# Sex and conflict in the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*)



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# ABSTRACT

Sexual selection is responsible for driving a diverse range of exaggerated traits across the animal kingdom, increasing the competitive ability of males that possess them, and consequently improving their reproductive success. Alternative reproductive tactics (ARTs) are expected to evolve when there is the potential for large disparity in reproductive success between groups of males, and when there is intense sexual selection due to competition between males for access to females. This study focused on the ecology and mating system of the New Zealand giraffe weevil, *Lasiorhynchus barbicornis* (Coleoptera: Brentidae), a species whose ecology and behaviour was largely unknown prior to the research presented herein. The main aim of this study was to determine the factors leading to the evolution of weaponry and alternative reproductive tactics in this species. To address this aim I used a combination of behavioural observations in the field, intensive morphometric sampling at a single site over three years, wider-scale sampling across the North Island of New Zealand, and a range statistical modeling techniques.

Firstly, I characterised the sex ratio, seasonal abundance, size variation and sexual dimorphism of *L. barbicornis* for a single population over three years. The population was found to be consistently male-biased in sex ratio, and both sexes showed high variability in size. There was a high level of sexual dimorphism, mostly due to the overall elongation of the male body, particularly the exaggeration of the male rostrum used as a weapon during male-male contests. A comparison of linear and non-linear regression models showed that the scaling relationship between body size and rostrum length in males was steeply, positively allometric, and was best described by a Weibull growth function. The non-linearity was due to an asymptote in the slope

towards the upper end of the distribution, suggesting that rostrum length for the very largest males is under constraint. Importantly, I found no evidence of dimorphism from the scaling relationship, and rostrum length was unimodal in distribution. In comparison, rostrum length in females was negatively allometric and linear in shape.

I found evidence of condition-dependent alternative reproductive tactics (ARTs) by conducting focal-male observations in the field. In addition to guarding and fighting behaviour, males less than 40 mm also used a sneaking tactic to gain access to females. Small males were more likely to sneak if the opponent male was larger than them, but would aggressively defend females if the opponent male was the same size or smaller than them. Mating frequency and duration was equal across all male body sizes, suggesting that the ability of small males to adopt a highly flexible set of tactics has allowed them to be as successful as larger males. Furthermore, there was no evidence of sexual selection on body size or rostrum length in males.

Focal observations determined the role of the rostrum as a weapon during male-male contests. I characterised the structure of antagonistic contests and correlated contest duration with the absolute and relative size of opponents to determine the competitive assessment strategy of *L. barbicornis*. Fighting was best described by the sequential assessment model (SAM), indicating that males use mutual assessment of their opponents to determine persistence during contests.

Finally, I determined geographical and temporal variation in mean body and weapon (rostrum) size by first measuring variation between 11 populations of *L. barbicornis* across the North Island of New Zealand, and then focusing on variation within a single population between and within three breeding seasons. The main finding from these studies was that there was significant variation in mean body size, rostrum size, and weapon allometry both within and between

populations. Most importantly, I found that while mean body size increased with latitude in accordance with Bergmann's rule, weapon allometry in males decreased with latitude. To my knowledge this is the first time weapon allometry has been shown to vary with latitude, and suggests that rostrum length is traded-off with increasing body size across latitude. I also speculate that temporal fluctuations in body and weapon size are due to developmental phenotypic plasticity in response to resource stress and environmental variation.

This study has contributed novel findings to the collection of literature on alternative reproductive tactics, competitive assessment behaviour and weapon evolution and variation. Furthermore, I have highlighted the giraffe weevil as a useful model species for behavioural ecological study. This thesis comprised the first major study of *L. barbicornis*' life history, and it provides a strong framework from which to base future single-species or comparative studies of the Brentidae.

