratory. Therefore, using both wild and laboratory measurements is ideal to gain an accurate understanding of variation in an individual's behaviour and the variables that may be affecting it.

Behavioural research is typically conducted using model organisms (e.g., *Drosophila,* honey bees, crickets) to formulate and test hypotheses (Zuk et al., 2014). Using model organisms has its advantages. They are usually easy to rear, observe, and experimentally manipulate. Furthermore, they allow knowledge to be built up rapidly because any confounding factors are known and can be controlled for in future research. However, using model organisms to generalize findings can be problematic. Many aspects of ecology and evolutionary biology are concerned with individual variation and how this influences fundamental processes such as speciation (Zuk et al., 2014). Therefore, research should choose organisms based on the question at hand and the behaviours sought to explain. More studies on wild populations using non-model species are required to understand the true extent of how sexual selection acts based on individual variation in a species.

The New Zealand giraffe weevil (Lasiorhynchus barbicornis)

The New Zealand giraffe weevil, *Lasiorhynchus barbicornis* (Fabricius), (Coleoptera: Brentidae) is endemic to New Zealand (Kuschel, 2003) and is found across the North Island and the north western regions of the South Island. This species has become a useful model for research on sexual selection and the evolution of exaggerated traits.

Brentidae

Brentidae are a diverse beetle family that consists of 1690 species with over 290 genera, most of which are commonly found in tropical rainforests (Sforzi & Bartolozzi, 2004). Weevils within the family Brentidae are commonly called straight-nosed weevils as unlike other families within the Curculionoidea which bear a curved rostrum, brentids have straight rostrums. The rostrum of male brentids are often exaggerated, resulting in size dimorphism (Sforzi & Bartolozzi, 2004). In addition, intrasexual size variation and competition between males has been reported for several species, including the New Zealand giraffe weevil (García-Cambronero, 1989; Johnson, 1982, 1983; Painting & Holwell, 2014a).

Ecology

Giraffe weevils are commonly found aggregating on dying standing, or fallen trees such as karaka (*Corynocarpus laevigatus*), māhoe (*Melicytus ramiflorus*), rangiora (*Brachyglottis repanda*), tarata (*Pittosporum eugenioides*), whau (*Entelea arborescens*), and tawa (*Beilschmiedia tawa*) (Meads, 1976; Painting & Holwell, 2014b). Females search for a suitable oviposition site where they will drill into the dying wood and deposit a single egg. While a female drills an oviposition hole, males congregate and attempt to copulate with them, often forming large aggregations (Meads, 1976; Painting & Holwell, 2014b).

Adult giraffe weevils have been observed consuming tree sap, and flower nectar, further reinforcing their attraction to dying or wounded trees (Meads, 1976; Painting & Holwell, 2014b). Mating and egg laying occur in warmer months between October and April, followed by at least a two-year wood-boring larval stage before the adults emerge (Meads, 1976; Painting & Holwell, 2014b). Peak population density occurs late summer (February) before the adults to die off in autumn (April/May) (Painting et al., 2014). After emerging, giraffe weevils live for several weeks, although large males and females have been observed to have an increased lifespan (LeGrice et al., 2019). Lastly, giraffe weevils are diurnal, retreating to the canopy when it gets dark and remaining on the underside of the leaves until dusk (Painting & Holwell, 2014b).

Morphology

Originally, male and female giraffe weevils were described as separate species due to the high level of sexual dimorphism (Kuschel, 2003). Male giraffe weevils possess an extremely elongated rostrum, which can be over half their total body length (**Figure 1.1**, Kuschel, 2003). Females use their rostrum to drill into dying wood to make an oviposition site, whereas males use their rostrum as a weapon in intense male-male competition for access to females (Meads, 1976; Painting & Holwell, 2014a). In males, the rostrum shows a positive allometric relationship with body size, suggesting that large males invest disproportionately more resources into their rostrum compared to their smaller male counterparts (Painting & Holwell, 2013). However, the wings, antennae, and legs showed positive correlations with rostrum length, suggesting males are compensating for their increased rostrum length (and possibly increased locomotory costs) (Painting & Holwell, 2013). Previous research exploring trade-offs between rostrum size and testes size in male giraffe weevils found no evidence of a trade-off (Painting & Holwell, 2013). However, there has been no research yet looking into if, and how, male giraffe weevils invest resources into other post-copulatory traits such as sperm length and concentration. Furthermore, there has only been one study (Painting & Holwell, 2013) exploring differences in investment between pre- and post-copulatory traits with respect to male body size.

Figure 1.1. A male (top) and female (bottom) New Zealand giraffe weevil. Note the position of the antennae which is further down the rostrum on the female to allow her to drill into the wood to lay her egg. Photos by M Lambert.

Furthermore, giraffe weevils show both extreme intra- and intersexual size variation, with males varying between 14-90 mm and females between 12-50 mm in total body length (**Figure 1.2**, Painting & Holwell, 2014b). This extreme size variability is likely to have driven the evolution of size-dependent alternative reproductive tactics (ARTs) in males. Males may employ either a fight and guard strategy, or sneaking strategy, for access to females (Painting & Holwell, 2014b). However, this ART is flexible in that small males can switch between sneaking and fighting based on the relative size of their opponents (Painting & Holwell, 2014). In addition, females do not appear to show any pre-copulatory preference for small or large males, and both sexes of various sizes have been found copulating (Meads, 1976).

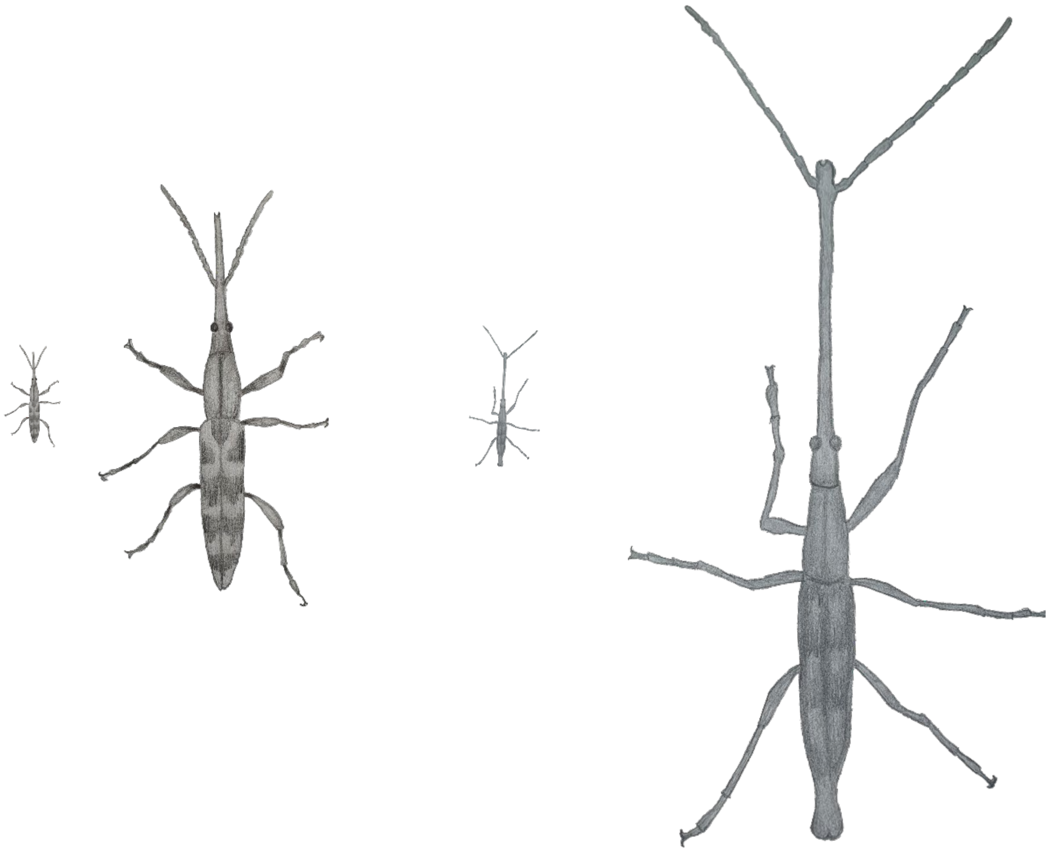


Figure 1.2. A comparison of female and male morphology and size variation in the New Zealand giraffe weevil, *Lasiorhynchus barbicornis.* Females are presented on the left, males on the right. These images are true to size, with the females being 12mm and 50mm, respectively, and the males 14mm and 90mm, respectively. Drawings by M Lambert.

Behaviour

Male giraffe weevils use their elongated rostrums in male-male competition for access to females (Painting & Holwell, 2014a). The use of the rostrum during intense competition highlights the importance of its use as a weapon, and suggests it may be under sexual selection (Painting & Holwell, 2013). Research by Painting and Holwell (2014b) found no evidence of directional or stabilising selection on rostrum length, suggesting that rostrum length has no influence over male mating success. However, a more recent study by Le Grice et al., (2019) found that larger males with longer rostrums have increased mating frequency and mate with larger (more fecund) females. Despite these studies, little is known about how body size interacts with population density to influence mating success in the New Zealand giraffe weevil. A study by Fisher et al., (2021) found that social selection was density-dependent for both males and females. Further, they found that small male mating success was influenced by the body size of their rivals but only when larger males were around and population density was high. However, they suggested further research with higher numbers of high-density populations was necessary to explore the interaction between body size and population density on mating success.

Study sites

Maungatautari Mountain

Male giraffe weevils were collected from Maungatautari (38°03'19.8"S, 175°33'38.5"E), a mainland ecological island that is a forested, extinct volcano surrounded by farmland in the Waikato, North Island, New Zealand (**Figure 1.3**). It is predominantly dense, podocarp-broadleaved native forest, covering approximately 3,400 ha (8,400 acres) (Speedy et al., 2007). It is significant in that is surrounded by a 47 km long predator-proof fence. Two smaller enclosures were set up as a pilot for the larger mountain, the northern (35 ha) and southern (65 ha) (Watts et al., 2020). The predator proof fence excludes introduced mammalian predators, including, but not limited to, brushtail possums (*Trichosurus vulpecula*), rats (*Rattus* spp.), mustelids (Mustelidae), and in the southern enclosure, mice (*Mus musculus*) (Watts et al., 2020). This field site is ideal for collecting and observing giraffe weevils as there is an abundance of native hardwood trees such as tawa (*Beilschmiedia tawa*) and māhoe (*Melicytus ramiflorus*), where they are known to aggregate over the summer period (Painting et al., 2014; Painting & Holwell, 2014c).

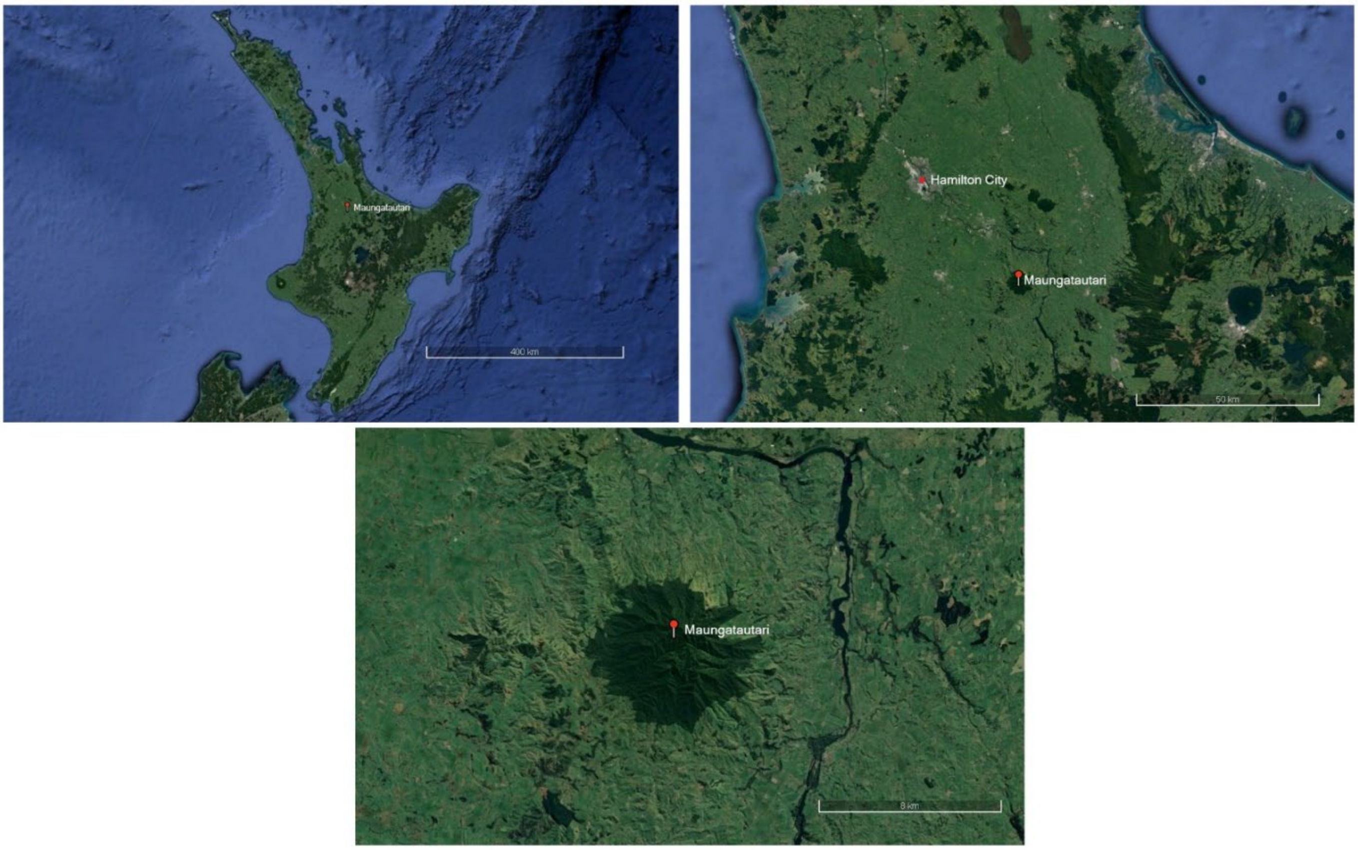


Figure 1.3. Field site, Maungatautari (red pin), south east of Hamilton (red dot) in the Waikato, North Island, New Zealand. Images taken from Google Earth.

Matuku Reserve

Behavioural observations were conducted at Matuku Reserve (36°51.92′S, 174°28.32′E), west of Auckland city in Waitakere Valley (Figure 1.4). Matuku Reserve is owned by the Royal Forest and Bird Protection Society and comprises 120ha of native coastal broadleaf forest (Staniland, 2009). This study site is ideal for carrying out observations on wild populations of giraffe weevils as they are found here in high densities during the warmer months, aggregating on trees suitable for oviposition (Painting et al., 2014; Painting & Holwell, 2014c). Aggregations of giraffe weevils have been found on a number of tree species, but most commonly found on karaka (*Corynocarpus laevigatus*) and māhoe (*Melicytus ramiflorus*), which are abundant at Matuku Reserve (Painting & Holwell, 2014c).

Figure 1.4. Field site, Matuku Reserve (yellow pin), west of Auckland city (green pin) in the North Island, New Zealand. Images taken from Google Earth.

Thesis outline

In this thesis, I aim to determine how sexually selected traits scale with body size, and if there are any trade-offs between such traits, as well as how demographic factors may influence male mating success. This thesis provides an extension of recent work on giraffe weevils that looked at how weapon size scaled with body size, along with other work that investigated the effect of body size on mating success. My research will contribute to a growing body of work exploring how sexual selection drives investment in traits that increase male mating success. Similarly, this research will also shed light on the reproductive ecology of natural populations, providing insight into how sexual selection acts on male New Zealand giraffe weevils. I present two studies on giraffe weevil morphology and behaviour to address my overall thesis aim.

In **Chapter Two**, I use allometric morphometrics and laboratory techniques to examine the relationship between body size and investment in pre- and post-copulatory traits. I use these results to discuss how males of different body and weapon sizes invest into pre- and post-copulatory traits, and if there are any trade-offs between these two types of traits. My findings in this chapter are novel, and address important implications that this research will have for understanding how sexually selected traits may differ based on the role they play to influence an individual's fitness.

Chapter Three investigates the interaction between population dynamics (density and sex ratio) and body size in relation to male mating duration and frequency. Here, I use two datasets: (1) a laboratory-based experimental dataset collected by myself, and (2) an observational field dataset collected by Dr Christina J. Painting and Dr Leilani Walker. The results from this chapter will allow us to build an understanding of how males of different body sizes in polygynous species may adjust their mating strategies in response to fluctuations in population density and sex ratio.

Finally, in **Chapter Four**, I summarise my findings and draw on these results to discuss how sexual selection acts on traits that pertain to male mating success. Additionally, I discuss how demographic factors can have complex interactions to influence male mating success and consequently, their fitness.

2

Allometry and trade-offs of pre- and post-copulatory traits in the New Zealand giraffe weevil

Abstract

Sexual selection is an important driver for the evolution of exaggerated traits such as weapons (e.g., horns) and ornaments (e.g., bright feathers). These exaggerated traits are expected to increase mating success, and consequently, net fitness. The production of these traits can come at a cost to other traits through trade-offs in insects that are holometabolous where resources are limited. Therefore, resources allocated to pre-copulatory traits may come at a cost of the development of post-copulatory traits such as testes size. Male giraffe weevils (*Lasiorhynchus barbicornis*) bear an extremely elongated rostrum used in competition with rivals for access to females. Here, I characterise the scaling relationship between body size and post-copulatory traits to determine their allometric relationship. Further, I explore the relationship between rostrum length and post-copulatory traits (testes diameter, testes weight, sperm length, sperm concentration, aedeagus length, and tegmen length) and the allocation of resources to these traits to determine if there are any trade-offs between pre- and post-copulatory traits. I found that post-copulatory traits were generally negatively allometric, but sperm concentration showed a positively allometric relationship with body size. I found no evidence of a trade-off between pre- and post-copulatory traits; however, relative testes weight showed a positive relationship with relative rostrum length. My results suggest that small males are investing relatively more into some post-copulatory traits that assist them during sperm competition compared to larger males. However, larger males may experience an accumulation of benefits that increase their reproductive success and ultimately, their fitness.

Introduction

Sexual selection through male-male competition and female choice is thought to be the main force driving the evolution of exaggerated structures such as ornaments and weapons (Andersson, 1994; Darwin, 1871). Females often mate with multiple males during the reproductive period (polyandry) (Arnqvist & Nilsson, 2000), and have the ability to store sperm (Parker & Pizzari, 2015), further extending male-male competition after copulation. Consequently, male sperm can compete within the female's genital tract or sperm storage organs for fertilization opportunities (Parker, 1970). In addition, females may be selective about which male fertilises her eggs (cryptic female choice, Eberhard, 1996). Competition for fertilisation success therefore drives post-copulatory selection on ejaculate traits such as sperm production and quality as well as seminal fluid (Perry et al., 2013). Theory predicts that males experiencing intense sperm competition should allocate more energy to sperm production, and there is growing evidence that this theory is supported (Simmons & Emlen, 2006). For example, *Drosophila melanogaster* males increase sperm number and sperm quality when perceiving the presence of rival males (Garbaczewska et al., 2013; Moatt et al., 2014). Similarly, in a comparative study of *Cataglyphis* desert ants, males experiencing greater levels of sperm competition produce more sperm (Aron et al., 2016). Female mate choice is the most well-studied area of sexual selection and as a consequence, the mechanisms underlying selection through male-male competition are poorly understood (McCullough et al., 2016). Furthermore, much of the work around male-male competition has focused on pre-copulatory traits such as weapons and ornaments and far less attention has been paid to mechanisms of post-copulation competition (Eberhard, 2009; McCullough et al., 2016; Parker & Birkhead, 2013).

Pre- and post-copulatory selection may occur simultaneously (Parker et al., 2013b), and although they are often treated as distinct processes, both influence net male fitness (Kvarnemo & Simmons, 2013). Producing and maintaining pre-copulatory traits (e.g. weapons) and post-copulatory traits (e.g. sperm) can be expensive (Lüpold et al., 2014). Sperm competition models predict that males have a limited resource pool, and those who allocate more energy to mate acquisition may experience a trade-off with limited investment in ejaculate production (Lüpold et al., 2014; Simmons, 2001). Thus, male fitness depends on how many resources are allocated to sexually selected traits and therefore can be influenced by environmental conditions and the amount of resources readily available (Reznick et al., 2000). For example, in the beetle genus *Onthophagus,* males that invest in horns used as weapons to compete for mating opportunities invest less resources in their testes (Simmons & Emlen, 2006). Based on this theory of condition-dependence, larger males can acquire and allocate more resources and therefore may suffer fewer trade-offs than their smaller male counterparts (van Noordwijk & de Jong, 1986).

One way of quantifying how much energy males are allocating resources to sexually selected traits is through allometry, the study of how the traits of an animal scale with body size (Huxley, 1932; Huxley & Teissier, 1936; Kodric-Brown et al., 2006). Allometry should reflect resource allocation trade-offs because initial resources are allocated to development, but following this, a fraction of the available resources are allocated to a secondary sexual trait (Bonduriansky and Day, 2003). Positive allometries ($b > 1$ on a log scale) suggest that larger males have disproportionately larger traits, whereas isometry ($b = 1$) suggests that traits scale in direct proportion to body size. Comparatively, negative allometry ($b < 1$) suggests that larger males have disproportionately smaller traits than smaller males (Kodric-Brown et al., 2006). Therefore, we would expect that larger males with disproportionately larger traits (positive allometries) have had access to more resources to allocate to sexually selected traits. This is seen in the white-tailed deer (*Odocoileus virginianus*), where males consuming higher quality nutrition allocated more to their antler mass compared to those consuming lower quality nutrition (Jones et al., 2018). Thus, allometry can help uncover size-dependent investment strategies and how males differentially allocate to pre- and post-copulatory traits.

Trade-offs between pre- and post-copulatory traits are commonly seen in species with alternative reproductive tactics (ARTs) (Simmons & Emlen, 2006). Alternative reproductive tactics are defined as alternative phenotypes such as size, age, condition, colour, or behaviour used to increase fertilisation success (Taborsky et al., 2008). They evolve due to a competitive disparity among groups of males, whereby one group are more successful at monopolizing females (Shuster, 2019). Thus ARTs are used because they offer less dominant or males of different body or trait sizes an opportunity to gain fitness by engaging in other methods of mate acquisition (Taborsky et al., 2008). ARTs may be flexible (condition-dependent), which can be beneficial should conditions change during the individual's lifetime (Taborsky et al., 2008). Body size is an example of a condition-dependent ART and is seen in several taxa. For example, in Atlantic salmon (*Salmo salar*), males mature as either small parr males or larger anadromous males and use either sneaking or fighting tactics, respectively (Gage et al., 1995). By nature of their mating tactic, sneakers are typically subject to increased sperm competition, whereas fighters are subject to sperm competition based on the number of sneakers in the population (Parker, 1990b). Because of this, males using different ARTs are under different selection pressures to increase their relative mating and fertilisation success. Examining the way in which males of different body sizes allocate resources to sexually selected traits based on their mating tactics can enable a deeper understanding of the interaction between developmental trade-offs and pre- and post-copulatory mechanisms of sexual selection.