*This thesis is dedicated to all the natural historians of the world who have inspired my curiosity in all that is weird and wonderful.*

*In particular, I would like to dedicate this thesis to Mike Meads, whose paper I based several of the questions from my study, and who I unfortunately was never able to meet.*

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# CHAPTER 1

# General Introduction

## 1.1 SEXUAL SELECTION AND WEAPON EVOLUTION

Sexual selection theory was originally developed by Darwin (1859, 1871) to explain the evolution of exaggerated traits most often displayed by males, such as bright colours, enlarged horns, and elaborate feathers. While it was initially difficult to understand how these elaborate traits which pose additional survival risks had evolved, Darwin recognized their importance in competition between males for access to mates, and therefore in influencing reproductive success. Sexual selection for exaggerated traits operates in two ways, either through intersexual or intrasexual selection (Andersson 1994). Intersexual selection (mate choice) influences traits that increase the attractiveness of males to females, resulting in the evolution of ornaments (e.g. male widowbirds, *Euplectes progne*, where males possessing the most elongated tail feathers are chosen as mates by females; Andersson 1982). Intrasexual selection (male-male competition) arises when males compete aggressively with each other for access to females, often resulting in selection for large body size or weaponry (e.g. large antlers used as weapons during aggressive encounters between male red deer, *Cervus elaphus;* Kruuk et al. 2002). The relative investment of males and females into gamete production has driven the distinct sex roles observed among most species (but see Eens & Pinxten 2000; Berglund & Rosenqvist 2003; Emlen et al. 2004 for examples of sex-role-reversal). Females produce a limited number of large, nutritious and non-motile eggs, in comparison to males who produce large numbers of small, motile sperm. Male reproductive success is therefore limited only by the number of females they have access to, and as such they will compete to mate as often as possible. Females on the other hand are expected to be choosier about mate selection because they are limited by the number of gametes that they can produce (Andersson 1994). By choosing the highest quality mates, females are therefore able to maximise their reproductive success and subsequently improve the viability and fitness of

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offspring (Andersson 1994).  These differences in sex-roles and consequent competition for mates are therefore the basis of sexual selection theory.

Weaponry shows an incredible diversity in form and function across the animal kingdom. Within the Coleoptera, weapon morphology is highly diverse, including enlarged mandibles (e.g. lucanids; Kawano 2000), horns (e.g. dynastid beetles; Eberhard 1979; weevils; Eberhard et al. 2000; scarabs; Emlen et al. 2005), fore-legs (e.g. harlequin beetle; Zeh 1992), hind-legs (e.g. leaf beetles; Eberhard 1996), and rostra (e.g. weevils; Johnson 1982; Eberhard 1983; Johnson 1983). However, an historic focus on the evolution of ornaments driven by female mate choice has resulted in a lack of understanding of how this diversity in weaponry has evolved (Emlen 2008).

Mate or resource guarding is expected to evolve when females are aggregated in space or time, usually around feeding or breeding sites, enabling males to monopolise and defend them (Emlen & Oring 1977). Weapons increase the ability of males to compete with other males, therefore it is expected that weapons evolve when there is the potential for males to defend resources or females in such a way that it increases their number of mating opportunities and subsequent reproductive success (Emlen 2008). For example, the elaborate horns of *Onthophagus* dung beetles have been suggested to have evolved partially due to the ability of males to defend tunnels in dung that females use as brood chambers (Emlen et al. 2005). This hypothesis may explain the prolificacy of weapons across the animal kingdom, but not necessarily why weapons are so diverse in form.

Under indicator models of sexual selection such as the handicap hypothesis, weapons are thought to be honest signals of quality, because only males in the best physical condition (and therefore highest genetic quality) are able to invest into costly, exaggerated traits (Zahavi 1975;

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Pomiankowski 1987). Exaggerated traits are highly condition dependent, and are sensitive to changes in environmental conditions such as nutrition and population density (Emlen 1994, 1997b; Kruuk et al. 2002; Cotton et al. 2004; Vanpé et al. 2007). Recent breakthroughs into understanding the developmental mechanisms responsible for the growth of exaggerated traits have also been made (Gotoh et al. 2011; Emlen et al. 2012). Hormones such as insulin/insulin-like-growth factor (IGF) and juvenile hormone (JH) are directly affected by environmental factors such as nutrient availability (Ikeya et al. 2002; Truman et al. 2006). Recently, horn growth in *Trypoxlus dichotomus* rhinoceros beetles was shown to be highly sensitive to circulating levels of insulin/IGF signals during development (Emlen et al. 2012). Similarly, the size of the enlarged mandibles of male *Cyclommatus metallifer* stag beetles were found to be highly sensitive to levels of juvenile hormone (Gotoh et al. 2011). During larval development, the effects of environmental conditions are communicated by circulating hormones, because the growth of imaginal discs that will eventually become exaggerated traits in adults are receptive and directly affected by the level of these hormones in the hemolymph. Environmental factors are known to be important in determining the condition of exaggerated traits, and it is through these hormonal pathways that the effects of environmental factors such as nutrition, stress and infection are imposed (Gotoh et al. 2011; Emlen et al. 2012; Shingleton & Frankino 2013). These findings provide evidence for the importance of weapons as reliable signals of quality or strength to potential rivals and/or mates. The use of weapons as reliable indicators of quality may go some way to understanding the incredible diversity of these traits (Emlen 2008).

Across animals, the most complex and elaborate weapons are rarely used to inflict serious damage on rivals during contests, despite being potentially lethal instruments. Instead, these weapons may be used to communicate size, status or condition during competitive assessment,

and therefore the most complex weapons may convey the most accurate information about the individual (Emlen 2008). Consequently, the evolution of increasingly complex weapons that act as signals during contests, could act as a mechanism driving diversification. In relation to this, exaggerated traits in some species (e.g. the enlarged claw of fiddler crab, *Uca* spp., males) have a dual purpose, as they are used as signals during male-male contests and also during courtship with females (Berglund & Bisazza 1996; Dennenmoser & Christy 2013). Female choice has been shown to be an important driver of the diversification of ornaments, and could therefore have an important role in driving weapon elaboration and diversification in some groups (Emlen 2008).

Several other mechanisms may be important in driving the diversification of weaponry. For example, animals may have evolved weaponry that are habitat-specific, or the volatility of sexual selection itself may drive diversity because of its ability to cause rapid evolution of trait size and shape (Emlen 2008). Most of the hypotheses proposed to explain weapon diversity remain untested at present, and studies of weapon evolution remain an exciting area of research.

1.**2 WEAPON ALLOMETRY**

The relationship between any part of the body and overall body size is commonly referred to as the scaling relationship or static allometry, and is highly variable in shape between species (Emlen & Nijhout 2000). Most morphological traits, such as wings or legs, are isometric, such that $b$ (the slope of the relationship between trait size and body size) is close to 1.0. This indicates that trait size scales proportionately with body size, showing that large individuals are simply "scaled-up versions" of smaller individuals (Shingleton & Frankino 2013). Slopes less than 1.0 indicate negative allometry, which is commonly observed for the genitalia of many

species because of stabilizing selection under the 'lock and key' hypothesis (Eberhard et al. 1998; Eberhard 2009). On the other hand, positive allometries expressed by slopes greater than 1.0 show that as body size increases, males invest increasingly more into relative trait size, indicating that larger males invest disproportionately more resources into trait size than smaller males. Sexually selected traits such as weapons and ornaments have been shown to almost always scale positively with body size, and subsequently positive allometry is often used as evidence that a trait is under sexual selection (Kodric-Brown et al. 2006). Positive allometries are proposed to arise in this way because larger males are expected to allocate more resources into weapon growth than small males as an honest signal of competitive ability, thereby reducing the number of antagonistic and potentially dangerous interactions with other males (Simmons & Tomkins 1996). Small males on the other hand are less likely to invest in large weapons because they will not increase their mating success by advertising low competitive ability, and the costs of developing the weapon outweighs any benefits of increased competitive ability.