There are numerous post-copulatory traits under selection in males due to sperm competition and cryptic female choice, including testes size, sperm quality, quantity, size and volume. Perhaps the most well-studied post-copulatory trait under selection is testes size. This is explained by the mating rate hypothesis, which predicts that larger testes are associated with a greater number of ejaculates and therefore males can engage in frequent copulations (Vahed & Parker, 2012). Testes size should therefore show a positive relationship with increasing levels of polyandry as frequent matings by males often demand more ejaculates. This was supported by a study of 21 bush cricket species (Tettigoniidae), where testes mass positively correlated with the degree of polyandry (Vahed et al., 2011). In contrast, the numerical sperm competition hypothesis suggests that larger testes have a higher number of sperm per ejaculate, giving the male an advantage during sperm competition (Vahed & Parker, 2012). Because males are under intense competition, they must invest into strategic sperm allocation (Birkhead & Pizzari, 2002). There are several ways they can do this. The first way is by increasing the number of sperm in the ejaculate (sperm concentration), commonly seen in crabs (Jivoff & Hines, 1998), birds (Hunter et al., 2000; Nicholls et al., 2001), insects (Kelly, 2008), and many other taxa. However, as sperm competition increases, individual males should invest less into each ejaculate because the probability of fertilisation is reduced (Stockley et al., 1997). For example, in the Australian field cricket (*Teleogryllus oceanicus*), males adjust their ejaculate size based on female mating status (Thomas & Simmons, 2007). Males transferred sperm of lower viability to unmated females and females that had mated many times, and sperm of higher viability to females that had mated once (Thomas & Simmons, 2007). Despite this, some taxa have shown the opposite effect and instead increase the number of sperm with increasing sperm competition (Aron et al., 2016; Garbaczewska et al., 2013).

Sperm size and quality also play an important role in strategic ejaculation. Sperm size is highly variable among species, ranging from a few micrometers to over 5 cm in length, and there is conflicting evidence on how sperm size is linked to increased competitive ability for fertilisation success (Snook, 2005). For example, in a study of 21 species of passerine birds, longer sperm were selected for under intense sperm competition, possibly because longer sperm are associated with increased swimming velocities (Briskie et al., 1997). However, in the dung beetle (*Onthophagus taurus),* shorter sperm actually increase fertilisation success, supporting the theory that sperm competition should favour the evolution of numerous smaller sperm (García-González & Simmons, 2007). Sperm viability is also important in intense sperm competition. Increasing the number of viable sperm in an ejaculate will increase the chance of fertilisation success, as seen in the cricket *Teleogryllus oceanicus* (García-González & Simmons, 2005). A comparative study suggested that promiscuous species produced more viable sperm than monogamous species, highlighting the importance of sperm viability in sperm competition (Hunter & Birkhead, 2002; Snook, 2005). Compared to pre-copulatory traits, there is much less work looking at how selection may drive differences in post-copulatory traits, however it is a growing area of study.

Sexual selection is thought to favour the evolution of positive allometries in pre-copulatory traits (Petrie, 1988, 1992; Tomkins & Simmons, 1996), and, in contrast, negative allometries are commonly associated with post-copulatory traits (Eberhard et al., 1998). A well-known example of this is the exaggerated antlers of the extinct Irish elk (*Megaloceros giganteus*) (Gould, 1974). In contrast to many pre-copulatory traits, the genitalia of insects and spiders typically show a negative allometric slope and it is predicted that genitalia show lower allometric values than other body parts (Eberhard et al., 1998). This negative allometric scaling of genitalia may be explained by the one size fits all hypothesis, originally proposed by Eberhard et al. (1998). According to this hypothesis, male genitalia are designed to fit the most common size or internal genital structures of females in the population. While traits used directly during copulation, such as aedeagus length and claspers, are expected to show negative allometry (Eberhard et al., 1998), there is a lack of studies exploring the allometry of post-copulatory traits such as testes and sperm size. However, one study that explored the allometry of testes in Scathophagidae dung flies has shown they exhibit positive allometry (Hosken et al., 2005), while sperm size is likely to show negative allometry based on stabilising post-copulatory selection which selects for less variable sperm (Fitzpatrick & Baer, 2011). Some studies have shown that genitalia such as the gonopodium may show positive allometry (Kelly et al., 2000; Lüpold et al., 2004), and such variation in allometry may suggest functional differences of male genitalia. This variation provides the opportunity to compare the allometry of post-copulatory traits to pre-copulatory traits under sexual selection and how they may differ based on their functional significance. Further, more research across species with different mating systems is required.

The New Zealand giraffe weevil, *Lasiorhynchus barbicornis* (Coleptera: Brentidae*)* is an ideal species to explore the interaction between pre- and post-copulatory sexual selection. It shows extreme sexual dimorphism and both sexes are highly variable in size where males can vary between 14-90mm, and females between 12-50mm in total body length (Painting et al., 2014). Female giraffe weevils use their rostrum to drill holes into dying, native trees such as karaka (*Corynocarpus laevigatus*), where they will oviposit their eggs (Meads, 1976). Males bear an extremely elongated rostrum, that comprises approximately half their total body length and shows steep positive allometry, which they use as a weapon in aggressive contests for access to females for copulation (Painting & Holwell, 2014a, 2013). Small male giraffe weevils have reduced success in acquiring mates compared to their larger male counterparts which mate more frequently over their lifespan (LeGrice et al., 2019). This size variation in males is thought to have driven the evolution of a conditional strategy in the form of alternative reproductive tactics (ARTs) where males use either mate guarding and fighting, or a sneaking tactic based on their absolute body size and the size of their opponent (Painting & Holwell, 2014b). Males less than 30 mm in size generally employ sneaking tactics, where they discreetly engage in copulations with females to avoid conflict with larger males. Female giraffe weevils are highly polyandrous and can store sperm, driving sperm competition and potentially cryptic female choice (LeGrice et al., 2019). While small males are likely always at risk of sperm competition because large males mate more frequently, there may be an interesting interplay between the level of polyandry and male body size due to size-assortative mating. Large males mate with the largest and most polyandrous females, suggesting that they too may suffer intense sperm competition (LeGrice et al., 2019). The combination of all of these unique traits presents an exciting opportunity to explore sperm competition in a promiscuous highly size variable beetle.

However, although the relationship between pre-copulatory weaponry, body size and mating success in the New Zealand giraffe weevil has been well described, the relationship between body size and post-copulatory traits that may be important for fertilisation success is currently largely unknown. Previous research found no evidence of a negative correlation (trade-off) between relative testes diameter and rostrum length (Painting & Holwell, 2013), but this method of measuring testes size has been under scrutiny (Tomkins & Simmons, 2002). This is because testes diameter can be affected by factors other than post-copulatory selection (Calhim et al., 2007). For example, testes size can co-vary with sperm depletion risk (Cartar, 1985). Furthermore, not much is known about sperm form and function (i.e., sperm concentration, sperm length) within the testes and how this may vary with different male body sizes. Therefore, with such a high variability in body size among males, there is likely to be different levels of investment in pre- and post-copulatory traits.

Here I use morphological measurements and allometry analysis to examine the interaction between pre- and post-copulatory selection on traits in male giraffe weevils. I first ask the question, how are body size variation and ARTs linked to investment in pre- and post-copulatory traits? Based on the findings by Voje (2016) and Kodric-Brown et al., (2006), I predict that post-copulatory traits (testes size, sperm form and quality) in male New Zealand giraffe weevils will be negatively allometric. Specifically, smaller male giraffe weevils will invest relatively more into their post-copulatory traits to compensate for their comparatively low rate of mate acquisition and increased risk of sperm competition compared to large males. Following this question, I explore the potential trade-offs between pre- and post-copulatory traits (based on the assumption that there is a limited resource pool). I predict that due to limited resource availability, males with larger weapons may be limited in the resources they can allocate to post-copulatory traits, suggesting a negative relationship between relative weapon and relative trait size. However, larger males are predicted to be better at acquiring resources and thus have more to invest across all traits (van Noordwijk & de Jong, 1986), which may prevent a trade-off between pre- and post-copulatory traits in males with large weapons as they are able to acquire enough resources to allocate to both types of traits.

Materials and methods

Specimen collecting

I collected 50 adult males of *L. barbicornis* between January and March 2021 from a wild population at Maungatautari Mountain in Waikato, New Zealand (approximately 38°03'06.9"S 175°34'08.6"E). Males were euthanised in the freezer at -20°C and stored individually in plastic vials.

Morphological measurements

Measurements were made using Rok digital calipers to the nearest 0.01 mm. Pronotum width was measured as the width of the pronotum in dorsal view. Weapon size (a pre-copulatory trait used in contests to acquire mates) was measured as the total length of the rostrum from the tip of the mandibles to the base of the head.

To dissect out internal structures the elytra and hindwings of males were first removed using micro-dissecting spring scissors. The abdomen was then carefully cut on each side, bearing as close to the outside of the abdomen as possible to prevent any damage to internal organs. Males were placed in a plastic vial along with their elytra and wings and the vial was filled with 0.9% saline solution. They were left to rehydrate for approximately 30 minutes before being dissected under an Olympus SZX7 microscope.

To measure variation in genitalia and sperm traits, I conducted dissections of the testes, vas deferens and aedeagus. The aedeagus, two bi-lobed testes and vas deferens were removed using micro-dissecting spring scissors and then placed onto a microscope slide with a small amount of saline solution to keep them hydrated. The vas deferens was carefully dissected from the testes and placed into a polymerase chain reaction (PCR) 0.2 mL tube along with 30 uL of saline for later use. The testes were then arranged in the same orientation on the microscope slide to ensure consistent measurement across individuals (**Figure 2.1**). The aedeagus was placed on the same slide (**Figure 2.1**). Olympus cellSens imaging software was used to take a photo of the testes and aedeagus. Image J was used to take measurements of testes diameter and aedeagus length. Testes diameter was measured as the short axis of the average diameter of each lobe in ventral view. Aedeagus length was measured from the tip of the aedeagus to the end of the aedeagus. Tegmen length was also measured, and is shown in Figure 2.1.

Following this, a disposable pipette was used to carefully remove the remaining saline on the slide, avoiding the testes. To prevent any dust particles from contaminating the samples, I used gloves to handle them and a 1 L Sistema container for storage. Using gloves, foil was cut up into approximately 5 cm x 5 cm squares and stored in an empty 1 L Sistema container that had been previously wiped with ethanol. All weights were taken using a Sartorius Quintix 35-1S balance to the nearest 0.01 mg. Before the testes were weighed, the foil number and date was written on the outside of the foil using a permanent marker. This was done prior to weighing as such a precise scale can be affected by marker fluid. The foil square was then placed onto the scale, and the weight recorded. The two bi-lobed testes were then placed onto the foil square while it was still on the scale, and the weight recorded again. Once weighed, the foil was folded carefully around the testes and placed into a clean 1 L Sistema container that had been wiped with ethanol. All tissue samples were stored in the fridge until I was finished sampling for the day, where they were then placed in a drying oven later the same day. To measure testes dry weight, I placed the foil packets into a Contherm digital series incubator at 55°C for approximately 2 days. Once samples had dried, they were transported from the oven to the balance in the 1 L Sistema container, handled using gloves, and re-weighed. The final measurement was recorded as the total dry weight of both bi-lobed testes combined.

To measure variation in sperm concentration and sperm length among males, the vas deferens (where mature sperm are stored) was carefully placed into a PCR tube with 30 microlitres uL of 0.9% saline solution, ruptured with tweezers, and then vortexed for 20s on a gentle cycle. To measure sperm concentration, 10 uL of the vas deferens solution was added to a haemocytometer and then it was placed under an Olympus BX43 microscope at 40x magnification, using phase contrast. The number of sperm heads per square for 5 large squares were counted (16 small squares per 5 large squares = 80 squares total). Sperm concentration was calculated as (number of sperm counted x dilution) divided by (number of squares x 4nL) to produce a final concentration of million/mL. To measure sperm length, 5 uL of the vas deferens solution was placed on a microscope slide with a cover slip. Ten randomly chosen individual sperm were photographed at 40x magnification, under phase contrast using an Olympus DP22 microscope camera. Sperm length was measured in ImageJ using the segmented line tool. Any unused tissue of the males was placed into 70% ethanol and back into a well-labelled vial in the freezer at -20°C for long term storage. The PCR tubes with the aedeagus and remaining vas deferens solution were stored in the vial with the other remaining tissue.

Figure 2.1. Photos showing the aedeagus, tegmen, testes, and sperm of male *Lasiorhynchus barbicornis*. Top left shows the aedeagus (measured between the black arrows), and the tegmen (measured between the red arrows) at 0.6x magnification. Bottom left photo shows the testes at 0.6x magnification. Right photo shows the sperm at 20x magnification, note the tail has become uncoiled.

Statistical analysis

All statistical analyses were conducted in R 3.6.3 (R Core Team, 2021). Histograms and scatterplots were first used to identify any potential outliers and measurement errors in the dataset for all traits. Because giraffe weevils have bilobed testes, symmetry was determined using paired t-tests to compare both the left and right lobes of each testis for each male. Testing for symmetry in the testes was necessary as it determined whether I could use just one of the lobes of one of the testes in subsequent analyses. I decided to use the average of all four lobes for testes diameter and area because the testes were not symmetrical.

The allometric relationships between body size (pronotum width) and seven traits (rostrum length, sperm concentration, sperm length, aedeagus length, tegmen length, testes diameter, and testes weight) were described using ordinary least squares (OLS) regression models. All traits were log-transformed prior to analysis. For the OLS regression where testes weight was the response variable, pronotum width was logged, and then cubed to ensure it was comparable to testes weight. I determined whether a trait deviated from isometry based on whether the slope and 95% confidence intervals encompassed 1.

I used two methods to determine possible trade-offs between weapon size (rostrum length, a pre-copulatory trait) and post-copulatory traits (testes diameter, testes mass, sperm length, sperm concentration). Firstly, I used a series of multiple regression analyses with the post-copulatory trait of interest as the response variable (i.e., testes weight, testes diameter, sperm length, sperm concentration), and body size (pronotum width) and weapon size (rostrum length) as explanatory variables. Due to the high level of correlation between pronotum width and rostrum size, I checked for possible collinearity using variance inflation factors (VIFs) with the 'vif' function in the *car* package (Fox & Weisberg, 2019). The VIF returned a result of 31.05 which is higher than the generally acceptable level of 10 (A. F. Zuur et al., 2010). Therefore, I also used residual analyses to compare relative weapon size to relative post-copulatory trait size (Emlen, 2001). I first conducted two OLS regression models using: (1) weapon size (log rostrum length) and body size (log pronotum width) and (2) the trait of interest (i.e., log sperm length, log testes diameter, log testes weight) and body size (log pronotum width). I then took the residuals from each of these models and did a third linear regression to get the relationship between the relative trait (i.e., log sperm length, log testes diameter, log testes weight) size and relative weapon size. This method, however, is also not without its problems and can lead to biased parameter estimates (Freckleton, 2002; Garcia-Berthou, 2001). I therefore present the results of both approaches, which found almost identical slope estimates and errors.

Results

Scaling relationships of pre- and post-copulatory traits

The mean, standard errors, and ranges of each trait for male giraffe weevils can be found in **Table 2.1**. The relationship between body size and each trait is presented in **Table 2.2** and **Figure 2.2**. As found previously (Painting & Holwell, 2013), rostrum length was steeply positively allometric. I also found a strong positive relationship between body size and sperm concentration (*b* = 1.78, df = 48, *SE* = 0.29, $p < 0.0001$, $R^2 = 0.44$) with larger males showing an estimated 155% increase in sperm per million/mL compared to the smallest male measured. However, all other post-copulatory traits (sperm length, aedeagus length, tegmen length, testes diameter, and testes weight) showed significant negative allometry. While sperm length showed a significant negative allometric relationship with body size (*b* = 0.02, df = 48, *SE* = 0.007, $p < 0.008$, $R^2 = 0.13$), the effect size was negligible; there was only a 2% predicted difference in sperm length between the smallest and largest males measured.

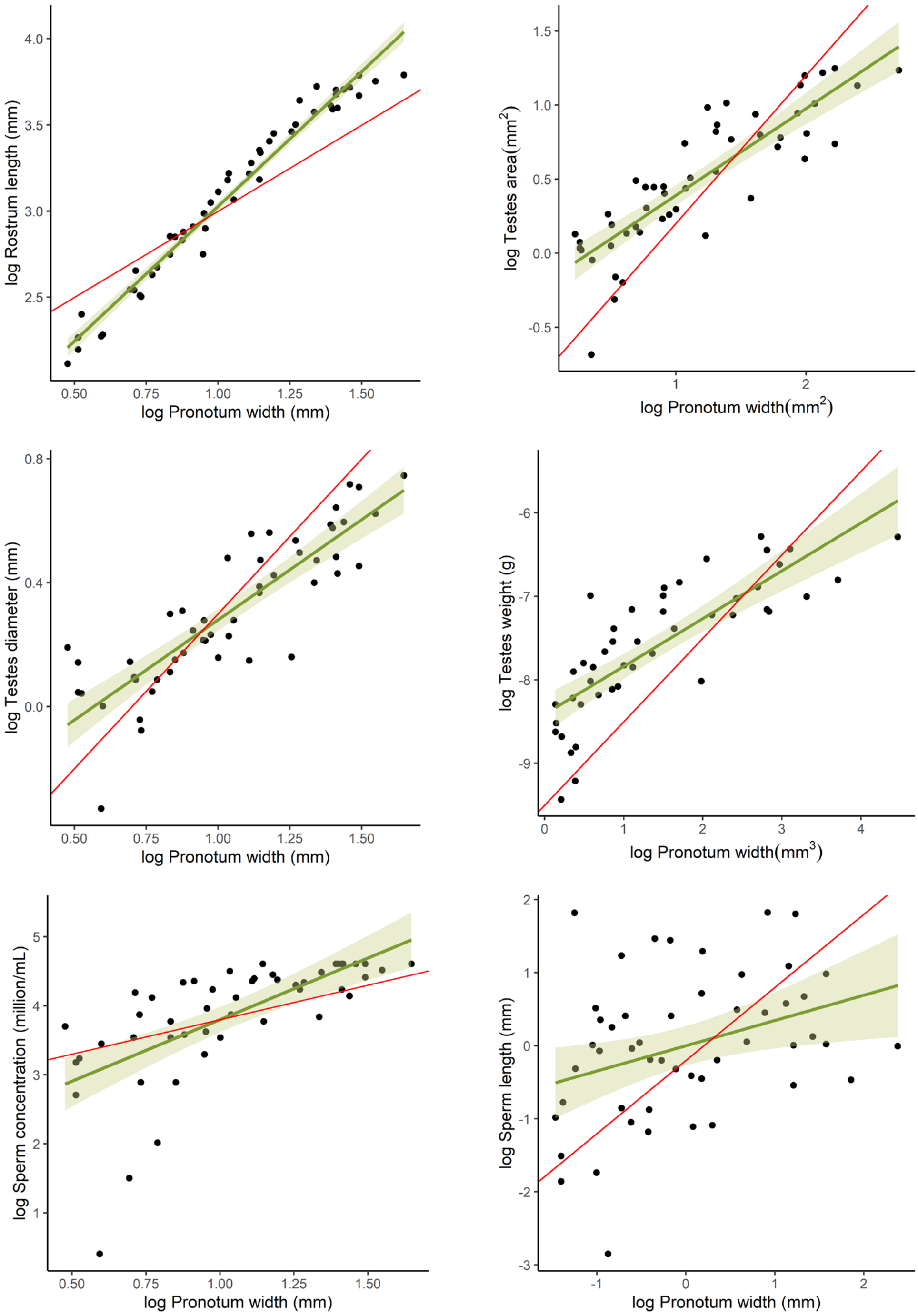
Table 2.1. Means, standard errors and ranges of trait size for male *Lasioryhynchus barbicornis*.

Trait	Mean size	*SE*	Min. size	Max. size
Pronotum width (mm)	2.98	0.13	1.61	5.19
Rostrum length (mm)	24.76	1.61	8.29	44.26
Total body length (mm)	50.28	2.84	20.23	86.44
Sperm length (um)	0.21	0.00000053	0.20	0.22
Sperm concentration (million/ml)	60.24	4.26	1.50	91.5
Aedeagus length (mm)	2.35	0.056	1.47	3.28
Tegmen length (mm)	2.11	0.051	1.44	2.98
Testes diameter (mm)	1.40	0.05	0.72	2.11
Testes weight (mg)	0.01	0.0042	<0.001	0.12

Table 2.2. Allometric values and confidence intervals for each trait (log transformed) regressed against log pronotum width. Significant *p* values indicate that the slope differs from isometry (slope of 1).

Trait	Slope (*b*)	lower CI	upper CI	R^2	*P*
Rostrum length	1.56	1.48	1.65	0.97	<0.001*
Sperm length	0.02	0.01	0.04	0.14	0.01*
Aedeagus length	0.41	0.32	0.50	0.64	<0.001*
Tegmen length	0.49	0.42	0.55	0.84	<0.001*
Testes diameter	0.65	0.54	0.76	0.75	<0.001*
Testes weight	0.57	0.45	0.70	0.65	<0.001*

*indicates significant *p* value


log Rostrum length (mm)
2.5
3.0
3.5
4.0
0.50
0.75
1.00
1.25
1.50
log Pronotum width (mm)
log Testes area(mm^2)
-0.5
0.0
0.5
1.0
1.5
1
2
log Pronotum width(mm^2)
log Testes diameter (mm)
0.0
0.4
0.8
0.50
0.75
1.00
1.25
1.50
log Pronotum width (mm)
log Testes weight (g)
-9
-8
-7
-6
0
1
2
3
4
log Pronotum width(mm^3)
log Sperm concentration (million/mL)
1
2
3
4
5
0.50
0.75
1.00
1.25
1.50
log Pronotum width (mm)
log Sperm length (mm)
-3
-2
-1
0
1
2
-1
0
1
2
log Pronotum width (mm)

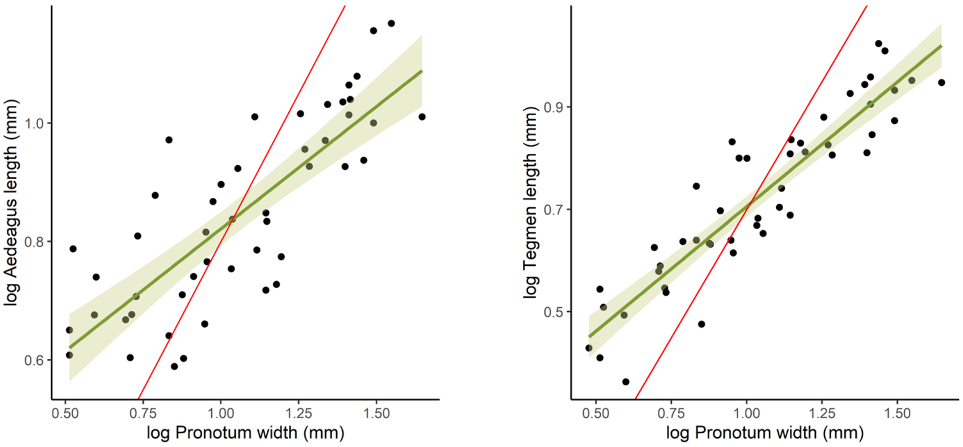


Figure 2.2. The allometric slope of each trait against body size with a confidence interval of 95%. Each black point represents a male New Zealand giraffe weevil. Red lines are a slope line of 1. Note that sperm length is mean-centred as the sperm length and pronotum width were on such different magnitudes of scale they could not be accurately plotted against each other.

Trade-offs between pre- and post-copulatory traits

There was no evidence of phenotypic trade-offs between weapon size (rostrum length) and any of the post-copulatory traits measured. The residual analyses found that relative rostrum length did not correlate with relative sperm length (*b* = 0.0149, df = 48, *SE* = 0.02, *p* = 0.58, R^2 = 0.006), relative sperm concentration (*b* = 1.6, df = 48, *SE* = 0.99, *p* = 0.113, R^2 = 0.05), or relative testes diameter (*b* = 0.15, df = 48, *SE* = 0.19, *p* = 0.42, R^2 = 0.01). However, testes dry weight (*b* = 0.0488, df = 44, *SE* = 0.02, *p* = 0.002, R^2 = 0.188) showed a significant positive relationship with relative rostrum length. The results of the multiple regression analyses were almost identical, with no qualitative differences when compared to the residual analyses (Table 2.3**,** Figure 2.3).

Table 2.3. Multiple regression outputs with rostrum length as the explanatory variable and each post-copulatory trait as the response variable. Each post-copulatory trait is log-transformed.