Problems with assuming a relationship between positive allometry and sexual selection have been raised by Bonduriansky (2007), who showed that an historic fascination with charismatic species has resulted in an overrepresentation of species with extreme exaggerated traits that all show positive allometry. In fact, when taking into account a wider range of species, it was found that not all sexually selected traits show positive allometries, and not all traits showing positive allometry are the result of sexual selection (Bonduriansky 2007). Simmons and Tomkins (1996) also showed that while earwig species with enlarged forceps showed positive allometry, so did elytra lengths, possibly as a means of compensating for the increased cost of flying in large individuals. Following this, van Lieshout and Elgar (2009) found that forceps size was not positively allometric in *Euborellia brunneri* earwigs, despite behavioural evidence that this trait

was under sexual selection in males. So, while positive allometries are certainly a common

characteristic of exaggerated traits, it is important to couple morphological analyses with

behavioural observations to provide evidence of the trait's importance in reproductive success. It

is also important to compare the allometry of any exaggerated male trait with the corresponding

trait in females or to other non-sexual traits.

*1.2.1 Analysis of allometric data*

Allometry is traditionally quantified by the power equation $Y = aX^b$, where $Y$ is the trait size of

interest (e.g. horn size), $a$ is the scaling constant or $Y$ intercept, $X$ is a measure of body size, and

$b$ is the allometric or scaling exponent (slope) (Huxley 1932; Huxley & Teissier 1936). Log-

transformation of both traits allows a linear regression model to be fit to the data, using the

equation $\log(Y) = \log(b) + a[\log(X)]$. These data are best fit using type II regression models such

as standardized major axis (SMA) regression, because ordinary least squares (OLS) regression

can underestimate the relationship between the two variables and does not take into account

measurement error on both axes (Warton et al. 2006).
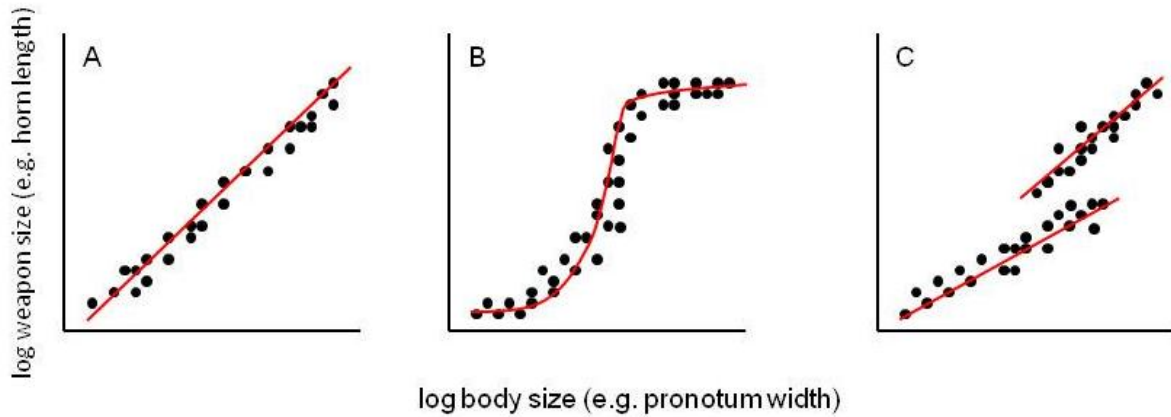
Figure 1. Hypothetical examples of weapon allometry. (A) Continuous, linear relationship between log body size and log weapon size, (B) sigmoidal relationship between log body and weapon size, (C) discontinuous relationship between body and weapon size, showing separation between two male morphs.

While many traits scale linearly with body size when plotted on a log-log scale (Figure 1A), there are also numerous examples of traits that are non-linear in distribution. These data require non-linear regression models to describe the scaling relationship (Knell 2009). The shape of a scaling relationship can be informative about the biology of a species. For example, many species show scaling relationships that are sigmoidal (Figure 1B) or discontinuous (Figure 1C) in shape, indicating the presence of at least two male phenotypes (Eberhard & Gutierrez 1991; Tomkins 1999; Moczek & Emlen 2000; Hanley 2001; Cook & Bean 2006; Okada et al. 2007; Buzatto et al. 2011; Zatz et al. 2011). For example, the presence of two male morphs was shown for *Serracutisoma proximum* harvestman, with major males displaying elongated second legs used in fights over territories compared to minor males that lack elongated second legs, and instead sneak copulations with females in major male territories (Buzatto et al. 2011).