Trait		Slope (*b*)	*SE*	t value	*P*
Sperm length	intercept	-8.496	0.040	-212.904	0.001*
	log pronotum width	-0.002	0.042	-0.057	0.954
	log rostrum length	0.010	0.027	0.559	0.579
Sperm concentration	intercept	-0.328	1.497	-0.219	0.828
	log pronotum width	-0.718	1.591	-0.451	0.654
	log rostrum length	1.600	1.001	1.599	0.116
Testes diameter	intercept	-0.593	0.287	-2.069	0.044
	log pronotum width	0.406	0.305	1.332	0.189
	log rostrum length	0.154	0.192	0.805	0.425
Testes weight	intercept	-9.124	0.249	-36.664	0.001*
	log pronotum width	-0.018	0.196	-0.093	0.927
	log rostrum length	0.049	0.015	3.155	0.003*

*indicates significant *p* value

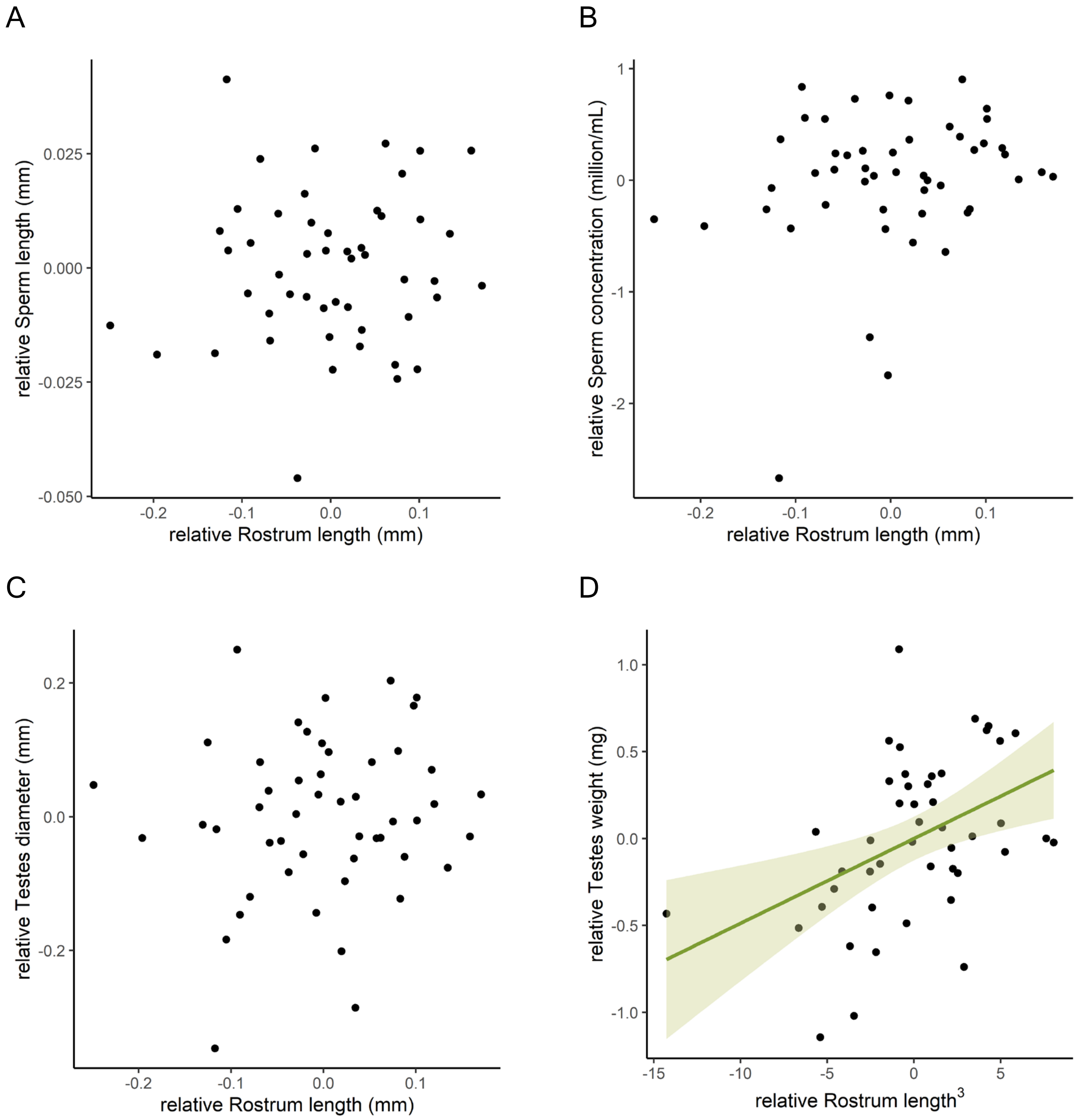


Figure 2.3. Relative trait size in relation to relative rostrum length for male *Lasiorhynchus barbicornis*. Relative trait size was calculated by taking the residuals of A) sperm length, B) sperm concentration, C) testes diameter, and D) testes weight from the best fit regression model of the trait against pronotum width. The line of best fit was added for any slopes that were significant ($p < 0.05$).

Discussion

My analyses show that rostrum length, a pre-copulatory trait, showed positive allometry and post-copulatory traits showed negative allometry. It is unsurprising that rostrum length showed a positive relationship with body size as previous research by Painting and Holwell

(2013) has found the same result, with large males investing proportionately more into their rostrum (weapon). In accordance with previous research (Eberhard et al., 1998; Voje, 2016), my results support the predictions that post-copulatory traits are generally negatively allometric. This suggests that smaller males may invest relatively more into post-copulatory traits than large males, possibly due to an increased risk and intensity of sperm competition. However, I found a strong positive relationship between sperm concentration and body size, which is surprising as sperm competition theory predicts small males should invest more into sperm traits as they experience higher levels of sperm competition (Parker, 1970, 1990b). This result does not support my prediction, and in fact, contradicts it, suggesting larger males have more resources to invest in sperm concentration compared to smaller males. Additionally, I found no evidence of a trade-off between pre- and post-copulatory traits in the New Zealand giraffe weevil. I found no relationship between relative weapon size and relative trait size except for testes weight. Testes weight showed a positive correlation with rostrum length, suggesting that males with relatively long weapons invest in absolutely larger testes. Thus, it appears that males with relatively large weapons have enough resources to invest in both weapons and testes weight to gain a reproductive advantage. Additionally, my results suggest that large males experience an accumulation of benefits such as more sperm to increase their reproductive success and fitness.

The post-copulatory traits I measured in this study generally showed negative allometry, consistent with the literature (Eberhard et al., 1998; Galicia-Mendoza et al., 2021; Voje, 2016). The one-size-fits-all hypothesis suggests that male genitalia are negatively allometric as they need to be able to fit the most common size or internal genital structures of females in the population (Eberhard et al., 1998). My results are consistent with this theory, whereby the aedeagus and tegmen were negatively allometric. However, this theory fails to address the extreme size variation in giraffe weevils, leaving the question of "How can males of varying sizes mate with females of varying sizes?", unanswered. Additionally, how testes size scales with body size is largely unknown. A study on dung flies, Scathophagidae*,* found that testes size showed positive allometry (Hosken et al., 2005). In contrast, my results show that testes size was negatively allometric, suggesting smaller males have disproportionately larger testes compared to larger males. This supports my prediction that small males invest more into post-copulatory traits that give them a competitive advantage when facing a higher risk of sperm competition. Similar results were found in Simmons and Emlen (2006), whereby males that had the biggest horns (weapons) showed the most shallow, or negative, testes allometry. Traits that are nutrition-sensitive (phenotypically plastic), generally show positive allometry compared to traits that are nutrition-insensitive (developmentally canalized) which generally show negative allometry (Simmons & Emlen, 2006). Therefore, it is likely that testes are not in direct competition for resources with surrounding traits, showing negative allometry.

Males that experience a competitive disparity are likely to invest more into post-copulatory traits according to sperm competition theory (Parker, 1970). Most of the post-copulatory traits I measured showed negative allometry, suggesting small males invest more into the post-copulatory traits measured, as I predicted. However, I found a positively allometric relationship between sperm concentration and male body size, suggesting large males have disproportionately higher concentrations of sperm. I expected to see a reduction in sperm concentration with increasing body size as smaller males experience higher levels of sperm competition (Parker, 1990b), but I found a 155% difference in sperm concentration beween the smallest and largest male. Therefore, this result is contrary to what theory predicts, that males should transfer more sperm when there is an increased risk of sperm competition (Parker et al., 1996, 1997). This finding could be explained if sperm production is condition-dependent (Zahavi, 1975). If ejaculate traits are costly to produce and maintain, then we would expect them to be condition-dependent (Andersson, 1994; Bonduriansky & Rowe, 2005; Rowe & Houle, 1996). There is empirical evidence to suggest that nutrient availability may affect ejaculate traits (Kahrl & Cox, 2015; Perry & Rowe, 2010; Rahman et al., 2013) and subsequently how many resources a male can invest into reproduction. High-condition males have been shown to produce larger numbers of sperm (Bunning et al., 2015; Perez-Staples et al., 2008), suggesting that this particular post-copulatory trait may be condition-dependent. Therefore, with the assumption that larger (i.e., higher-condition) males can acquire and allocate more resources (van Noordwijk & de Jong, 1986), increasing sperm concentration with body size may be an indication of having increased resources to invest into post-copulatory traits. Large males may therefore be accumulating more matings during their lifetime, and may also be able to engage more effectively in sperm competition for fertilisation opportunities compared to small males, making them competitively superior.

However, although sperm concentration was measured, ejaculate size was not. Ejaculate size is worth including in studies examining post-copulatory traits as sperm only constitutes a small part, the rest being seminal fluid which contains components that influence sperm motility and activity and also substances responsible for several behavioural and genetic changes in females (i.e., increased oviposition) (Chapman &

Davies, 2004; Poiani, 2006; Reinhardt et al., 2009). Ejaculate traits are usually influenced by sperm competition, with evidence to suggest individuals that face increased sperm competition transfer bigger ejaculates (Wedell & Cook, 1999; Wylde et al., 2019). However, there is empirical evidence to suggest larger males produce bigger ejaculates (Blanco et al., 2006; Fedina & Lewis, 2004). This is likely due to condition-dependence, but how males alter their ejaculate composition or volume in response to sperm competition is not well known. We would expect that, because larger males typically have higher mating rates (Bonduriansky & Brassil, 2005; LeGrice et al., 2019), they may evolve strategic ejaculate allocation strategies to take advantage of frequent matings. On the other hand, small males may invest maximally in all matings because their probability of mate acquisition is lower, and they do not have the resources to elevate ejaculate expenditure when facing increased sperm competition risk (Wylde et al., 2019). Exploring the relationship between ejaculate size and body size will provide further information on the extent to which male New Zealand giraffe weevils are investing in post-copulatory traits.

My allometric analyses produced contrasting results. For most post-copulatory traits, I found negative allometry (e.g., testes size), but sperm concentration showed positive allometry. Thus, my results do not show a consistent pattern across males of different body sizes and all the traits measured. Small males have bigger testes relative to their body size, which may allow them to have bigger ejaculates to combat sperm competition. On the other hand, large males have higher numbers of sperm, which will also help them during sperm competition. Such differing results mean there is no clear answer as to which males will benefit from these traits, but it is interesting to consider that males of different body sizes may be engaging in sperm competition in different ways. Future research looking at the relationship between ejaculate size, testes size, and ARTs may aid in our understanding of how males of different body sizes are engaging in sperm competition.

Contrary to my prediction that males may trade-off investment between pre- and post-copulatory traits, I found that males with relatively larger weapons (irrespective of body size) had relatively heavier testes. Larger testes are thought to be indicative of increased sperm-producing tissue (Hosken, 1997; Kusano et al., 1991; Lüpold et al., 2009; Møller, 1988; Schultz, 1938), consistent with my findings where large males have higher quantities of sperm. My findings suggest that there was no trade-off between weaponry and testes in this study, rather that the expression of the trait is condition-dependent, and those that have enough resources can allocate to both types of traits.

It is possible that there is indeed a trade-off between pre- and post-copulatory traits, but I was unable to detect any in this study as I only looked at phenotypic correlations which offer little support for evidence of trade-offs (Simmons et al., 2017). Experimental manipulations are a powerful way to test for trade-offs between pre- and post-copulatory traits as all other variables are held constant, which prevents environmental factors from having an effect (Simmons et al., 2017; Simmons & Emlen, 2006). For example, leaf-footed bugs, *Mictis profana*, that drop their weapon grow larger testes in an experimental study, however, in the wild, a positive correlation was found between testes size and weapons (Somjee et al., 2018). Studies that have tested condition-dependence in traits subject to post-copulatory competition have demonstrated that nutrition influences sperm quality (Gage & Cook, 1994; Simmons, 2012), ejaculate size (Ferkau & Fischer, 2006; Jia et al., 2000; Lewis & Wedell, 2007; Perry & Rowe, 2010), and testes size (Ward & Simmons, 1991). However, this result is contradictory to theory which predicts that smaller males are under higher sperm competition and thus are likely allocate more resources to testes size (Gage, 1994; Hosken, 1997; Møller, 1988; Stockley et al., 1997). Although, not all species with small male morphs increase testes size in response to sperm competition, which may be explained by variation in the costs of gaining mates (Munguía-Steyer et al., 2012). Therefore, large males are better at acquiring mates than smaller males, but also might have an advantage during sperm competition by having larger testes and producing more sperm, contrary to my initial predictions.

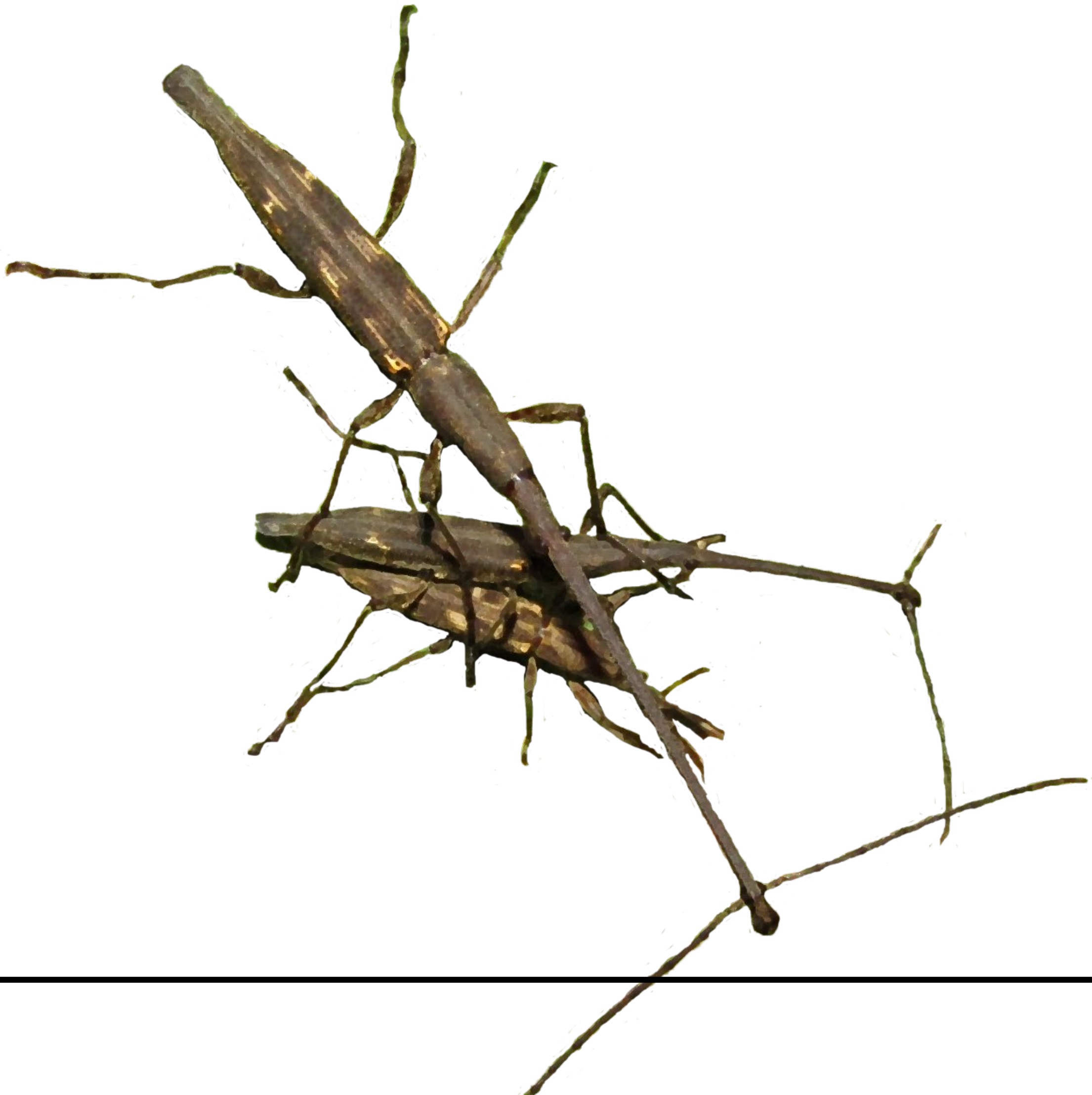
Trade-offs are expected based on the assumption that holometabolous insects have a limited resource pool along with adjacent developmental traits which use resources from the same pool for growth (Emlen, 2001). Previous research investigating trade-offs between pre- and post-copulatory traits in animals have shown mixed results (Lüpold et al., 2015; Simmons & Emlen, 2006), demonstrating that there is no universal trend among animals with exaggerated traits. For example, in the *Onthophagus* dung beetles, males that develop without horns grow larger bodies and invest more in testes growth, suggesting a trade-off between weapons and testes (Simmons & Emlen, 2006). In contrast, a study by Lupold et al. (2015) showed that there was no evidence of a trade-off between weaponry and sperm length in five taxa (primates, ungulates, pheasants, stalk-eyed flies, and dung beetles). Many correlational (Pizzo et al., 2012; Simmons, Emlen, et al., 2007b; Simmons et al., 1999) and experimental studies manipulating weapon size have shown trade-offs with post-copulatory traits such as testes size (Moczek & Nijhout, 2004; Okada & Miyatake, 2009; Simmons & Emlen, 2006; Somjee et al., 2018; Yamane et al., 2010). However, trade-offs are typically only apparent in environmental conditions that evoke stress (e.g., food shortages) (Metcalfe, 2003; van Noordwijk & de Jong, 1986), and studies that only examine phenotypic correlations (as I do here) may not be able to reveal the extent of trade-offs among traits.

Sperm length showed a significant positive relationship, but was negatively allometric with body size, albeit the effect size was small. There is contrasting evidence in the literature regarding sperm length variation in response to sperm competition (Briskie et al., 1997; Gage, 1994; Hosken, 1997; Stockley et al., 1997). Several studies have shown that sperm length increases in response to sperm competition (Byrne et al., 2003; Fitzpatrick et al., 2009; Immler & Birkhead, 2007). Longer sperm is thought to be selectively favoured during sperm competition as longer sperm may generate more power and swim faster (Birkhead, 1998). For example, in the snail, *Viviparus ater*, males with the longest sperm conferred higher fertilisation success and sired the most offspring (Oppliger et al., 2003). Long sperm are also better at displacing shorter sperm from the site of fertilisation, suggesting a competitive advantage (Colegrave et al., 1995; Pattarini et al., 2006). In contrast to fewer, longer sperm, sperm competition theory predicts that tiny, numerous sperm are favoured (Parker, 1982), and this is supported by numerous studies (García-González & Simmons, 2007; Moatt et al., 2014). For example, in the cricket, *Gryllus bimaculatus,* males that produced relatively small sperm and relatively numerous sperm, won competitions for fertilisation (Gage & Morrow, 2003). Such variation in sperm length in response to sperm competition may be explained by how the benefits of sperm size change with sperm density (Immler et al., 2011). A species with a low risk of sperm competition may show a positive relationship between sperm competition risk and sperm size, whereas a species with a high risk of sperm competition may show little or no relationship between sperm competition risk and sperm size (Immler et al., 2011). My results show that large male New Zealand giraffe weevils had longer sperm, likely due to a decreased risk of sperm competition, consistent with theoretical predictions by Immler et al., (2011). Larger males having longer sperm, higher sperm concentration and larger testes suggests that large males may be competitively superior in pre-copulatory competition and post-copulatory competition.

Male giraffe weevils had positively allometric pre-copulatory traits, and majority of post-copulatory traits were negatively allometric. Interestingly, small males invested disproportionately more into their post-copulatory traits (e.g., testes weight), except for sperm concentration, which was much higher in large males. Therefore, my results suggest males of different body sizes may be engaging in sperm competition in different ways. With exaggerated traits being costly to produce and maintain, it seems sensible to assume there may be a trade-off with the investment in and maintenance of other traits. However, I did not find evidence of trade-offs between pre- and post-copulatory traits in the New Zealand giraffe weevil. Previous research has shown that large male giraffe weevils mate more frequently, suggesting large males are likely to be facing a high intensity of sperm competition which may result in increased sperm concentration, longer sperm, and larger testes (LeGrice et al., 2019). Painting and Holwell (2014) also found that larger males won the majority of fights over smaller males and therefore gained access to and monopoly of oviposition sites ensuring a relatively high number of mating opportunities. However, research by Painting and Holwell (2014b) found that small males may mate as frequently as large males. Therefore, further research is needed to quantify individual variation in mating success and the factors that may be affecting mating success In the New Zealand giraffe weevil, being large may be beneficial due to advantages both during mate aquisition and fertlisation, but the reproductive success of different sized males is still unclear. Overall, giraffe weevils offer an exciting opportunity to explore the interaction between pre- and post-copulatory trais, and how these may affect total reproductive success.

3

Investigating the effects of population density and sex ratio on male mating success



Abstract

Population dynamics such as density and sex ratio can strongly affect the intensity of competition for mates and consequently, the opportunity for sexual selection to occur. Perceived differences in the density of competitors and available mates, and thus sperm competition, may result in changes to a male's reproductive strategy and success. Whilst several studies have examined the effects of population density and sex ratio on sexual selection in a controlled laboratory environment, few have attempted to quantify its effects in natural populations, particularly in insects. Here, I use the endemic New Zealand giraffe weevil (*Lasiorhynchus barbicornis*), a highly polyandrous and sexually dimorphic beetle, to examine how population density and sex ratio can influence male mating behaviour and sexual selection, both experimentally and in the wild. I find that temporal fluctuations in population density, sex ratio, and body size can have an important influence on the mating dynamics of the giraffe weevil in a natural population. Male giraffe weevils had density-dependent mating success, with small males doing best at high densities, and large males at low densities. Further, my laboratory-controlled behavioural assays also show that males employ a reproductive allocation strategy where increased investment in copulation is associated with lower levels of mate-guarding. These findings suggest that male giraffe weevils have evolved several adaptive mate strategies that are density-dependent. Overall, this work emphasises the importance of using both laboratory and field studies to increase our understanding of how sexual selection can shape the life-history of an insect.

Introduction

Sexual selection is a driving evolutionary force, shaping much of the phenotypic diversity seen in nature (Rieseberg et al., 2002; Ritchie, 2007; Schluter, 2009; van Doorn et al., 2009). Sexual selection is dynamic, and can vary in strength (e.g. strong or weak; Hereford et al., 2004; Kingsolver et al., 2001), direction (e.g. positive or negative; O'Brien et al., 2017), form (e.g. linear or nonlinear; Brodie et al., 1995), space (Monteiro & Lyons, 2012; Thompson, 2005), and time (Grant & Grant, 2002). Research examining how the environment, such as temperature or population dynamics, influences the agents of sexual selection — mate choice, sperm competition, cryptic female choice, and male-male competition — is becoming increasingly common (Cornwallis & Uller, 2010; Cotton et al., 2006; Kasumovic et al., 2008). For example, population density (Jann et al., 2000a; Punzalan et al., 2010; Rittschof, 2010), sex ratio (Jann et al., 2000a; Punzalan et al., 2010) and the presence of heterospecifics (Rundle et al., 2008) can all change the strength, form, and direction of sexual selection (Miller & Svensson, 2014). Despite empirical evidence to show that sexual selection is influenced by environmental factors, research on sexual selection in insects has been predominantly laboratory based, and therefore research surrounding variation of sexual selection in natural populations is lacking (Svensson, 2019).

Sexual selection has the opportunity to influence fitness components such as mating success (often through female choice) and survival (costly and damaging interactions through male-male competition), both of which are strongly influenced by variation in spatial and temporal environmental conditions (Jann et al., 2000a). An important demographic factor is population density, which has been shown to have important impacts on sexual selection (Aronsen et al., 2013; Evans & Garcia-Gonzalez, 2016). However, it is important to recognise that population density is not a distinct environmental influence, and interacts with sex ratio and many other population parameters to influence total sexual selection (Kokko & Rankin, 2006). Operational sex ratio (OSR), the ratio of sexually competing males to sexually receptive females (Emlen & Oring, 1977), increases the mate encounter rate for one sex while decreasing the other (Kokko & Rankin, 2006). The effects of sex ratio and population density are closely intertwined, thus often making it very difficult to resolve the effects of the individual parameters on sexual selection (Head et al., 2008). For example, Alonso-Pimentel and Papaj (1996) showed that mating success in the walnut fly, *Rhagoletis juglandis*, was determined by both the density of each sex as well as the OSR. However, the interaction between sex ratio and population density are expected to increase the variance in mating and reproductive success (Emlen & Oring, 1977; Shuster & Wade, 2003).

Indeed, there is empirical evidence to show that population density and OSR affect both male and female mating behaviours (Arnqvist, 1992a; Borgia, 1980; Conner, 1989; Jann et al., 2000b; Jirotkul, 1999; Mills & Reynolds, 2003). It is predicted that as the population increases, males are more likely to encounter other males and as a result, male-male competition for access to females is likely to increase (Emlen & Oring, 1977; Knell, 2009). In these instances, being a large male can confer a significant advantage in male-male competition for access to females (Andersson, 1994), and there is evidence that relatively large males in a population have increased mating success (Biedermann, 2002; Krishna & Hegde, 2003; LeGrice et al., 2019). At high densities, large males may be better at monopolising females by excluding smaller males from securing mating opportunities (Rittschof, 2010). In contrast, at high densities, large males may be unable to successfully guard females or valuable resources (i.e., oviposition sites), resulting in scramble competition (Conner, 1989; Herberstein et al., 2017; McLain, 1992). When population density is low and females are widely dispersed, however, the frequency of male-male competition is likely to decrease (Legrand & Morse, 2000) eroding the benefits that large body size may confer (Bertin & Cézilly, 2005). Therefore, small males may have an advantage whereby they can find mates more quickly (Conner, 1989; McLain, 1992), and efficiently (Fairbairn, 2007). Determining the threshold of mating success at different population densities can provide information about how selection favours different behaviours, or in this case, body sizes, as density changes (Knell, 2009).

Two major agents of sexual selection, female mate choice and male-male competition are expected to be density-dependent. For females, the opportunity to exercise preference for quality mates is expected to increase as the proportion of males increase in the population (Kokko & Rankin, 2006; Shelly & Bailey, 1992). For example, in the katydid, *Xederra charactus*, females rejected male mating attempts more often in high population densities (29% of copulations) than in low densities (8% of copulations) (Lehmann, 2007). On the other hand, male-male competition is also expected to be density-dependent as harassment and aggression increases with increasing numbers of males in the population (Knell, 2009; Smith & Sargent, 2006). Therefore, variation in population density is likely driving traits that enhance male reproductive success. For example, male earwigs (*Forficula auricularia*) compete in intense contests for access to females, and at high densities, male fighter morphologies are more common (Tomkins & Brown, 2004). This supports the theory that increasing population density increases the opportunity, and intensity of, sexual selection on female choice and male-male competition (Darwin, 1871; Jann et al., 2000a; Sutherland, 1987; Wilkinson, 1987).

Similarly, a bias in the sex ratio can lead to intense competition between individuals of the sex in excess (Kokko & Rankin, 2006; Kvarnemo & Ahnesjo, 1996). A male-biased sex ratio is expected to increase competition and aggression between males (Smith & Sargent, 2006; Weir et al., 2011), influence the use of alternative mating tactics (Zamudio & Chan, 2008), and decrease mating success (Arnqvist, 1992b). Alternatively, a female-biased sex ratio is expected to decrease male-male competition and as a result, males are more likely to be choosier in mate selection (Painting et al., 2014). Furthermore, with fewer competitors, males are more likely to achieve full paternity and therefore should invest more into mating multiple times rather than guarding females (Berger-Tal & Lubin, 2011). There is some empirical evidence to support these predictions (Jirotkul, 1999; Kvarnemo & Ahnesjo, 1996), however the fitness consequences of such interactions are still not well known, especially in insects where it is often difficult to observe and track their behaviours in the wild (Rodriguez-Munoz et al 2010).

Variation in several mating behaviours such as copulation duration and mating frequency is likely to be strongly influenced by the OSR and density of a population. Several models have assumed that a male-biased sex ratio leads to an increase in copulation duration (Parker, 1970; Parker & Simmons, 1994; Simmons, 2001), and there is empirical evidence to support this assumption (Alonso-Pimentel & Papaj, 1996; Barbosa, 2011; Bretman et al., 2010; Horton, 2013; Klemme & Firman, 2013; Lizé et al., 2012; Wang et al., 2008). Increasing copulation duration is thought to increase the probability of successful egg fertilisations which could be due to the transfer of more sperm, decreasing receptivity of the mated female to future males by the transfer of seminal fluid proteins (Wigby et al., 2009), and the deposition of a mating plug to prevent females from remating (Eberhard, 1996; Simmons, 2001). Based on this, increasing male density elevates perceived sperm competition risk to influence reproductive allocation strategies. Several theories have attempted to explain the adaptive significance of increased copulation duration. The in-copula guarding hypothesis suggests that increasing copulation duration is a form of post-insemination mate guarding to reduce or avoid sperm competition with future ejaculates (Alcock, 1994). For example, in the bug, *Triatoma brasiliensis*, males extended their copulation duration only in the presence of other males, suggesting a postinsemination mate guarding strategy (Vitta & Lorenzo, 2009). Similarly, the sperm loading hypothesis suggests that copulation duration is associated with sperm transfer and the longer a male copulates the more sperm he transfers (Parker, 1990b). Empirical evidence to support the sperm loading hypothesis has been observed in the milkweed beetle, *Labidomera clivicollis*, where males sired more offspring by increasing copulation duration and transferring more sperm to delay females from mating with other males (Dickinson, 1986). However, there is also evidence that increasing density does not result in increased copulation duration. For example, in the Australian polydesmidan millipede, *Gigantowales chisholmi*, males decreased copulation duration when male density was higher due to scramble competition (Holwell et al., 2016). Most studies exploring the adaptive significance of copulation duration in response to density are laboratory-based, however there is still a lack of empirical evidence exploring this in wild populations.

Males that encounter a disparity in their ability to acquire mates may employ alternative reproductive tactics (ARTs), a type of phenotypically plastic mating strategy (Oliveira et al., 2008; Taborsky et al., 2008). ARTs are often condition-dependent (e.g. body size), and can be influenced by the nutritional environment experienced during development (Bonduriansky & Rowe, 2005; Delcourt & Rundle, 2011; Plaistow, 2004; Simmons & Kotiaho, 2002). For example, Moczek (1998) found that larval diet quality influences adult body size in the dung beetle *Onthophagus taurus,* determining the presence or absence of a horn, and consequently the ART that males employ. Alternative reproductive tactics can also be dynamic responding to ephemeral changes in local conditions including competitor density, size of competitors, and body condition (Oliveira et al., 2008; Taborsky, 1998). Alternative reproductive tactics usually occur when the population density experiences fluctuations over a shorter timescale than the lifespan of the individual (Kokko & Rankin, 2006; Schlichting et al., 1998). This suggests that demographic factors play a central role in the evolution and maintenance of ARTs (Oliveira et al., 2008; Taborsky, 1998). For example, the red spider mite (*Tetranychus urticae*) employs three ARTs that are context-dependent (chosen based on the number of competitors present) to gain access to females - fighting, sneaking, and opportunistic behaviour (Sato et al., 2013), and as the number of fighter males increases, so does the proportion of sneakers (Sato et al., 2014). Similarly, in the European bitterling (*Rhodeus sericeus*), the number of sneak fertilisations has been shown to positively correlate with increasing male density (Reichard et al., 2004).

Insects are underrepresented in studies aiming to understand how sexual selection drives evolution in the wild (Rodriguez-Munoz et al., 2010). Given that there is empirical evidence to suggest that population density and sex ratio influence sexual selection in laboratory conditions (Alonso-Pimentel & Papaj, 1996; Flay et al., 2009; Wang et al., 2009), it would be particularly useful to study this in wild populations where there is natural variation and a less likelihood of artefacts from a laboratory setting that may misconstrue how sexual selection operates in the wild (Bonduriansky & Brassil, 2005; Kawasaki et al., 2008; Lande & Arnold, 1983). A wealth of sexual selection research has been conducted on insects as they typically have short generation times, are easy to study in the laboratory, and offer potential for large sample sizes (Miller & Svensson, 2014). However, because insects are often small-bodied and particularly mobile, conducting research on population density in wild populations is often difficult. Studying both natural populations in conjunction with laboratory populations will provide a broader understanding of how sexual selection can drive variation in insect populations and shape the life-history of an insect.

The New Zealand giraffe weevil (*Lasiorhynchus barbicornis,* Coleptera: Brentidae) is an ideal species to explore the effect of population density and sex ratio on mating success. Both sexes are highly variable in size: males can vary from 14-90 mm, and females between 12-50 mm in total body length (Painting et al., 2014). Because of this high size variability, males have evolved flexible ARTs, switching between 'sneaking' and 'fighting' tactics depending on their body size and the size of their opponent, to access females and gain mating opportunities (Painting & Holwell, 2014a, 2014b). Male giraffe weevils bear an extremely elongated rostrum, which can comprise up to half their total body length that is used as a weapon in male-male competition (Meads, 1976; Painting & Holwell, 2014a). Females use their rostrum to drill holes into dying native trees where they lay their eggs (Meads, 1976). Males often aggregate on these trees, mating with and guarding a female while she searches for a suitable oviposition site (Meads, 1976; Painting & Holwell, 2014c). Females are polyandrous and can store sperm, leading to intense pre- and post-copulatory competition between males (LeGrice et al., 2019). The unique behaviours of male giraffe weevils, and their tendency to aggregate, make them particularly useful for longitudinal study in the wild.

Previous research on mating success in the New Zealand giraffe weevil has shown that small males that commonly use sneak tactics mate as frequently as larger males (Painting & Holwell, 2014b). However, longitudinal research by LeGrice et al., (2019) found that large males experience higher average mating frequencies with no apparent trade-off in survival; in fact, they live longer than smaller males. A recent study exploring the effect of population density and body size on social selection by Fisher et al., (2021) showed that large male giraffe weevils had a negative effect on small males fitness at higher densities. My study looked at fluctuations in population dynamics both over a full breeding season and also between-tree variation in abundance and sex ratio within a season. However, many of the fine-scale mating behaviours in this species have yet to be examined in detail including how demographic factors influence copulation duration and mate guarding. The overall aim of this study was to gain a deeper understanding of how sexual selection acts on New Zealand giraffe weevils using (1) an experimental approach with mating assays, and (2) an observational dataset collected in the wild. More specifically, my aim was to determine how population density, sex ratio and body size effects male mating frequency, copulation duration and mate guarding duration. I predict that at low densities, small males will be less successful at achieving matings than large males due to large males being competitively superior. In contrast, at high densities, small males will likely be more, or just as successful at achieving matings due to their sneaking tactics. I also predict that at higher densities, males will have reduced mating duration and frequency irrespective of body size due to increased pre-copulatory competition.

Materials and Methods

Laboratory experiment

I collected male and female *L. barbicornis* between January and March 2021 from a wild population at Maungatautari Mountain in Waikato, New Zealand (38°03'19.8"S, 175°33'38.5"E). Males and females were transported in separate 12” insect rearing and observation cages (BioQuip Products). All weevils had their total body length, pronotum width, and rostrum length measured. Total body length was measured as the tip of the mandibles to the end of the elytra. Pronotum width was measured as the width of the pronotum in dorsal view, and rostrum length was measured as the total length of the rostrum from the tip of the mandibles to the base of the head. Their containers were misted every few days so the humidity levels replicated approximately what it was in the wild at the time of collection. They were placed in a temperature-controlled room at 22°C on a 12h light: dark cycle. All weevils were marked with a unique identification number on their pronotum using a Sharpie oil-based white paint pen and permanent marker.

To examine how perceived sperm competition risk and body size may influence ejaculate allocation strategies, I used rhodamine B, a non-toxic fluorescent dye that binds to proteins in the body (Hayashi & Kamimura, 2002). Male giraffe weevils were fed a 0.1% Rhodamine B (Sigma Aldrich, 95% dye content, HPLC) dissolved in sugar water solution

(1:10). Rhodamine B was used to stain the male's ejaculates for analysis after mating to compare ejaculates between males housed at different densities. It is a histological stain that is commonly used as a biomarker in insects (Beija et al., 2009; P. Fisher, 1999). For example, Blanco et al. (2006) used rhodamine B to stain the spermatophores of tobacco budworm moths (*Heliothis virescens*). The rhodamine B solution was soaked in a dental swab or cotton ball to make it easy for the weevils to drink. However, females were fed regular sugar water (1:10).

To measure how population density and body size effect ejaculate size and copulation duration, I conducted mating trials. To set up conditions where males would perceive different levels of sperm competition risk, males were randomly separated into three density treatments – low density (1 male, N = 33), medium density (2 males, N = 38), and high density (5 males, N = 65) for a minimum of one week before trials commenced. The total sample size was 136 males across the three treatments.

Before the mating trial began, males and females were weighed on a Sartorius Quintix 35-1S balance to the nearest 0.01 mg. The aim of this was to measure ejaculate size as there is thought to be a correlation between male weight loss and female weight gain after mating (Edvardsson & Tregenza, 2005). A male was placed in the mating trial arena (1L Sistema container) for 10 minutes prior to introducing a randomly chosen female. Between three and four trials took place simultaneously and were conducted for two hours or until a male had copulated and then stopped copulating for at least one minute. If the male and female did not mate, they were returned to their containers to be used at a later date. However, if the male mated, he was weighed again and then placed in a freezer at -18°C to be euthanised. The female was then placed back into the container to allow the sperm to travel from the bursa copulatrix to the spermatheca. After 48 hours the female was weighed again and then euthanised. Males were then dissected to remove the testes for weighing for morphological measurements in **Chapter 2**. Females were dissected to remove the single, sickle-shaped spermatheca. The spermatheca was placed on a concave microscope slide with Prolong Diamond antifade mountant (Life Technologies) and placed in a dark drawer to cure for 24 h. After curing, the slide was placed in the freezer and analysed within one week. The slide was placed under the Olympus BX43 confocal microscope using a UV filter to determine the amount of ejaculate deposited by the male giraffe weevils. Unfortunately, the spermatheca appeared to auto-fluoresce and therefore it was not possible to determine how much the male had ejaculated during mating using the reflectance of the Rhodamine B dye. Further, the sperm/accessory glands did not appear to absorb any of the dye either. Therefore, using ejaculate as a measure of response to sperm competition level was not useful. Thus, only copulation duration was used as a measure of mating investment in response to perceived sperm competition risk in the laboratory component of this study.

Field study

Study site

The data was collected at Matuku Reserve (36°51.92'S, 174°28.32'E), west of Auckland city, New Zealand. Matuku Reserve is owned by the Royal Forest and Bird Protection Society, occupying 120 hectares, and consisting predominantly of coastal lowland broadleaf forest. *Lasiorhynchus barbicornis* are commonly found on sick or dying native trees such as karaka (*Corynocarpus laevigatus*) and māhoe (*Melicytus ramiflorus*). All observations were conducted on standing, dying karaka and mahōe trees and were recorded by Dr Christina J. Painting and Dr Leilani Walker between November 2011 and May 2012. During each sampling period, five or six trees were chosen that were currently attracting giraffe weevils and used as observation sites. A total of nine trees were used for the observations, eight were karaka and one was māhoe.

Before observations began, all giraffe weevils were removed from the host tree and morphological measurements were made to the nearest 0.01 mm using digital calipers (Shenzhen Rok Precision Instruments Co., Shenzhen, China). Total body length was measured from the tip of the rostrum at the mandibles to the bottom of the elytra. All weevils were given an individual number and marked with Queen Bee marking paints (Lega SRL, Faenza, Italy) on the pronotum and elytra (as in Painting & Holwell, 2014) and returned to their host tree. Any unmarked weevils discovered on subsequent observation days were measured and marked before observations began.

Observations

There were four sampling periods over the season with approximately 6 weeks between each period. The sampling periods were recorded as months (November, January, February, April/May). Giraffe weevils are common in the warmer months (November – April) and steadily increase in abundance from November, peaking in February before declining again. However, the sex ratio is almost always male-biased to varying degrees (Painting et al., 2014). Each sampling period was made up of between 20 and 25 individual days of observations. The exact number of observations for each tree during each sampling period can be seen in **Table 3.1**. On each day of observations, the total number of males and females present were recorded allowing me to calculate male, female, and total abundance, as well as the sex ratio. Sex ratio was calculated as the number of males divided by the number of females.

Continuous scan sampling was used to record mating behaviour as this method allows the behaviour of numerous individuals to be recorded simultaneously (Altmann, 1974). All male giraffe weevils present on a tree were observed for a 1-hour period between 8:00 AM and 16:00 PM and the duration and frequency of all mating and guarding behaviours were recorded. The start and end times of all mating and guarding behaviours were recorded along with the identification number of the male, and any females that the male mated or guarded. There was a total of 88 observation days over the whole season (**Table 3.1**). Four hundred and thirty-three individual males and 289 females were recorded present across the season.

Table 3.1. The number of individual observation days at each tree for each sampling period.

	Sampling period			
Tree	November	January	February	April/May
T5	5	5	5	5
T6	2	0	4	2
T9	3	1	0	0
T16	4	5	0	0
T18	5	5	5	5
T20	0	0	0	5
T22	0	0	1	1
T29	0	3	5	0
T30	5	6	1	0
Total	24	25	21	18

Data analysis

Data preparation

All statistical analyses were conducted using R 4.1.1 (R Core Team, 2021). Firstly, due to the nature of collecting observational data in the wild, there was some missing data for male body sizes. There was missing body size data for four males (0.92% of the total number of observations). This is because some giraffe weevils that were recorded during observation days were unmarked and disappeared into the canopy before they could be captured, measured, and marked. Often researchers deal with missing data by removing it from the dataset completely. However, this can lead to biased parameter estimates and also reduce statistical power (Harrison et al., 2018; Nakagawa & Freckleton, 2011). Therefore, I used multiple imputation (MI) as recommended in Nakagawa and Freckleton (2011) to increase the accuracy of my model selection procedure. Multiple imputation can protect against the mistaken rejection of the null hypothesis (type 1 error) as variation in parameter estimates can be incorporated in pooled estimates, accounting for imputation uncertainty (McKnight et al., 2007; Noble & Nakagawa, 2018). Male body sizes were imputed with mean values using the 'aregImpute' function from the Hmisc package in R (Harrell Jr et al., 2021).

Model building procedure

For the laboratory experimental data, I used simple linear regressions to determine the effects of population density on male mating duration and the interaction between mating and guarding duration. Mating duration was used as the response variable, and the density treatment (low, medium, high) and male body size was used as the predictor variable. Then, to test the interaction between mating duration and guarding duration, I used a simple linear regression with the guarding duration as the response variable and mating duration as the predictor variable.

For the field-based observational data, I used a model selection procedure to determine which predictors and interactions to include in each model. The relative fit of each model was determined using the Akaike Information Criterion (AIC) (Akaike, 1973). Models with Δ AIC > 7 were considered as fitting the data significantly worse than the best fitting model (Burnham et al., 2011). Furthermore, I utilised a simulation approach to check each model for misspecification, dispersion, zero-inflation, and autocorrelation using the *DHARMa* package (Hartig, 2021).

To investigate population density fluctuations over time, I used a generalized linear mixed model (GLM) with a Poisson distribution. Two separate models were fit where the number of males, and the number of females were used as the response variables, respectively, that both included sampling period as the predictor. Using a GLM with a Gaussian distribution, I ran another two separate models with male body size and female body size as the response variables, respectively, with sampling period as the predictor. Male and female body size were logged to reduce skew in the data and approximates a normal distribution to avoid violation of model assumptions. Finally, a GLM with a Gaussian distribution was used to determine sex ratio changes over each sampling period, with sex ratio as the response variable and sampling period as the predictor. The percentage of each sex in the sampling period was calculated as the number of males divided by the number of females, then divided by the total abundance, multiplied by 100 to give a percentage.

To investigate the effect of body size and population density on mean male mating duration, I fit a linear mixed model using the *lmerTest* package (Kuznetsova et al., 2017). Only males that mated were included in these analyses to avoid problems with zero inflation as many males did not mate but were still recorded in the dataset. The mean mating duration was calculated as the average mating duration for each individual male on the same tree on the same day. Log mean mating duration was used as the response variable, and the number of males, number of females, male body size, and male mating frequency were used as fixed effects, along with the interaction between the number of males and male body size, the number of males and number of females, male body size and male mating frequency, and the number of females and male mating frequency. The date, observation tree, and male identification were included as random effects.

To investigate the effect of male body size and population density on total male mating frequency, I used a zero-inflated GLMM with a Poisson distribution, in the *glmmTMB* package (Brooks et al., 2017). Both males that mated and did not mate were included in these analyses for mating frequency. Mating frequency was defined as the number of times a male mated, irrespective of if it was with the same female (i.e. a male could mate more than once with the same female during the observation period). Total mating frequency was used as the response variable, while the number of males, male body size, number of females, mean mating duration and male body size rank were used as fixed effects, along with the interactions between number of males and male body size, number of males and number of females, male body size and mean mating duration, male body size and number of females, number of females and mean mating duration, and male body size rank and number of males. Because individual males were observed on individual trees, male identification was nested within date and tree to account for this data structure as a random effect.

Due to some issues with multicollinearity and violations of distributional assumptions, frequentist methods of regression were found to be unsuitable for models examining the number of unique females a male mated with, and guarding duration. Unique females was defined as the number of different females a male mated with during an observation period. I therefore used a Bayesian approach with the *brms* package to fit GLMMs (Bürkner, 2018). To select the best model for each response, I used the Leave One Out

Cross-Validation (LOO-CV) procedure to ascertain the relative weight and fit of each model where a difference of < 4 was considered insignificant (Vehtari et al., 2017). I used the default student-T priors to fit four parallel MCMC chains each with 6000 iterations and 2000 burnin that resulted in low autocorrelation between samples for both model structures described below. To model the number of unique females a male mated with I included male body size, number of males and male body size rank as fixed effects. Body size rank was based on males that were present on a tree during a single observation period. The 'cut' function was used to rank males from smallest to largest body size. The best model of male mate guarding duration included the number of males, number of females, male body size, copulation duration as predictors, and the interactions between the number of females and number of males, male body size and copulation duration. Date and tree were used as random effects for both models.

Results

Experimental assay

Mating duration

Forty-six mating trials were run, but only sixteen resulted in successful copulation. Five of these were in the low-density treatment, 6 of these in the medium density treatment, and 5 in the high-density treatment. Male density was almost significant (p = 0.08), but there was no relationship between mating duration and body size (**Table 3.2**).

Table 3.2. Summary statistics for the linear model estimates of density treatment, mating duration, and male body size.