Some allometric slopes have also been shown to asymptote at the upper end of the distribution, such that there is actually a decrease in slope for the largest males (Knell et al. 2004; Tomkins et al. 2005; Zatz et al. 2011). This declining allometry has been interpreted as evidence of resource limitation and physiological constraint (Knell et al. 2004), because in holometabolous insects such as beetles the allocation of resources to the imaginal discs that eventually become adult body parts occurs after the larva has ceased feeding (Nijhout & Wheeler 1996). This results in competition between traits as resources must be shared from a limited pool, and in very large males any further exaggeration of trait size would deplete resources available to the rest of the body (Knell et al. 2004).

*1.2.2 Variation in weapon allometry*

Exaggerated traits such as weapons represent a balance between natural and sexual selection, and a shift in either of these selection pressures can cause a subsequent shift in trait expression (Endler 1980; Andersson 1994; Svensson & Gosden 2007). Sexually selected traits are theoretically expected to evolve rapidly in response to change in selection pressures (West-Eberhard 1983; Svensson & Gosden 2007). However, studies documenting temporal or spatial variation in trait size have, until recently, been rare, and so currently this is an exciting area of evolutionary biology. Despite the paucity of evidence, scaling relationships do evolve, and this has been shown in a dramatic example between populations of *Onthophagus taurus* beetles (Moczek & Nijhout 2003). The switchpoint in the horn scaling relationship (indicated by the inflection point on the sigmoidal curve) has shifted to the degree normally only seen when comparing between species, but in this case was shown between native and exotic populations of *O. taurus* over a short period of less than 40 years. Although the cause of this change is not

known for *O. taurus*, the authors hypothesise that divergence in selection pressures between the populations such as population density may drive the evolution of the scaling relationship. Scaling relationships also show fine-scale temporal fluctuations, and in several insect species, decrease over a breeding season (Hardersen 2010; Hardersen et al. 2011). This is interpreted as evidence of the costliness of exaggerated trait production, because animals emerging later in the season should have less energy to allocate to resource-intensive traits such as wing spots in *Calopteryx splendens* damselflies (Hardersen 2010). Therefore, exaggerated traits not only evolve rapidly over time and space, but are also highly plastic and condition dependent in relation to environmental change.

## 1.**3 ALTERNATIVE REPRODUCTIVE TACTICS**

Alternative Reproductive Tactics (ARTs) are widespread in animals, and are expected to evolve whenever there is intense competition for mates, and a disparity between individuals in their relative reproductive success (Gadgil 1972; Shuster & Wade 2003). ARTs are described as alternative ways to obtain fertilisation, and these traits must be distinctive and show a discontinuous distribution (i.e. discrete behaviours and/or polymorphisms; Taborsky et al. 2008). Although ARTs can and do evolve in females (Henson & Warner 1997; Rüppell & Heinze 1999), they are much more likely to evolve in males because of the increased costs imposed on females through investment in gamete production and the higher levels of sexual selection on males (Taborsky et al. 2008).

ARTs have been described within a game theory framework, and classified under three strategies: alternative, mixed, and conditional strategies (Gross 1996). ARTs under the

alternative strategy are characterised by a polymorphism under frequency-dependent selection, where there are at least two genetically distinct morphs following alternative tactics (Gross 1996). Polymorphisms are maintained in these species because fitness is equal between the morphs, and they therefore remain stable in the population. Although there are numerous strategies in nature, one of the most well-known species is the marine isopod *Paracerceis sculpta*, which has three genetically distinct male morphs that employ different but equally successful mating tactics (Shuster & Wade 1991). Large, dominant males defend harems in intertidal sponges, smaller males mimic females and deceive dominant males, and tiny males hide in the harem and sneak copulations with females (Shuster 1987). Mixed strategies, although theoretically possible and described as a single strategy under probabilistic allocation to alternative tactics, were not known from any species when Gross (1996) described the potential for them to occur. To date I am only aware of one study that shows some evidence of a mixed strategy in the mating behaviour of the African striped mouse *Rhabdomys pumilio* (Schradin & Lindholm 2011).

By far the most common type of ARTs described from nature fall under the conditional strategy (for hundreds of examples see Oliveira et al. 2008), which is defined by status-dependent selection, such that an individual 'chooses' the phenotype most appropriate to its condition that will return the highest possible fitness (Gross 1996). Under status-dependent selection, males using different tactics are not required to have equal fitness to maintain multiple phenotypes because the tactic is determined by the status (e.g. body size or age) of the individual rather than a genetic polymorphism, and consequently the individuals employing the inferior tactic are expected to have lower reproductive success. However, there has been much debate regarding the relative fitness of individuals using conditional ARTs, with Shuster and Wade (2003) in

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particular arguing that fitness is rarely measured correctly and that the fitness of the dominant phenotype is usually over-estimated. They argue that although the average fitness of the inferior phenotype may not be as high as the most successful individuals of the dominant phenotype, the overall average fitness for each phenotype should be equal. While Shuster and Wade's (2003) critique of the status-dependent selection models raises legitimate problems, some of their points are flawed, especially because their argument for equal fitness is supported by examples of polymorphic species, not those with conditionally-expressed tactics (Tomkins & Hazel 2007). Regardless of this debate, there have been few studies that determine the relative fitness of each phenotype, despite its importance for understanding the evolution and maintenance of ARTs.

*1.3.1 ARTs in insects*

In insects, ARTs are highly diverse in form, and are present in species from most orders and from many types of mating systems. Most commonly, insect ARTs are employed during mate searching, and are divided between high investment, stationary tactics versus low investment, active tactics (Brockmann 2008). Many ARTs have evolved in species with distinct morphological differences between groups of males employing different mating tactics, often due to the presence of a secondary sexual trait which is enlarged in one group of males. For example, male *Perdita portalis* bees can be divided into two morphs according to head size, with large-headed males using aggressive and frequently fatal fighting tactics, while small-headed males copulate away from nesting sites while females forage on flowers (Danforth 1991). However, morphological polyphenism is not necessary for ARTs to evolve, and alternative behaviours are frequently related to size (Alcock 1996; Pitnick et al. 2009; Schradin & Lindholm 2011) or age (Miller 1984; Neff & Gross 2001) without being associated with a dimorphism.

Yellow dung flies (*Scathophaga stercoraria*), for example, use ARTs associated with male body size, in the absence of a sexually selected exaggerated trait and subsequent dimorphism (Pitnick et al. 2009). The ecology of a species can also have important implications for the opportunity for ARTs to evolve, and it is in species where females are spatially segregated in the environment (i.e. between oviposition vs. feeding sites) when alternative tactics are frequently employed by males (Thornhill & Alcock 1983).

*1.3.2 Sneaking versus fighting as ARTs*

ARTs are commonly divided between males that rely on aggressive, defensive behavior to secure resources or females, versus smaller, less dominant males that rely on opportunistic tactics such as sneaking to secure mates. This combination of tactics has been shown in many species across multiple taxa, but is particularly well known in the *Onthophagus* dung beetles. This genus is incredibly diverse, with more than 2000 species described, and several species have become model organisms for studying the evolution of weaponry, male dimorphism, and alternative mating tactics (Simmons & Ridsdill-Smith 2011). *O. taurus* and *O. acuminatus* males are characterised by two morphs in association with the size and presence of a pair of thoracic horns used as weapons during male-male contests. Major males with large horns guard tunnels used by females as brood chambers, and fight aggressively with competing males to prevent their access to the female (Emlen 1997a; Moczek & Emlen 2000). In both species, small, hornless males have been shown to rely on sneaking tactics to gain access to females, which they sometimes achieve by digging side-tunnels to intercept brood tunnels and avoid being noticed by the guarding male. The relative fitness of each male morph has been determined for *O. taurus* in

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several studies, but there is conflicting evidence for both equal and unequal fitness between

morphs (Tomkins & Simmons 2000; Hunt & Simmons 2001; Simmons et al. 2004).