	Estimate	*SE*	t value	*P*
Intercept	2670.65	873.80	3.06	0.01
Treatment (low)	-1138.88	586.68	-1.94	0.08
Treatment (medium)	157.62	505.05	0.31	0.76
Male body size	5.90	14.25	0.41	0.69

Guarding duration

Using the experimental data I collected in the laboratory, I examined the relationship between mating duration and guarding duration. I was able to detect a negative relationship where males that copulated with females for a longer duration, spent less time mate guarding (*b* = -0.24, df = 14, *SE* = 0.11, p = 0.04, R^2 = 0.25, **Figure 3.1**).

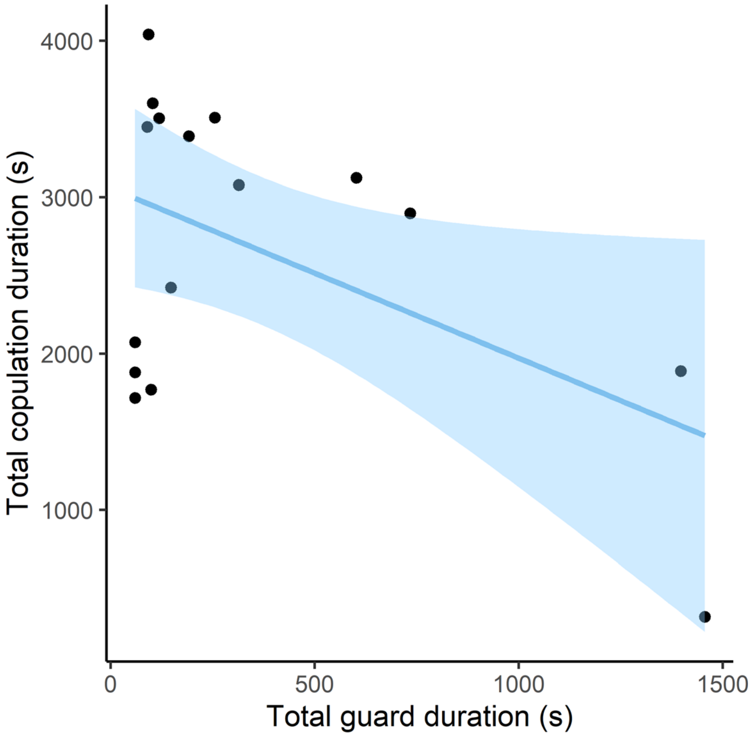


Figure 3.1. Total copulation duration plotted against total guard duration. Solid black points represent each individual male, and the blue error ribbon represents a 95% confidence interval.

Observational data

Population dynamics

i. Population density

Male density was higher than female density across all sampling periods (**Figure 3.2**). For both sexes, population density was highest in February (male; *b* = 0.54, *SE* = 0.028, *z* = 19.44, p < 0.01 and female; *b* = 0.64, *SE* = 0.037, *z* = 17.25, *p* < 0.01) and January (male; *b* = 0.25, *SE* = 0.19, *z* = 8.99, p < 0.01 and female; *b* = 0.25, *SE* = 0.039, *z* = 6.45, *p* < 0.01), which are the months considered to be the peak of the mating season.

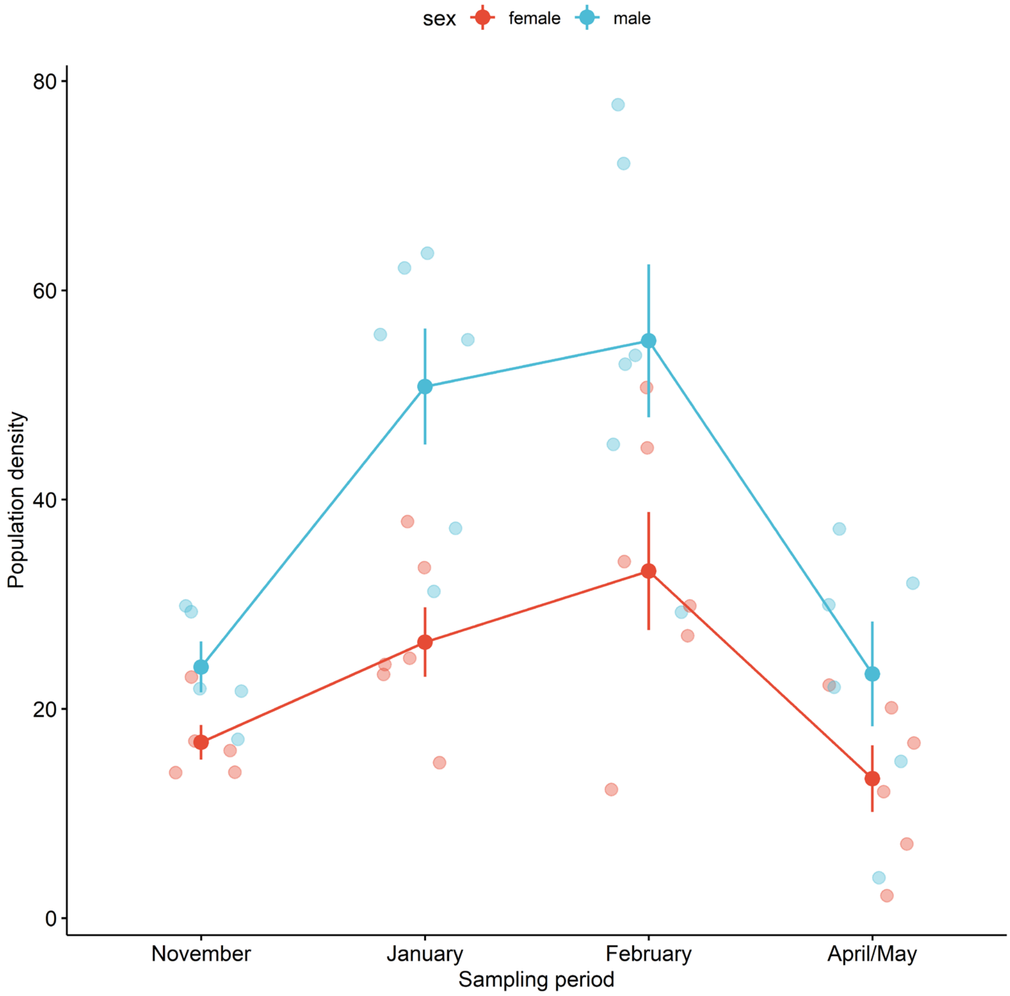


Figure 3.2. Variation in population density of both male (blue) and female (red) giraffe weevils over the four sampling periods (November 2011 – April/May 2012). Each translucent point signifies the sum of observed males and females seen on that day across all trees. The bold points are the means from that month and the vertical lines represent Standard Errors of the Mean (SEM).

ii. Body size

There was significant variation in male and female body size across the season. The largest males and females were most common in the sampling period of April/May (**Table 3.3,** Figure 3.3).

Table 3.3. Summary statistics for the generalised linear model estimates of log male body length, log female body length and sampling period.

	Sampling period	Estimate	*SE*	t value	*P*
log Male body length	November	-0.06	0.03	-2.30	0.02*
	January	-0.20	0.02	-9.38	<0.01*
	February	-0.05	0.02	-2.60	0.01*
	April/May (intercept)	3.71	0.02	209.94	<0.01*
log Female body length	November	0.02	0.02	1.59	0.11
	January	-0.12	0.01	-9.61	<0.01*
	February	-0.05	0.12	-3.83	<0.01*
	April/May (intercept)	3.29	0.01	322.47	<0.01*

* indicates significant p value at the 95% level

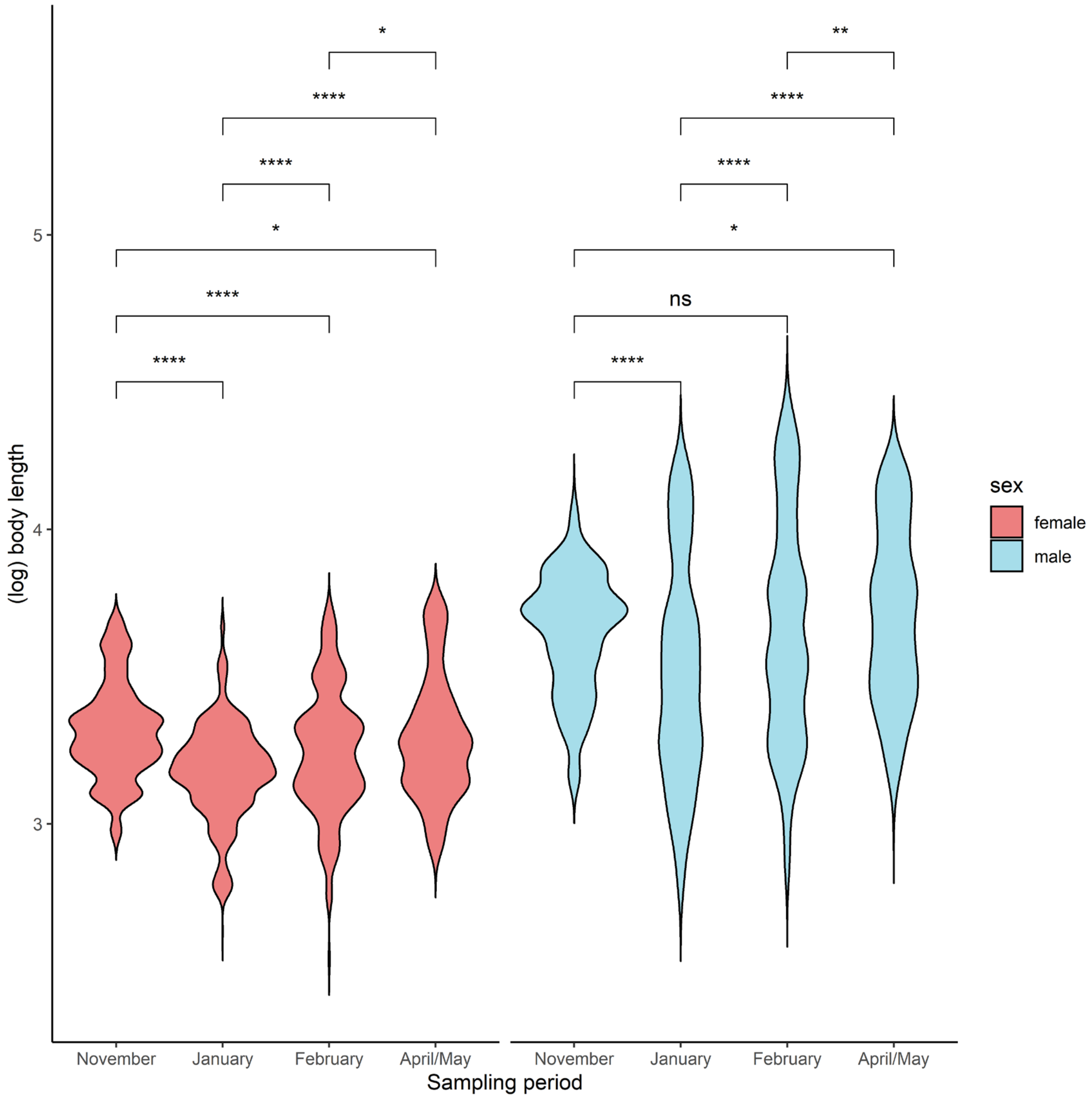


Figure 3.3. Changes in log body length of female (red) and male (blue) giraffe weevils over the four sampling periods (November 2011 – April/May 2012). * : p <= 0.05, ** : p <= 0.01, *** : p <= 0.001, **** : p <= 0.0001. Significance was determined using the Kruskal-Wallis test.

iii. Sex ratio

Sex ratio was always male-biased but peaked during January (**Figure 3.4)**. There was a significant difference in sex ratio between the sampling period April/May and January (**Table 3.4**, **Figure 3.4**).

Table 3.4. Summary statistics from the generalised linear model estimates of sex ratio and sampling period.

Sampling period	Estimate	*SE*	t value	*P*
November	0.03	0.13	0.26	0.80
January	0.38	0.11	3.52	<0.01*
February	-0.11	0.11	-1.00	0.32
April/May (intercept)	1.96	0.09	22.21	<0.01*

* indicates significant p value at the 95% level

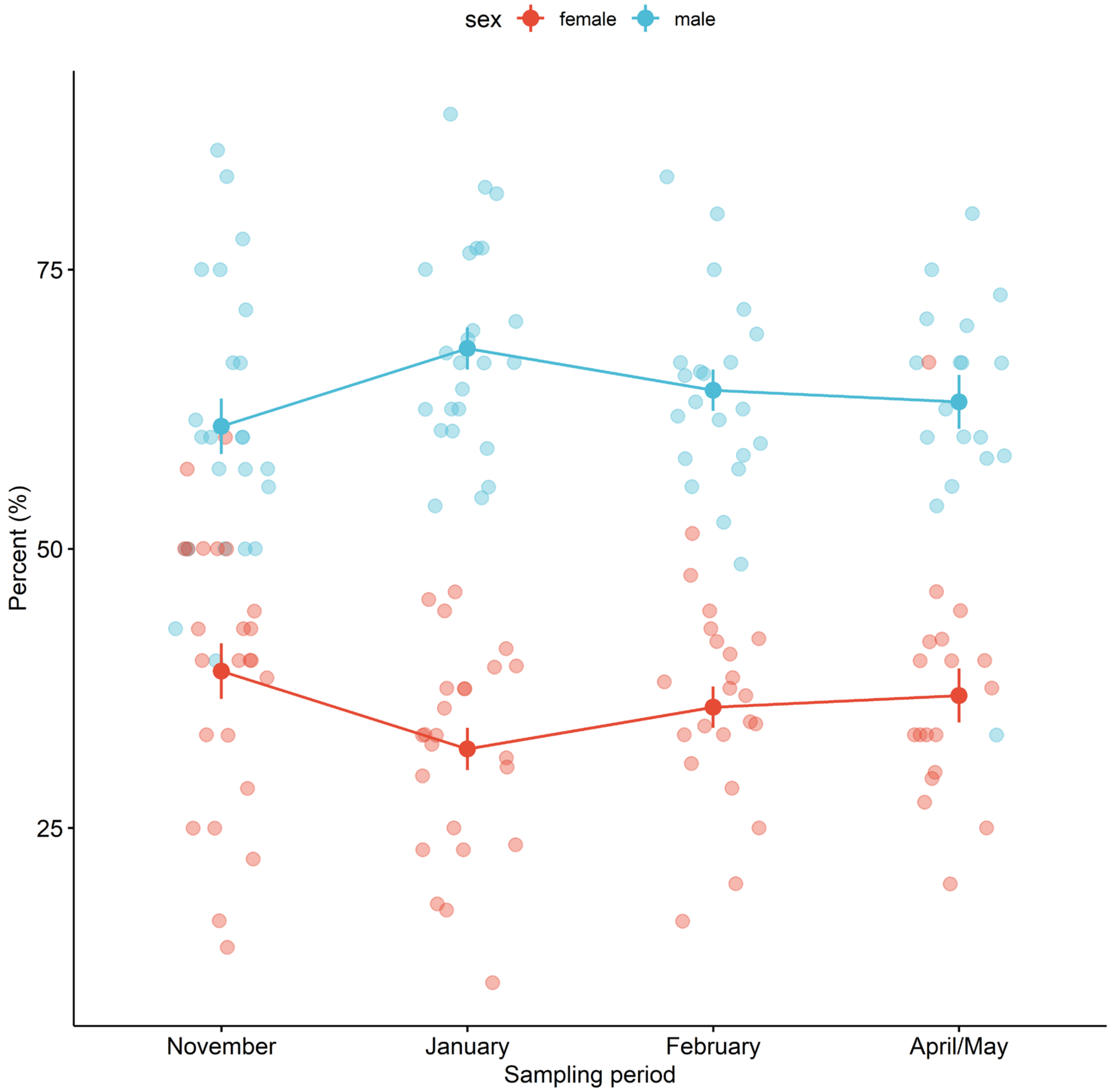


Figure 3.4. Percentage of males (blue) and females (red) over the four sampling periods (November 2011 – April/May 2012), expressed as a percentage.

Mating dynamics

i. *Mating duration*

Mating duration was significantly affected by the number of males, the number of females, mating frequency, and the interaction between the number of females and mating frequency (**Table 3.5**). The number of males and the number of females negatively affected the mating duration, therefore with more males or females around, males mated for less time. Although the number of males had a significant negative effect on male mating duration, the effect size was relatively small when compared to the effect of female density (**Figure 3.5**). Mating frequency was negatively related to mating duration where males that mated for less time had higher mating frequency (Figure 3.6). However, as female density increases, mating frequency declines as well as mating duration (**Figure 3.6**). There was no significant relationship between male body size and mating duration.

Table 3.5. Summary statistics for the generalized linear model estimates of mating duration using the dataset that only contained males that mated.

	Estimate	*SE*	t value	*P*
Intercept (Mating duration)	7.185	0.450	15.956	<0.001*
Number of males	-0.049	0.025	-1.966	0.050*
Male body size	-0.002	0.010	-0.250	0.803
Number of females	-0.120	0.036	-3.336	0.001*
Mating frequency	-0.308	0.104	-2.974	0.003*
Number of males:Male body size	0.000	0.000	0.415	0.679
Number of males:Number of females	0.003	0.002	1.783	0.076
Male body size:Mating frequency	0.003	0.002	1.272	0.204
Number of females:Mating frequency	0.015	0.005	2.849	0.005*

* indicates significant p value at the 95% level

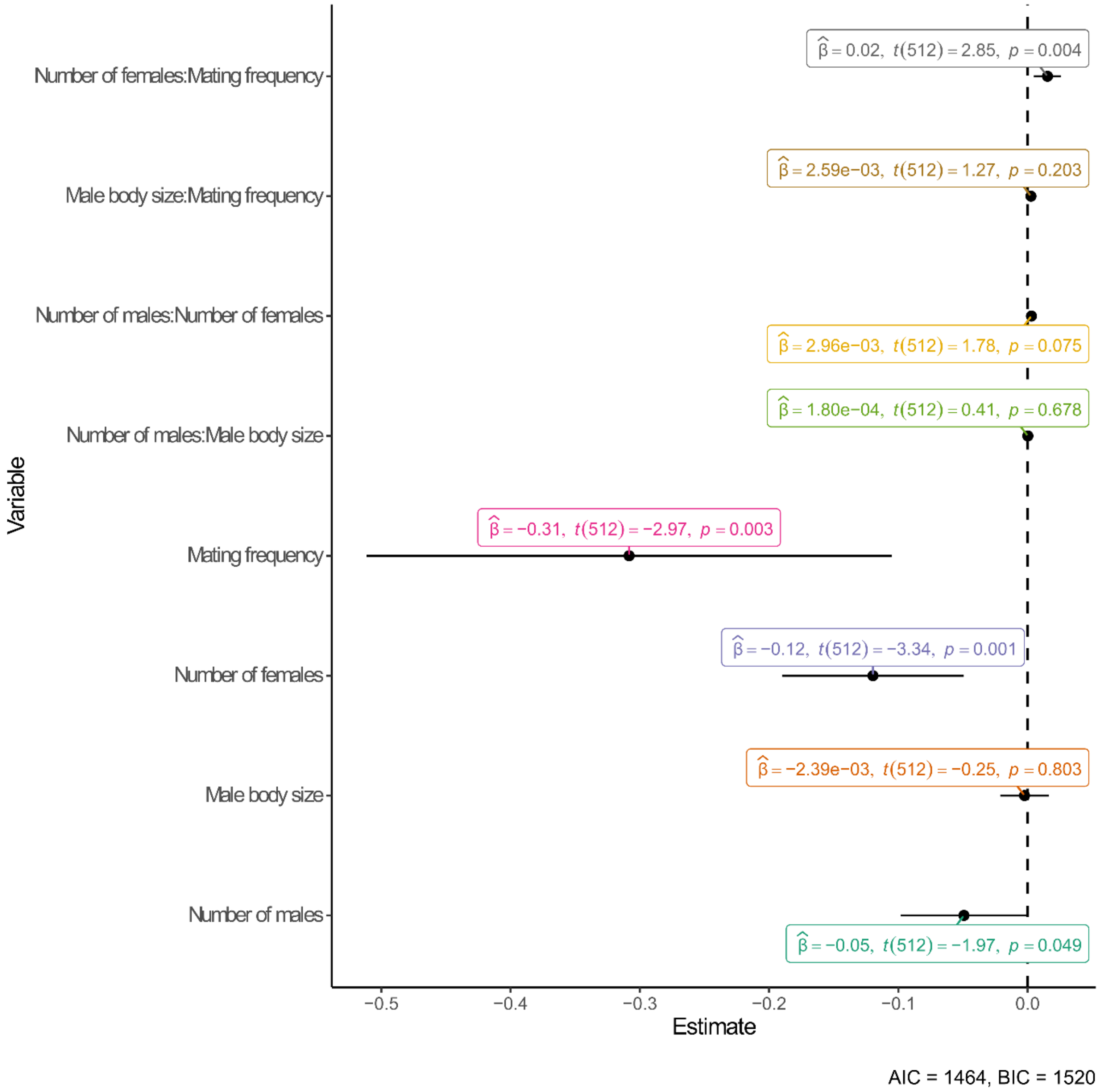


Figure 3.5. The effect size plot for the generalized linear model of mating duration. The black points represent the estimate value, and the solid black lines represent 95% confidence intervals.

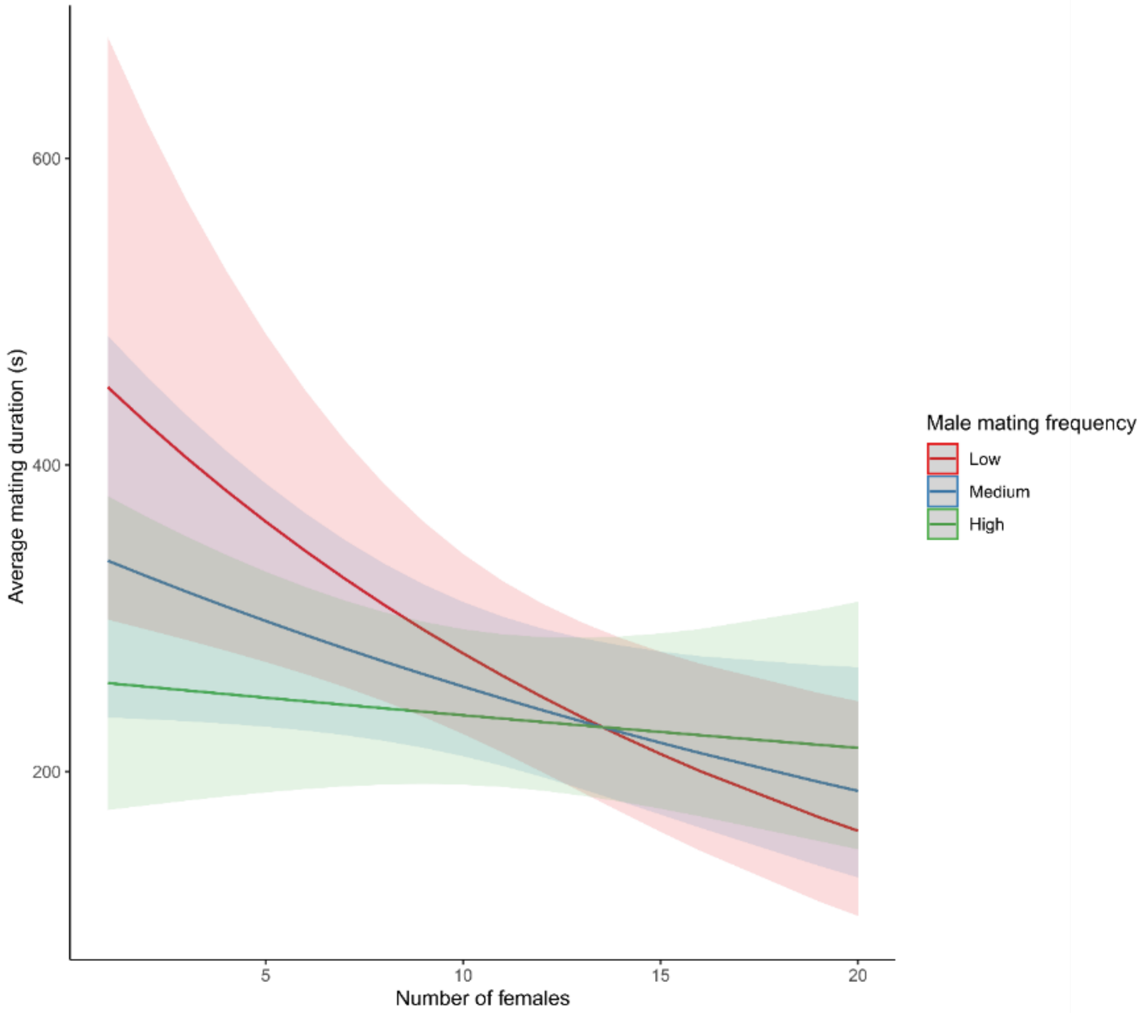


Figure 3.6. The interactive effects of number of females and male mating frequency on predictions of average male mating duration. The solid, coloured lines represent the population-level estimates of average male mating duration across different levels of mating frequency and the number of females. Shaded coloured areas represent 95% confidence intervals.

ii. Total mating frequency

Male mating frequency was significantly related to the number of females, average mating duration, male body size rank (small), and the interaction between the number of males and number of females (i.e., total population density) (**Table 3.6**). The number of females had a strong positive effect on mating frequency (**Figure 3.7**), where the more females that were present, the more times a male mated. Similarly, average mating duration had a strong effect on mating frequency (**Figure 3.7**), where the longer a male mated for, the more times he mated. Male body size rank (small) negatively influenced mating frequency, showing that smaller males copulate relatively less than larger males. However, male body size did not significantly affect mating frequency. The interaction between the number of males and number of females negatively influenced mating frequency.

Table 3.6. Summary statistics for the generalized linear model estimates of mating frequency using the dataset that contained both males that mated and did not mate.

	Estimate	*SE*	*z* value	*P*
Intercept (Male body size rank (large))	-0.279	0.628	-0.444	0.657
Number of males	-0.017	0.039	-0.439	0.660
Male body size	-0.425	0.230	-1.851	0.064
Number of females	0.228	0.039	5.847	<0.001*
Average mating duration	0.302	0.057	5.292	<0.001*
Male body size rank (medium)	-0.216	0.507	-0.426	0.670
Male body size rank (small)	-1.482	0.717	-2.069	0.039*
Number of males:Male body size	0.023	0.014	1.682	0.093
Number of males:Number of females	-0.007	0.002	-3.057	0.002*
Male body size:Average mating duration	0.004	0.034	0.111	0.912
Male body size:Number of females	-0.006	0.010	-0.604	0.546
Number of females:Average mating duration	-0.010	0.007	-1.419	0.156
Number of males:Male body size rank (medium)	0.008	0.027	0.283	0.777
Number of males:Male body size rank (small)	0.071	0.039	1.800	0.072

* indicates significant p value at the 95% level

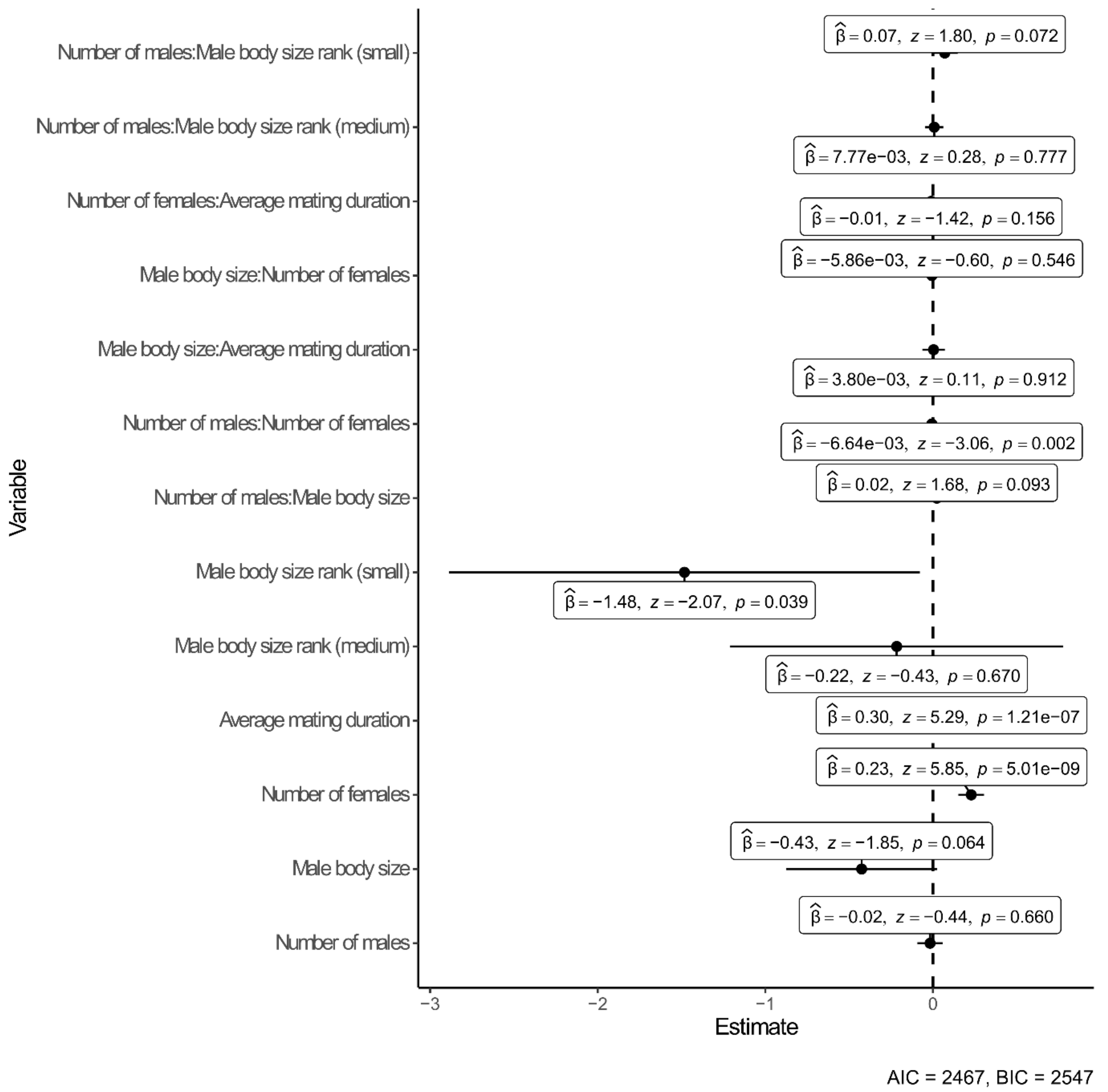


Figure 3.7. The effect size plot for the generalized linear model of male mating frequency. The black points represent the estimate value, and the solid black lines represent 95% confidence intervals.

At low female densities, the smallest ranked males mated less frequently, with the largest ranked males showing the greatest variation in mating frequency (**Figure 3.8A**). However, at medium female densities, the smallest ranked males mated most frequently compared to the medium ranked males and largest ranked males. The smallest ranked males mated almost as frequently as the largest ranked males at high female densities (**Figure 3.8A**). The same relationship was identified when looking at the relationship between male body size rank and male density (**Figure 3.8B**).

When the number of males was low, and the number of females was high, mating frequency was high (**Figure 3.9**). However, as the number of males increased, male mating frequency declined significantly regardless of the number of females (**Figure 3.9**).

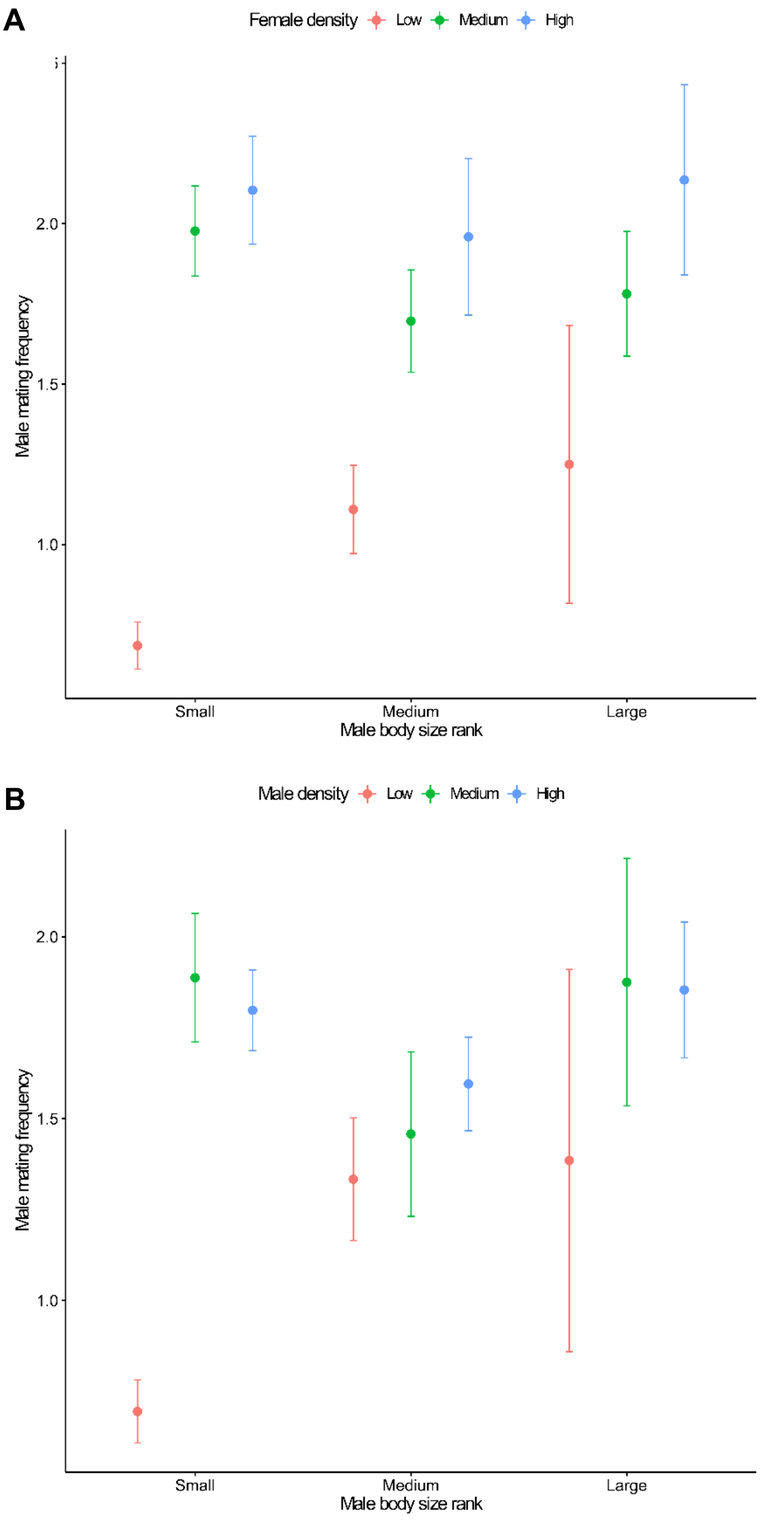


Figure 3.8. Mating frequency grouped by male body size rank at differing densities. Female density is on the left, and male density is on the right. Each point represents the mean, and the error bars represent Standard Errors of the Mean (SEM).

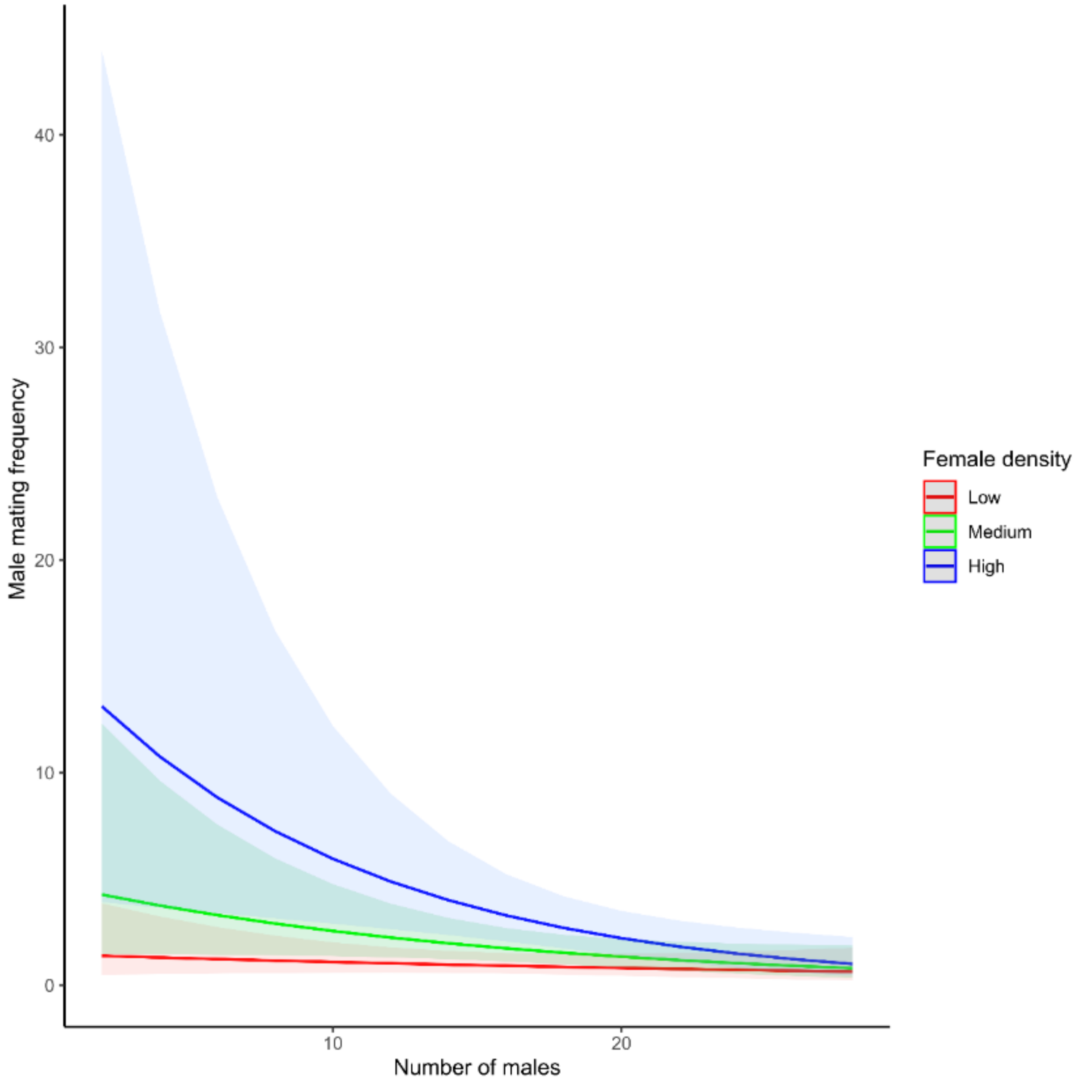


Figure 3.9. The interaction between number of males, and number of females and its effect on male mating frequency. The solid, coloured lines represent population-level estimates of male mating frequency across different levels of female density and the number of males. Shaded coloured areas represent 95% confidence intervals.

iii. Mating frequency with unique females

The interaction between number of males and male body size rank (medium) has a probability of 94.35% [pd] of being positive (Median = 0.04, 95%CI([-0.01, 0.09]). However, it is uncertain if it is influencing the number of unique females a male mated with (**Table 3.7**, **Figure 3.10**). The interaction between male body size and number of males has a probability of 93.91% [pd] of being positive (Median = 0.001, 95%CI([-0.000, 0.003]), however it is uncertain if it is influencing the number of unique females a male mated with according to the pd value (**Table 3.7**, **Figure 3.10**). The number of unique females a male mated with decreased as male body size increased (Figure 3.11). The number of unique females a male mates with correlates with male body size rank, and male density. At lower densities, the largest ranked males mate with the most unique females (**Figure 3.12**). However, as male density increases, the smallest ranked males mate with the most unique females (**Figure 3.12**).

Table 3.7. Variation in unique female matings by male *Lasiorhynchus barbicornis*. I present fixed (β) and random (σ^2) effects with 95% confidence intervals. These values are median estimates of the posterior distribution. pd is the probability of direction representing the certainty associated with the most probable direction (Makowski et al., 2019).

Fixed effects	β (95% CI)	pd (%)
Intercept	1.99 (-0.27, 4.41)	95.05*
Male body size	-0.01 (-0.05, 0.01)	84.64
Number of males	-0.07 (-0.20, 0.05)	87.99
Male body size rank (medium)	-0.26 (-1.18, 0.69)	70.46
Male body size rank (small)	-0.18 (-1.57, 1.21)	60.40
Number of males:Male body size rank (medium)	0.04 (-0.01, 0.09)	94.35
Number of males:Male body size rank (small)	0.04 (-0.02, 0.12)	89.10
Male body size:Number of males	0.001 (-0.000, 0.003)	93.91

Random effects	σ^2 (95% CI)
Date	0.14 (0.03, 0.26)
Tree	0.23 (0.11, 0.47)

* indicates probability of direction above 95% level

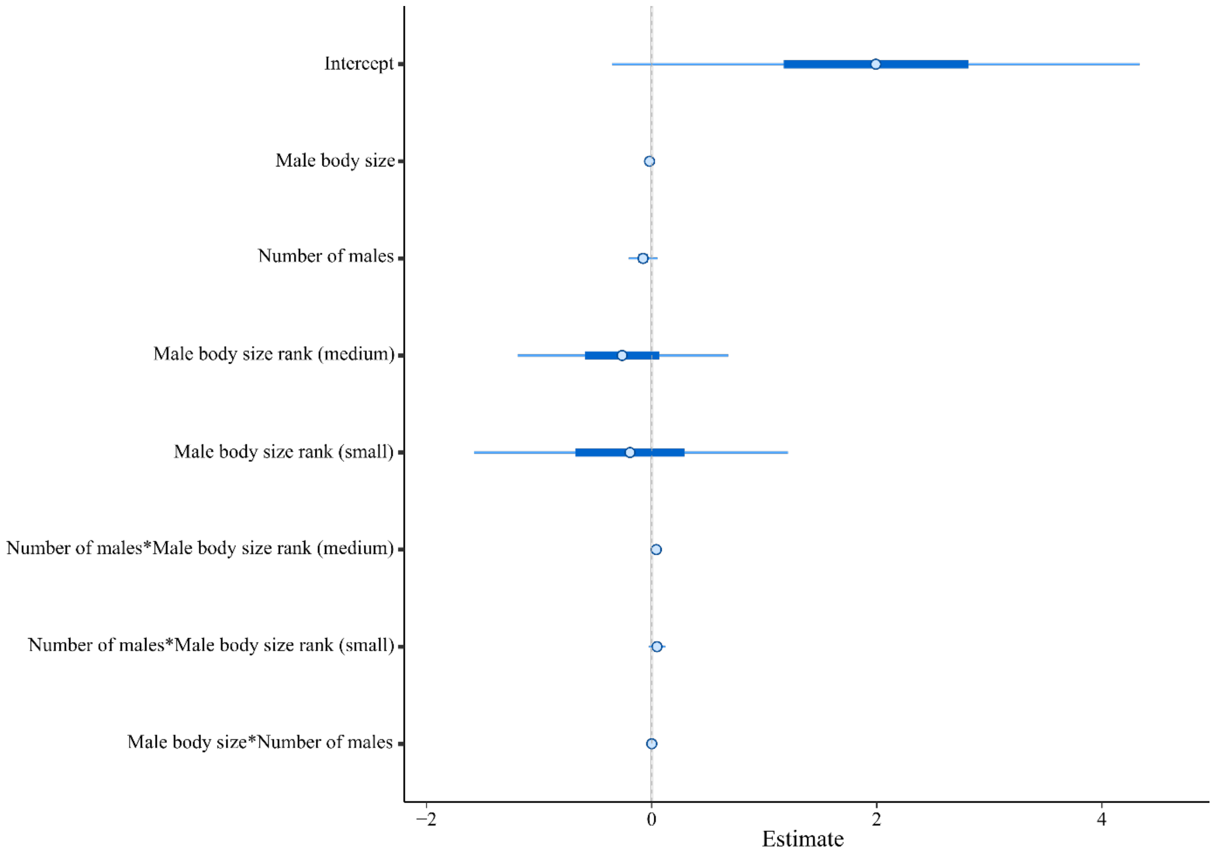


Figure 3.10. The effect size of factors that influence the number of unique females a male mates with. The thick and thin lines describe the 50% and 95% credible intervals (CI) of each parameter, respectively. The small transparent dots are the median of the posterior distribution.

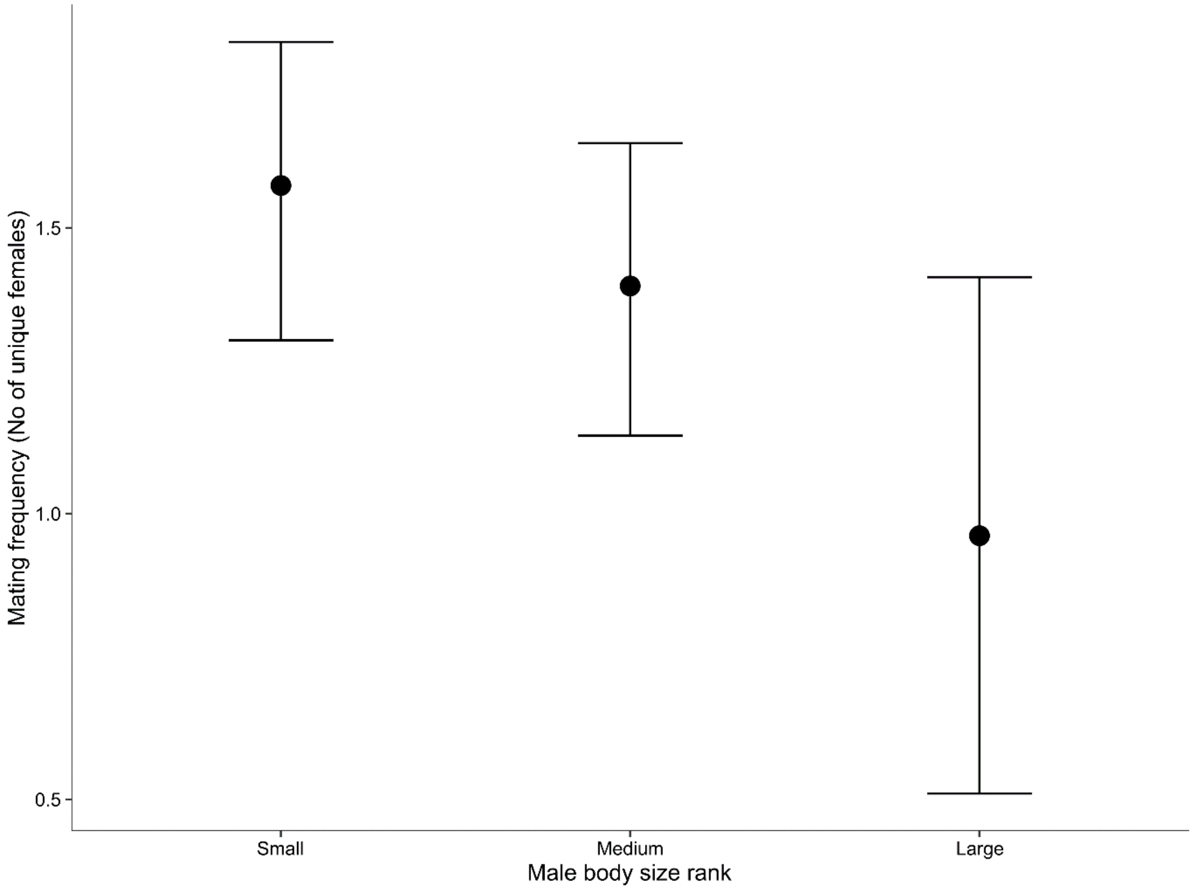


Figure 3.11. The relationship between male body size rank and the number of unique females a male mated with. The solid points represent the mean, and the error bars represent Standard Errors of the Mean (SEM).

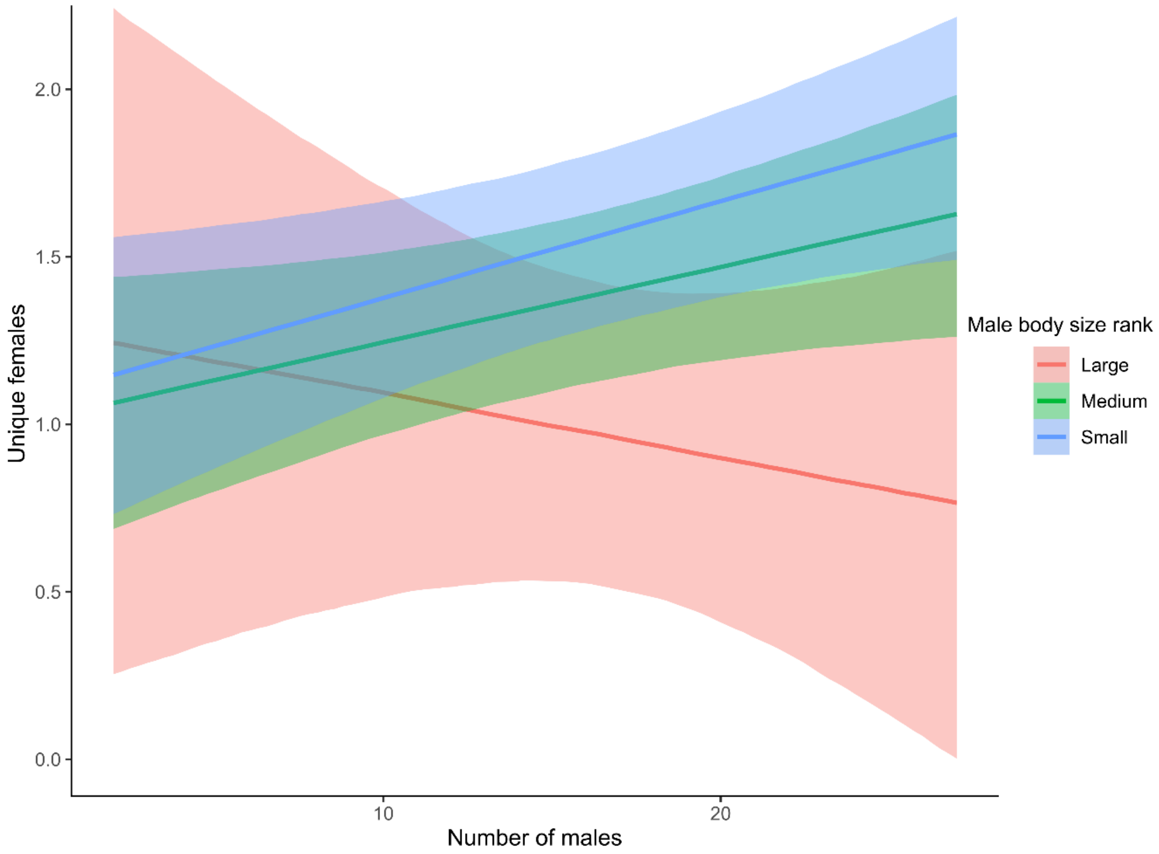


Figure 3.12. Interaction between number of males and male body size rank on the number of unique females a male mates with. Lines represent the posterior fits from Bayesian generalized linear mixed models. Shaded areas represent the lower and upper bounds of 95% uncertainty interval of the response.

iv. Guarding duration

The model examining factors that influence guarding duration showed several interesting results. In support of my experimental data, males that guarded for longer, mated for shorter durations (**Figure 3.14**). The number of males has a probability of 96.5% [pd] of being positive (Median = -18.54, 95%CI[−40.5, 2.13]), and can be considered to possibly influence male guarding duration (

Table 3.8, **Figure 3.13**). Number of females had a probability of 97.57% [pd] of being positive (Median = -33.55, 95%CI[-60.76, -5.15]), and can be considered to likely influence male guarding duration (

Table 3.8, **Figure 3.13**). The interaction between number of males and number of females has a probability of 99.63% [pd] of being positive (Median = 2.46, 95%CI[0.75, 4.14]), and can be considered to significantly influence male guarding duration (

Table 3.8, **Figure 3.13**). Guarding duration increased drastically as the number of males increased and female density was high (**Figure 3.15**). If female density was low, guarding duration decreased as the number of males increased (**Figure 3.15**). As male body size increased, male guarding duration increased, regardless of mating duration. However, males that mated for less time, guarded for longer and were larger (**Figure 3.16**).

Table 3.8. Variation in guarding duration in male *Lasiorhynchus barbicornis* for the best fitting model; I present fixed (β) and random (σ^2) effects with 95% confidence intervals. pd is the probability of direction representing the certainty associated with the most probable direction (Makowski et al., 2019).

Fixed effects	β (95% CI)	pd (%)
Intercept (Guarding duration)	466.78 (179.97, 751.36)	100*
Number of males	-18.54 (-40.50, 2.13)	96.5*
Male body size	2.09 (-1.49, 5.98)	85.13
Number of females	-33.55 (-60.76, -5.15	97.57*
Mating duration	-0.01 (-0.34, 0.30)	56.375
Number of males:Number of females	2.46 (0.75, 4.14)	99.63*
Male body size:Mating duration	-0.00 (-0.01, 0.01)	53.38

Random effects	σ^2 (95% CI)
Date	73.39 (16.53, 144.46)
Male identification	40.46 (1.81, 104.99)
Tree	37.05 (1.57, 122.38)

*indicates the probability of direction above 95% level

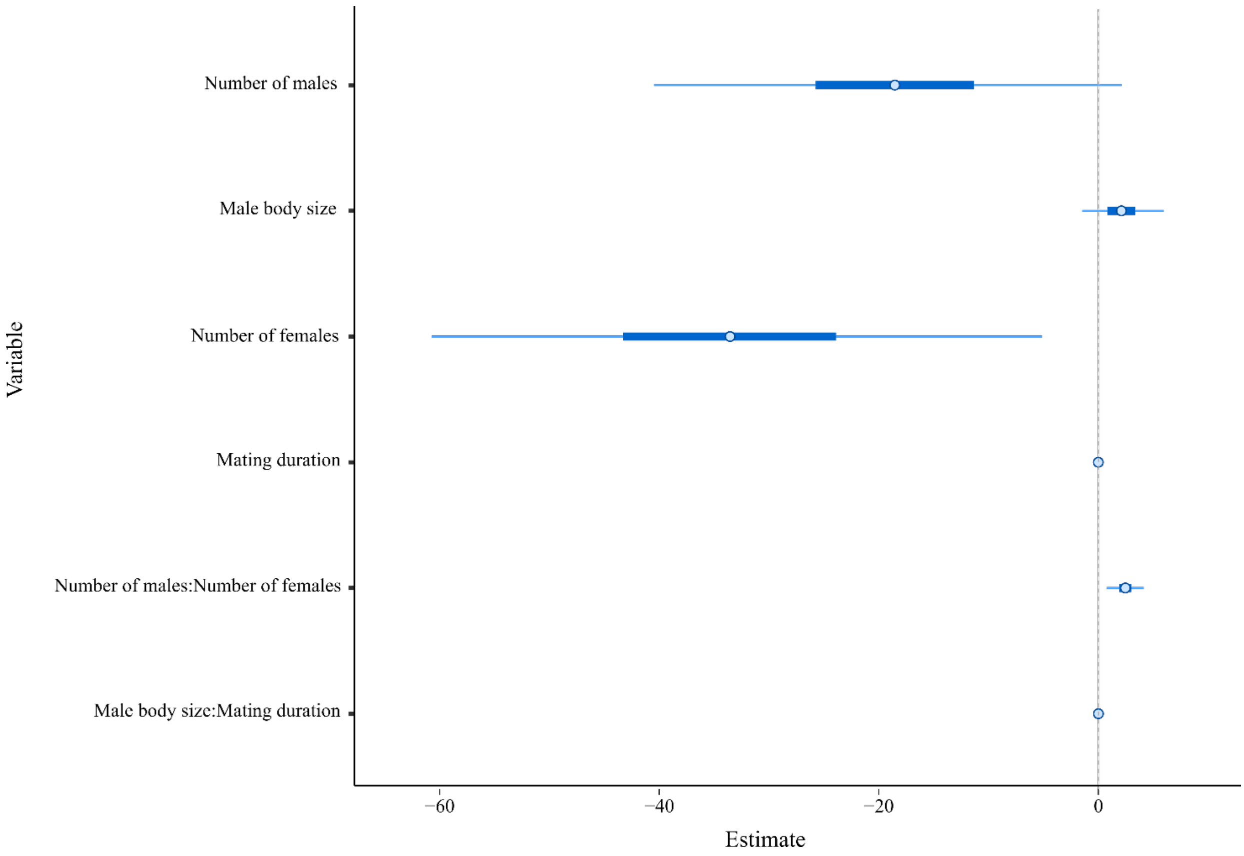


Figure 3.13. The effect size plot for the Bayesian model for guarding duration. The thick and thin lines describe the 50% and 95% credible intervals (CI) of each parameter, respectively. The small transparent dots are the median of the posterior distribution

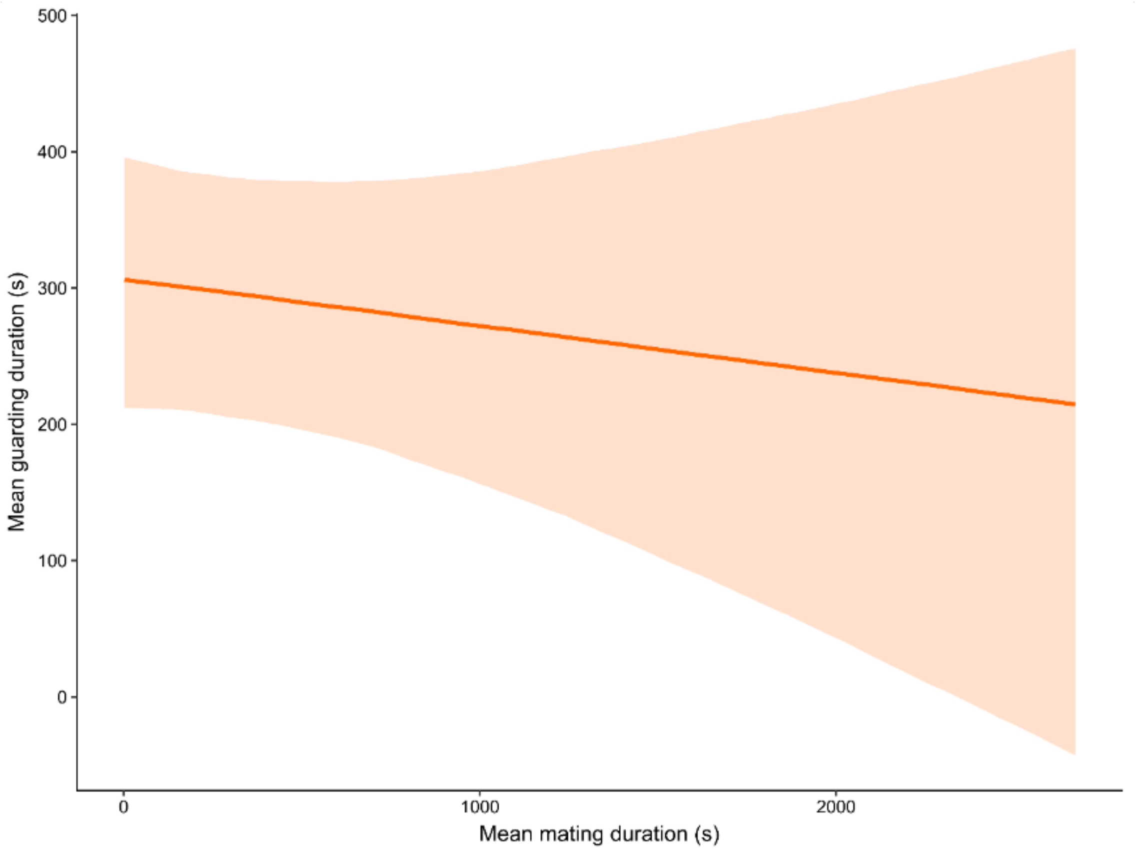


Figure 3.14. The relationship between mean guarding duration, and mean mating duration. The solid line represents the line of best fit, and the shaded area represents the upper and lower bounds of a 95% confidence interval.

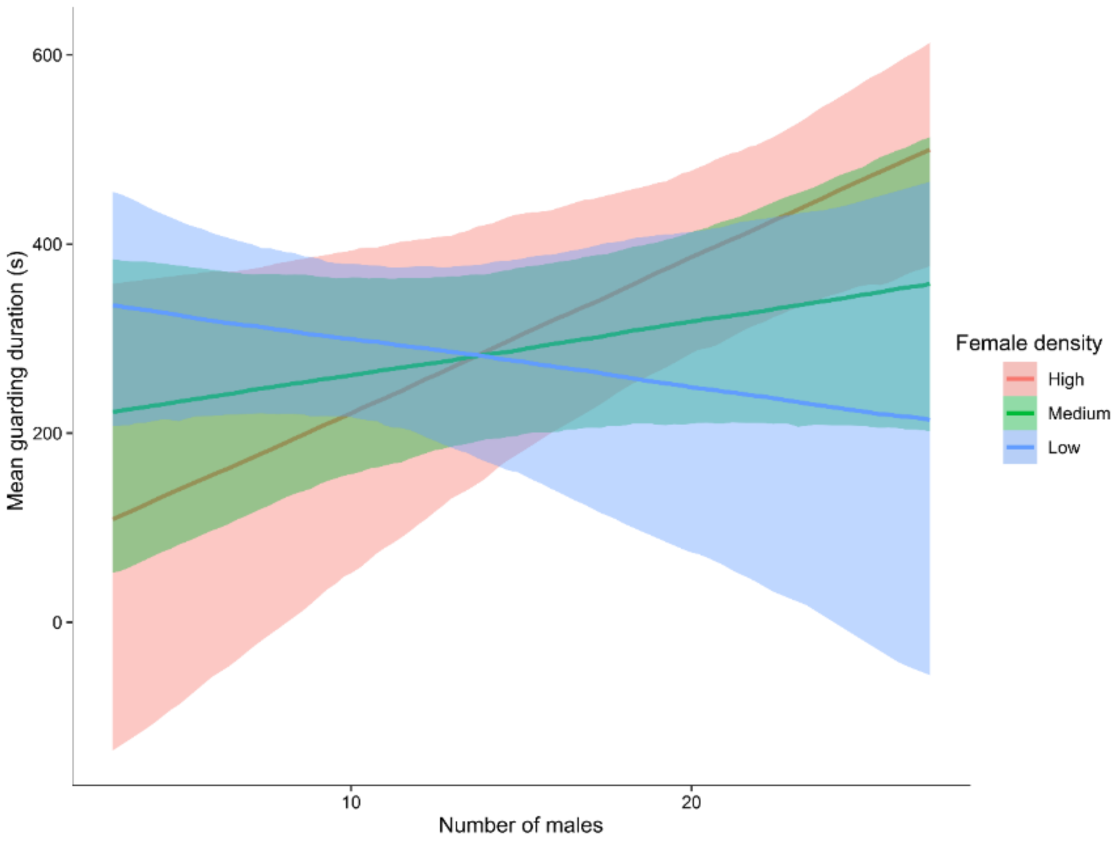


Figure 3.15. Interaction between mean number of males and female density on mean guarding duration. Lines represent the posterior fits from Bayesian generalized linear mixed models. Shaded areas represent the lower and upper bounds of 95% uncertainty interval of the response.

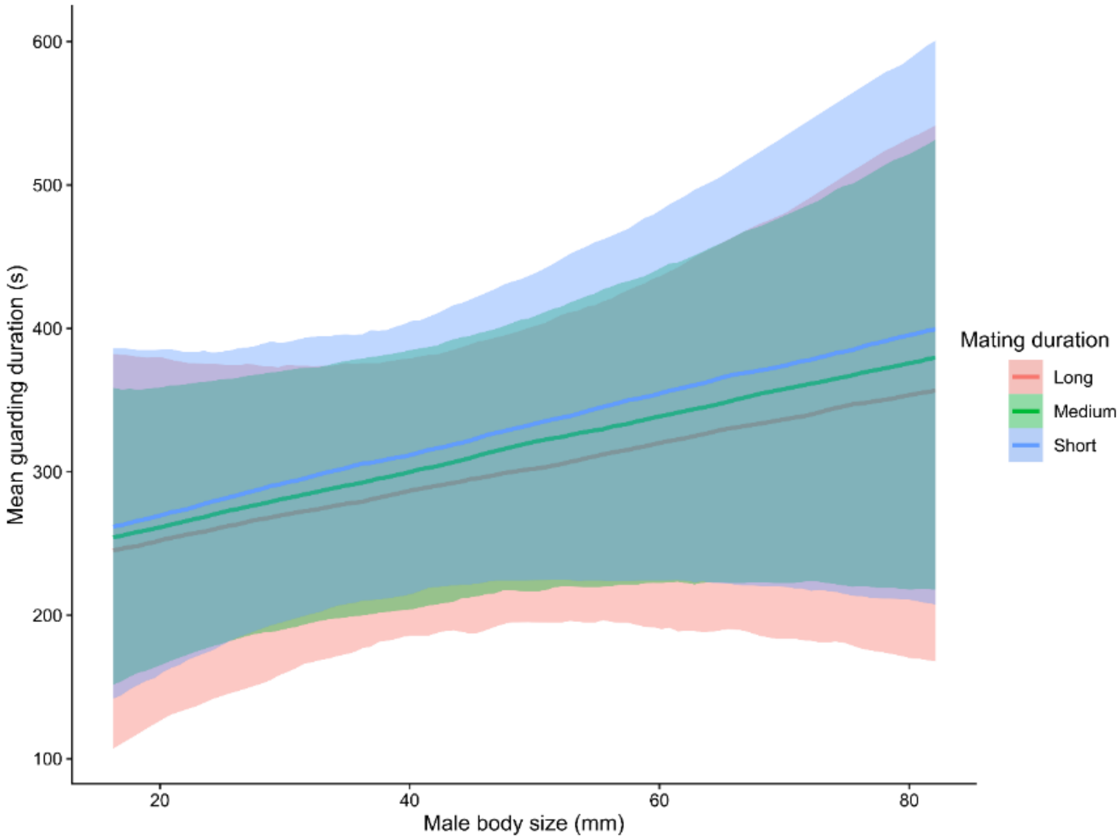


Figure 3.16. The interaction between mean male body size and mating duration on mean guarding duration. Lines represent the posterior fits from Bayesian generalized linear mixed models. Shaded areas represent the lower and upper bounds of 95% uncertainty interval of the response.

Discussion

In this study, male New Zealand giraffe weevils responded to the dynamics of population density by adjusting their copulation duration, mating frequency, and guarding duration. In support of my prediction, males mated for shorter periods in response to increasing numbers of males in the population. However, a complex interaction between male mating duration, mate frequency, and female density suggests that males may be strategically allocating their ejaculates in response to the availability of mates (**Figure 3.6**). In addition, mating frequency was significantly affected by population density. With an increase in male density, male mating frequency declined, but with an increase in female density, male mating frequency increased (**Figure 3.9**). This study will contribute to our understanding of how mating dynamics are affected by changes in demography. Further, it will contribute to a growing area of research exploring how sexual selection can influence the life-history traits of insects.

The presence of competitor males and their effect on mating duration is possibly due to interference from rivals removing them from the female (Bretman et al., 2009; Dore et al., 2021). However, a reduction in mating duration may also be a strategy, whereby males are responding to sperm competition intensity instead of sperm competition risk. In highly polyandrous species such as *L. barbicornis*, risk is expected to be less important as females will likely have already mated and will regularly re-mate, hence the risk of sperm competition is almost guaranteed (Bretman et al., 2009; Engqvist & Reinhold, 2005). On the other hand, sperm competition intensity, or the number of males competing for fertilisation of the female ova, is likely to be more variable. If males were responding primarily to sperm competition risk, we would expect them to increase their mating duration as a way of increasing investment (Bretman et al., 2009), but that was not the case. Instead, males decreased mating duration, suggesting male giraffe weevils may show adaptive mating behaviours based on sperm competition intensity. Additionally, males mated for less time but increased their mating frequency in response to increasing numbers of females in the population, suggesting a potential trade-off between current and future reproductive opportunities (Parker & Pizzari, 2010). This is the opposite of what I found in my second model looking at the effect of density on mating frequency, where males that mated for longer had higher mating frequency. The cause of this contrast may be due to the way the data was prepared or the way the models were specified, with the mating duration model using only males that mated, and the mating frequency model using both males that mated and males that did not mate. The reason I did not exclude males that did not mate from the mating frequency model is because ignoring zeros in ecological datasets can lead to biased parameter estimates and overdispersion of the data (Blasco-Moreno et al., 2019; Martin et al., 2005; A. Zuur et al., 2009). Despite this, the more likely result is that males are reducing their copulation duration and increasing their mating frequency. This may be evidence of a context-specific male investment strategy (Wedell et al., 2002), where males maximise fitness by decreasing copulation duration and increasing mate frequency. In this context, a decrease in copulation duration may enable males to avoid sperm depletion (Arnqvist & Danielsson, 1999; Berrigan & Locke, 1991; Parker & Simmons, 1994) whilst taking advantage of the influx of mating opportunities or help to offset the energy expenditure associated in mate searching when females are scarce (Kasumovic et al., 2007; Rowe, 2010). This may be particularly important to the mating system of *L. barbicornis*, where the sex ratio is often male biased, female distribution is patchy, and oviposition sites are ephemeral (Painting et al., 2014). Mating duration does not, however, necessarily correlate with increased sperm transfer or fertilisation (Eady, 1994; Kelly & Jennions, 2011), and therefore at higher densities, it may be beneficial for males to invest further into mate searching. On the contrary, my experimental data showed no relationship between male density and copulation duration. However, a different result may have been found if my sample size was larger.

Similarly, male New Zealand giraffe weevils appeared to have flexible responses of mating duration and mating frequency in response to the number of females in the population (**Figure 3.6**). When the number of females was low, mating duration was relatively long whereas when the number of females was high, mating duration was relatively low regardless of male mating frequency. On the other hand, for successful males who had high mating frequency, the decrease in mating duration was negligible, regardless of how many females were in the population. This variation could suggest a reproductive tactic that is context-dependent. This was evident by the apparent increase in mating duration when female density was low. In this context, males that are less likely to encounter or attain as many mates may be choosing to maximally invest in their current mate. For the more successful males, mating duration was more consistent, suggesting the presence of a condition-dependent ejaculate allocation strategy where males of high-condition may have lower marginal costs to ejaculate production when compared to low-condition males (Perry & Rowe, 2010). Because selection favours judicious ejaculate allocation strategies (Wedell et al., 2002), one would expect high-condition males to elevate their ejaculate expenditure only when perceiving an increased risk of sperm competition (Wylde et al., 2019). A previous study found that large male giraffe weevils mate more frequently than their smaller male counterparts (LeGrice et al., 2019). Therefore, if large males are a sign of high-condition, have reduced variation in mating duration and higher mating frequency, then this suggests that large males are able to invest equally into all matings. A study by Wylde et al. (2019) found that large male neriid flies, *Telostylinus angusticollis*, transferred greater ejaculate when mating second (i.e., increased sperm competition risk). On the other hand, small male neriid flies transferred relatively the same amount of ejaculate regardless of sperm competition risk, providing support for the theory that ejaculate allocation strategies may be condition-dependent. However, my finding here that increased mating duration is condition-dependent should be treated with some caution due to the type of sampling method used. Focal animal sampling was used for 1-hour sampling times, neglecting the possibility that at other times of the day, males that were unsuccessful during the sampling hour may have been successful when not being observed, and *vice versa.* Similarly, there was likely demographic stochasticity, whereby at one point, a small male may have been the largest male in the population and attain more matings, then at other times he may have been the smallest male in the population and competitively excluded.

As expected, male mating frequency increased with female density. With more females in the population, the likelihood of a male obtaining one or more mates increases. Small males were able to achieve almost, if not, the same number of matings as large males. This may be a case of different life-history strategies, whereby smaller males may achieve similar lifetime fitness when compared to larger, more dominant males, but over different time scales. For example, large males may have sharper reproductive peaks where they accumulate many matings over a short period of time compared to smaller males which may have shallower reproductive peaks where they accumulate few matings over an extended period of time (Andersson, 1982; Bonduriansky & Brassil, 2005; L. Rowe & Houle, 1996). Although previous research suggests large males live for longer than smaller males (LeGrice et al., 2019), there is yet to be research exploring the reproductive ageing of males of different body sizes. For example, in the neriid fly, *Telostylinus angusticollis*, large males 'live fast, die young', reaching their reproductive peak earlier in life, however also experiencing accelerated reproductive ageing compared to their smaller male counterparts (Hooper et al., 2017). A similar pattern was observed in antler flies, *Protopiophila litigata*, where large males had higher mating rates earlier in life, but aged faster than smaller males (Bonduriansky & Brassil, 2005). Thus, despite large male giraffe weevils generally living for longer (LeGrice et al., 2019), they may reach their reproductive peak earlier in life, and as such may have similar overall reproductive success than smaller males.

When total population density increased, males were less successful at securing mates and as a result had lower overall mating frequency. However, small males mated as frequently as medium and larger males, but only at higher densities (**Figure 3.8**). This suggests that the benefits of being large are eroded by the presence of smaller males, but only when population density is high. Interestingly, previous research on giraffe weevils by Fisher et al., (2021) found that large males negatively affected the mating success of small males at high densities, the opposite of what I found. At lower densities, large male body size can confer a significant advantage when large males are able to competitively exclude other males from securing mating opportunities (Rittschof, 2010). However, at higher densities, large males may be unable to successfully defend females, resulting in a breakdown of resource defence (McLain, 1992). The fact that small giraffe weevil males may be mating just as frequently as large males at high densities suggests they are using their sneaking tactic to gain matings. This was observed in the seed bug, *Neacoryphys bicuris*, where at high densities, large males were less likely to monopolize patches of host plant where females aggregate (McLain, 1992). Furthermore, at higher densities, males of the quacking frog, *Crinia georgiana,* allocate less time to mate calling and spend more time as satellites or searching for mates (Byrne & Roberts, 2004). This suggests that there may be thresholds in population density that are a key determinant of which ART an individual employs. Additionally, this may be an example of negative frequency-dependent selection, where small males, the less common morph, experience higher fitness than larger males, the more common morph. If ARTs are maintained by frequency dependent selection, then male mating success, and consequently male fitness, will vary based on the social environment (e.g., population density) (Bleay et al., 2007). However, experimental evidence to suggest ARTs are balanced by negative frequency dependent selection is lacking and further research in this area is needed.

Although male body size did not interact with the number of males to influence male mating frequency, it was trending towards significant (p = 0.07), which may be because there are so many factors all interacting to influence male mating frequency. This result suggests that the determinants of male mating success are complex and are influenced not only by body size, but the many other demographic factors I examined in this study. Additionally, this may suggest that there is divergent selection for male body size, where it is beneficial to be small or large, but not intermediate sized. Interestingly, there was no evidence of sexual selection based on mating success driving large body size in male giraffe weevils (Painting & Holwell, 2014b). However, research by Le Grice et al. (2019) found opposing evidence (albeit with a small effect size), suggesting there is indeed directional selection on large male body size based on increased mating frequency and survival. Because of such contrasting results, more extensive observations are necessary to depict the extent to which selection is acting on male body size with regards to mating success.

Males that mated for longer guarded for less time, with no significant effect of body size. This result suggests that extending mating duration is a form of post-insemination mate guarding (Alcock, 1994). Post-insemination mate guarding may function to prevent other males from mating with the female whilst his sperm fertilises her eggs, however it does not necessarily mean the male is investing in more sperm or ejaculate, rather just attaching to her for longer (Alcock, 1994). Males who guard lose the opportunity to inseminate more females, therefore mate guarding is only beneficial when there is a high probability of losing fertilisations when sperm competition is high (Parker, 1970), or a low probability of finding more females if a male chooses to search instead of guard (Parker, 1974a). In this study, sperm precedence and male mating order was not explored as a potential factor contributing to male guarding duration. Previous research on sperm precedence in male giraffe weevils showed that 50% of the time, the last male to mate with the female was the one to fertilise her eggs (Hockings, 2019). However, because I did not analyse mating order, I could not determine if there was a relationship between mating order and guarding duration. I would expect, based on the study by Hockings (2019), that males who mated last might mate for longer and guard for less time as they have a 50% chance of fertilising the females eggs. This represents an interesting avenue that should be examined in future studies. However, prolonged copulation accompanied by shorter guarding duration may be a sign that males are 'sperm-loading'. The sperm-loading hypothesis was originally proposed by Dickinson (1986) and suggests that males that mate for extended periods of time benefit by transferring lots of sperm which is likely to outcompete rival males during sperm competition. Several studies have shown that copulation duration correlates with sperm number (Arnqvist & Danielsson, 1999; Birkhead et al., 1995; Dickinson, 1986; Parker et al., 1990). The sperm-loading hypothesis is plausible when sperm compete numerically, however it is unknown how male giraffe weevils' sperm compete in the female genital tract and if number, viability, or size are better predictors of reproductive success.

Guarding duration was context-dependent in that it depended on the relative density of male competitors, but also on the number of females and consequently, the opportunity to mate with multiple females. Classic sexual selection theory and anisogamy suggests that male fitness is better optimised by mating with as many females as possible (Andersson, 1994; Bateman, 1948; Parker et al., 1972). Because males would do well to mate with as many females as possible, at higher densities, there may be a trade-off occurring between siring more offspring per mating (by increasing guarding duration), or siring less offspring per mating, but more offspring to different females. Such a strategy may be termed as bet-hedging (Seger & Brockmann, 1987; Slatkin, 1974; Starrfelt & Kokko, 2012). Bet-hedging is typically studied from the females point of view, with the theory that polyandry evolved to reduce the risk that all of a females eggs may be sired by a single, unsuitable male (Yasui & Garcia-Gonzalez, 2016). However, bet-hedging is likely common in males as well, as they may want to reduce the risk of investing all of their ejaculate into a single, unsuitable female. Bet-hedging has been explored with respect to ARTs in previous models proposed by Parker (1990b) and subsequently extended on by

Gage et al., (1995). In the models, if the relative cost of producing sperm is lower for sneakers, then they are expected to allocate less sperm per mating and maximise their mating opportunities. This was supported by my results, with smaller males mating with more unique females than large males. My experimental data also showed that males that mated for longer guarded for less time, supporting these predictions. However, although the giraffe weevils were kept in the presence of competitors until the mating trials began, they did not experience interruptions or conflict from other males as they might in the wild. Therefore, I would expect to see this negative relationship because they are able to mate for longer without risk of conflict or interruption, and thus will likely guard for less time too. In future, an experimental study with a larger sample size and trials conducted with rivals present will be beneficial to support the observational data in this study. Experimental studies provide the opportunity to control variables that otherwise may not be accounted for in observation studies. Therefore, experimental studies may show relationships between variables more clearly and allow such relationships to be disentangled.

Population density varied greatly over the season. Population density was lowest during November and highest during February. The sex ratio was almost always male-biased throughout each of the four sampling periods. However, the changes in sex ratio are more likely attributed to changes in overall abundance rather than there being more of one sex present. Both sex ratio and population density can influence a species' mating system, particularly by affecting the relative levels of intrasexual competition. Importantly, in a polygynous species such as the New Zealand giraffe weevil, both the OSR and population density are likely to affect the intensity and probability of sperm competition (Gage et al., 1995; Wang et al., 2008). During the peak of the season in February, up to 27 males and 20 females were found aggregating on the survey trees with females clumped together at optimal sites for oviposition. This behaviour displayed by females has likely led to the evolution of guarding behaviour in males to maximise their reproductive success, particularly because females can mate multiple times (Painting & Holwell, 2014b), and therefore there is a high risk of sperm competition. In species where prolonged associations occur, or females are a limited resource, higher densities of male competitors can influence mating duration and post-copulatory mate guarding (Holwell et al., 2016; Kokko & Rankin, 2006).

Because my study was based on observations of natural populations, it provides a more realistic and accurate picture of what actually influences male mating success. However, because studying natural populations does not allow for all possible conditions to be controlled, variation is expected, and makes it difficult to determine the complex interactions that may drive sexual selection without oversimplifying them. This study used an experimental and observational approach to explore my original research question which is ideal to gain an accurate understanding of variation in an individual's behaviour and the variable's that may be affecting it. There were some differences in individual behaviour in the observational and experimental data, however many variables in the experimental data were not accounted for as they were in the observational data. Artificial manipulations can be misleading, and many factors in the laboratory may have been affecting male giraffe weevils mating behaviour such as the lighting and surface textures. For example, mating trials were conducted in a plastic 1 L Sistema container, but naturally, mating events happen on trees. Further, these manipulations are static, whereas in the wild, social environments are dynamic and have a large element of stochasticity to them that is difficult to replicate in a laboratory environment. Therefore, it would have been better to collect a small tree trunk to conduct mating trials on in the lab as this is most natural to them. Similarly, the difference in mating duration between observational and experimental data may have been due to the difference in female density. The observational data showed that female density was important in determining male mating success, however this was not manipulated in my experiments as males were only exposed to a single female. In future, examining the same variables both in the field and the laboratory will provide better estimates of natural variation in male giraffe weevils mating behaviour.

Overall, my study shows that population dynamics can have complex effects on the mating behaviours of male *Lasiorhynchus barbicornis*, that are not captured by manipulative or artificial laboratory environments. Additionally, my experimental study shows that mating duration can be influenced by perceived sperm competition, but also that mating duration appears to trade-off with guarding duration, where the longer a male mated, the less time he is likely to guard for. These observations provide us with insight into the maintenance of ARTs, behaviour, and how sexual selection operates in male New Zealand giraffe weevils. Population density and other demographic factors can influence male mating and guarding duration and mating frequency, which are all important in our understanding of sexual selection and the mating system of this species.

4

General discussion

This thesis demonstrates the importance of body size as a predictor of investment in pre- and post-copulatory traits and the implications this has for sperm competition and fertilisation success in the New Zealand giraffe weevil. I found that small males may be investing more into their post-copulatory traits, possibly to gain a competitive advantage in sperm competition (**Chapter 2**). Additionally, this thesis has shown population dynamics have important implications for male mating success and the maintenance of alternative reproductive tactics (ARTs) in males of different body sizes. My approach used field and laboratory-based methods to answer unique questions pertaining to sexually selected traits and male mating success. I found that male New Zealand giraffe weevils responded to variation in population density by adjusting their copulation duration, mating frequency and guarding duration (**Chapter 3**). Overall, these findings demonstrate that (1) males of different body sizes invest differently into their pre- and post-copulatory traits, and (2) sexual selection is likely driving complex interactions between demographic factors and male mating success.

How are males of different body sizes investing into their pre- and post-copulatory traits?

Male giraffe weevils show differential investment into their pre- and post-copulatory traits based on weapon size. Weapon size can be advantageous for increasing reproductive success through male-male competition. However, because competition persists even after mating has occurred, males should invest in traits that allow them to compete post-copulation (Parker et al., 2013a; Parker & Pizzari, 2010). Males that are disadvantaged during pre-copulatory competition may invest more into traits that provide them with an advantage during post-copulatory competition to ensure they have a chance at fertilising the female's egg(s). In **Chapter 2**, I found negative allometry for all post-copulatory traits except sperm concentration. This suggests that small male giraffe weevils are investing disproportionately more into their post-copulatory traits (sperm length, aedeagus length, tegmen length, testes diameter, and testes weight) than large males. Investing into post-copulatory traits is a key way to increase reproductive success (Birkhead & Pizzari, 2002; Parker, 1998). For this reason, in species where body size is so variable, and resources are limited, investing more into post-copulatory traits is likely most common in smaller males.

Further, I found that males with relatively larger weapons (irrespective of body size) are not investing more into their post-copulatory traits, except for testes dry weight. This suggests that males with relatively large weapons have larger testes and therefore may have enough resources to allocate to both pre- and post-copulatory traits. It is interesting that large males have higher sperm concentration, and males with relatively larger weapons have larger testes. Large testes are thought to be indicative of increased sperm-producing tissue (Hosken, 1997; Kusano et al., 1991; Lüpold et al., 2009). Therefore, it is reasonable to assume that large males are experiencing an accumulation of benefits by having large testes and more sperm, as well as higher mating success (Le Grice et al 2019, but see **Chapter 3**), which may act together to increase their reproductive success.

How do population dynamics affect the mating success of males of different body sizes?

This study suggests that population density affects male mating success by influencing male mating duration, frequency, and guarding duration (**Chapter 3**). The effect of body size on mating success has previously been explored in the New Zealand giraffe weevil (LeGrice et al., 2019; Painting & Holwell, 2014b), and how population density and other demographic factors affect social selection has also been explored in one study (Fisher et al., 2021). Population density and the operational sex ratio has been shown to influence male mating success, particularly in species that are highly polyandrous (Bertin & Cézilly, 2005; Buzatto et al., 2015; Holwell et al., 2016; Niemelä et al., 2021; Rittschof, 2010; Wang et al., 2009). In species that are polyandrous, population density is likely to increase sperm competition risk and intensity, likely increasing the variation in male fertilisation success and consequently, reproductive success. However, it is difficult to base mating success solely on population density as both intrinsic (e.g., body size) and other extrinsic factors (e.g., sex ratio) interact to influence a male’s mating success. In **Chapter 3**, I found that male mating success was influenced by complex interactions between mating duration, mating frequency, population density, and male body size. Interactions between intrinsic and extrinsic factors that affect mating success are not uncommon. For example, in the walnut fly, *Rhagoletis juglandis*, mating duration was affected by both the density of each sex and the ratio of one to the other (Alonso-Pimentel & Papaj, 1996). Similarly, in the pipefish, *Syngnathus typhle*, population density and sex ratio interact to influence selection on body size, a determinant of mating success in both males and females (Aronsen et al., 2013). My findings clearly demonstrate that male mating success cannot be attributed to a single factor whether it be intrinsic or extrinsic.

I have shown that mating success can be credited to variation in population density and the operational sex ratio. Smaller males can secure as many matings as large males, but only when population density is high. This may be because at higher densities, the ability of large males to defend resources (oviposition sites and females) breaks down and they can no longer competitively exclude smaller males as efficiently as when population density is low (becomes scramble competition). Previous research found that large males have higher mating success (measured as mating frequency) than smaller males (LeGrice et al., 2019), however my findings suggest that they are likely to have similar mating frequencies at higher densities. It is important to incorporate population demography into sexual selection studies because it can influence the opportunity for, and strength of, sexual selection (Crowley et al., 1991; Emlen & Oring, 1977; Hubbell & Johnson, 1987; Kvarnemo & Ahnesjo, 1996). A recent study by Fisher et al., (2021) also found that population density affected the interactions between individuals (social selection), with social selection becoming more negative at higher densities. My finding was consistent with theoretical predictions by Painting and Holwell (2014b), where through the use of ARTs, smaller males may engage in equivalent numbers of copulations as large males. Furthermore, because smaller males engage in equivalent or higher numbers of copulations as large males at high densities, this may suggest that there is negative-frequency dependent selection maintaining this polymorphism in male body size.

Studying behaviour in the wild

Studying behaviour in the wild provides the opportunity to observe unaltered behaviour of natural populations. However, studying natural populations means that conditions cannot be controlled when trying to understand an aspect of an individual’s behaviour. Therefore, some relationships may not be clear due to uncontrolled variation and confounding variables (Bell et al., 2009; Kingsolver & Pfennig, 2007; Lambrechts et al., 1999). Studies exploring the influence of population density on mating success are predominantly conducted in the laboratory (Bretman et al., 2010; De Nardo et al., 2021; Wang et al., 2009), probably due to convenience. However, observational studies arguably provide more accurate representations of the biological variation in behaviour. For example, in **Chapter 3**, I found a large amount of variation between large individuals mating success. This variation was likely due to the sampling method used which only encapsulated 1-hour periods in an individual’s lifetime. I also found some contrasting results between the laboratory experiment and observational data, however this is likely due to the small sample size in the laboratory experiment. Despite the variation in the data, I believe I was still able to draw important conclusions about mating success in the New Zealand giraffe weevil. Therefore, I would argue that this observational study provides stronger evidence for a relationship between density and mating success because it was found in a natural population where all possible variation is present.

Limitations of my research

My research showed some interesting and exciting results that will aid in our understanding of how sexual selection acts on males of different body sizes. However, there were some limitations to my study that could be addressed in the future to make this research more robust. Firstly, in the experimental component of my population density study (**Chapter 3**), my sample size was very limited. Unfortunately, many of the giraffe weevils were not willing to mate, although I am not entirely sure of the reason. I suspect it may have been due to the plastic container the mating trials were conducted in. In the wild, females search for suitable oviposition sites on the host tree before drilling into the wood to lay their eggs (Meads, 1976). Perhaps females release pheromones when they are searching for suitable oviposition sites which signals to males that she is receptive to mate. This has been seen in many spider species, where females release airborne pheromones and contact pheromones to stimulate attraction and courtship (Costa et al., 2000; Gaskett et al., 2004; Herberstein et al., 2002; Kasumovic & Andrade, 2006). Similar findings have also been observed in *Drosophila* (Averhoff & Richardson, 1976; Greenspan & Ferveur, 2000; Savarit et al., 1999). Therefore, I propose that future studies address this by providing a small piece of wood from one of their host trees for the mating trials to take place on. Further, more research on their chemical ecology would aid in our understanding of their mating system.

One of my initial goals for this study was to measure ejaculate size to determine if males adjusted their ejaculate based on perceived levels of sperm competition. In my mating experiments, I attempted to look at the weight of the individuals before and after mating to measure ejaculate size as there is evidence of a correlation between male weight loss and female weight gain after mating (Edvardsson & Tregenza, 2005; Rönn et al., 2008). However, the amount of ejaculate was so small that it was not detectable despite the extremely precise scales (0.01 mg) in our laboratory. Furthermore, I aimed to dye the ejaculate *in vivo*, using Rhodamine B, a procedure used in Wylde et al., (2019), to measure ejaculate volume. Rhodamine dye is a thiol-reactive fluorescent dye that emits a bright red-orange colour when fluoresced, and has been successfully used in other studies to assess mating events (Beija et al., 2009; Blanco et al., 2006; Fisher, 1999; Johnson et al., 2017). Unfortunately, the sperm/accessory glands did not absorb the dye. Due to the limited time I had to conduct my research and challenges with COVID-19 lockdowns, I had to discontinue this approach. If the sperm absorbed the dye, I would have been able to measure how males of different body sizes adjust their ejaculate volume based on perceived levels of sperm competition (population density). When analysing the female sperm storage organ (spermatheca), I also found that the capsule auto-fluoresces in the same spectral range as Rhodamine B making it difficult to determine the volume of sperm transferred. Future research should explore different dyes available that would best work to dye male sperm and simultaneously overcome the obstacle of the auto-fluorescing spermatheca. One method I propose is the dying of ejaculate after the mating event has occurred. For example, Triton X-100 (TX) has been used to dye sperm post-copulation to measure ejaculate size or sperm in several taxa, including butterflies (Bissoondath & Wiklund, 1995), rats (Pattij et al., 2005), and leopards (Tipkantha et al., 2017). However, this approach is only useful if there are several colour variations of the dye to measure multiple ejaculates, or the females are previously unmated and there is no likelihood of previous ejaculates being stored in the spermatheca.

Future directions

Because there appears to be so much variation in mating success irrespective of body size, I suggest the use of genetic techniques to better understand the predictors of paternity share in *L. barbicornis*. By using genetic techniques such as microsatellite markers, patterns of sperm utilization and sperm precedence can be determined (McCullough et al., 2017), which is useful for the advancement of sexual selection research. Previous research looking at sperm utilization patterns in the New Zealand giraffe weevil found that males who mated last sired an egg only 50% of the time, however the other 50% were sired by unidentified males who were not observed to mate with the female (Hockings, 2019). In addition, there has been no research looking at female choice in New Zealand giraffe weevils, and such a high variability of paternity share suggests there is potential for cryptic female choice. By extending this area of research, we can determine the effect that body size may have on male reproductive success in the New Zealand giraffe weevil.

In addition, being able to manipulate the development of giraffe weevils will be useful to determine if there are any trade-offs between pre- and post-copulatory traits that I did not detect in this study. In my research, I only looked at phenotypic correlations, neglecting the possibility that genetic differences may explain some variation in the ability of individuals to acquire and allocate resources (Metcalfe, 2003; van Noordwijk & de Jong, 1986). Furthermore, trade-offs may only be detected during experimental manipulations in which the development of one trait is compromised to determine how this affects the development of another trait (Simmons & Emlen, 2006; Somjee et al., 2018). There can be high levels of genetic variation in an individual's ability to acquire resources which can lead to differences in resource allocation (Robinson & Beckerman, 2013). Variation in how individuals acquire resources can show positive correlations between the size of two phenotypic traits. However, this may be due to the ability of larger individuals to acquire more resources, therefore having more resources available to allocate to both traits when compared to small low-condition individuals (van Noordwijk & de Jong, 1986). This was seen in my research, where males with relatively large rostrums had relatively large testes, suggesting they had enough resources to allocate to both traits. Furthermore, it is possible that trade-offs are only apparent when individuals experience stressful environmental conditions, such as food shortage (Messina & Fry, 2003; Simmons & Emlen, 2006). Future research manipulating environmental influences such as larval diet will allow us to explain how different life-history traits are influenced by body size, environmental, and genetic factors.

Ejaculate size was not measured in this study due to the sperm not absorbing the rhodamine dye and weighing inaccuracies. However, ejaculate size is an important measure when looking at sperm competition and mating tactics, as it can allow us to see whether males of different body sizes (and ARTs) are adjusting their ejaculate size when facing different levels of sperm competition. In most situations, males that employ sneaking tactics are expected to invest more into their ejaculate than males that employ fighter tactics (Engqvist & Taborsky, 2016; Gage et al., 1995; Parker, 1990b). Similarly, males in a disfavoured role are expected to invest more than males in a favoured role (Ball & Parker, 2003; Parker, 1990a). As the population density increases, large males may find themselves in a disfavoured role, where they cannot defend females and oviposition sites effectively, and may adjust their ejaculate in response to this. On the other hand, small males may choose to invest maximally into all matings. However, further research is needed to explore these predictions. Therefore, looking at ejaculate size in male giraffe weevils of different body size (and ARTs) will improve our understanding of how sperm competition shapes the evolution of ejaculate traits.

Implications for behavioural ecology research on the New Zealand giraffe weevil

This thesis has extended areas of previous research on giraffe weevils, but has also provided a new understanding of how sexual selection may be acting on this species. In

Chapter 2, I explored how males adjust their investment into pre- and post-copulatory traits which may indicate strategies to increase their mating success. Further, by using an observational method, I was able to determine in **Chapter 3** how population density, body size, and sex ratio interact to affect male mating success. Giraffe weevils present an outstanding model organism for studying sexual selection, particularly because of their extreme size variation, exaggerated traits, and potential for sperm competition. My research will contribute to a growing body of research focusing on how sexual selection can shape the life-history of an insect.

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