## 1.4 FIGHTING AND ASSESSMENT STRATEGY

Across the animal kingdom fighting is a widespread phenomenon in many species used to

increase reproductive success. In species with ARTs fighting is often the tactic employed by

dominant males to secure females or defend resources. To date most studies on agonistic

behaviour and competitive assessment have focused on species where fighting is a ubiquitous

behaviour across all males. However, a logical extension to studies of ART evolution is

determining how assessment influences fights between dominant males.

While most studies have shown that contests are won by males that have the highest resource

holding potential (RHP; Parker 1974), much research in recent years has been focused on

determining the assessment strategy used by males during contests (Hack 1997; Hofmann &

Schildberger 2001; Taylor et al. 2001; Pratt et al. 2003; Jennings et al. 2005; Morrell et al. 2005;

Kelly 2006; Prenter et al. 2006; Hsu et al. 2008; Egge et al. 2011; Godsall & Smallegange 2011;

Kasumovic et al. 2011; Jennings et al. 2012; Yasuda et al. 2012). Several models have been

designed to describe the assessment strategy used in antagonistic contests between animals, with

the main aim to distinguish between species that use mutual assessment of their opponent, versus

species that fight to the limit of their own threshold (self-assessment). The sequential assessment

model (SAM) assumes that when individuals first encounter each other they have limited

information about their opponent's RHP, but gain information about the relative strength and

ability throughout a contest (Enquist & Leimar 1983; Leimar & Enquist 1984; Enquist et al.

1990). Fights are therefore based on mutual assessment of the opponent in relation to the individual's own strength and ability, and contests endure until the weaker individual determines that it cannot win and terminates the interaction. The energetic war of attrition model (eWOA; Mesterton-Gibbons et al. 1996; Payne & Pagel 1996, 1997) and cumulative assessment model (CAM; Payne 1998) have however been developed for species that persist in contests only in relation to their own ability. In these models contest duration or escalation is determined by the weaker individual's own threshold rather than an assessment of the opponent in relation to their own ability.

Each model has specific predictions about the relationship between fight duration and the absolute size of the smaller and larger contestants. Distinguishing between these models can be done by using linear regression modeling (Taylor & Elwood 2003). Under the energetic war of attrition model, contest duration should correlate positively with the size of both the smaller and larger individuals, but the slope for the larger male will be less steep. The cumulative assessment and sequential assessment models both predict that the relationship between contest duration and the size of the smaller male will be positive, and the relationship between the size of the larger male and duration will be negative at approximately the same magnitude. To further tease apart the differences between these models, one must determine the structure and intensity of contests such as the presence/absence of escalation and de-escalation of phases, infliction of injuries, and behavioural matching (Briffa & Elwood 2009).

## 1.5 THE BRENTIDAE

The Brentidae (Coleoptera: Curculionoidea) are a highly diverse family within the weevil superfamily, with more than 290 genera and 1690 species currently described (Sforzi & Bartolozzi 2004). Brentids are commonly referred to as the straight-nosed weevils, because they lack the curved rostrum characteristic of other families within the Curculionoidea. The majority of species are xylophagous and live in tropical rainforests, although some species are also found in temperate, arid and sub-desert areas, including some myrmecophilous species (Sforzi & Bartolozzi 2004).

Despite the lack of behavioural ecological research, brentid weevils are ideal as models for exploring weapon evolution, mating and fighting behaviour as many species exhibit high levels of sexual dimorphism, often due to the possession of exaggerated traits (rostrum and mandible size) in males (Sforzi & Bartolozzi 1998). High levels of intrasexual phenotypic variation, and aggressive fighting behaviour between males has been reported for several species (Johnson 1982, 1983; Sanborne 1983), including *Ithystenus angustatus* males observed by Wallace during his journey around the Malay Archipelago (Wallace 1869). Although very little is known about the ecology and life history of most species within this family, the mating systems of several of the more charismatic species have been described. Evidence for sexual selection on male rostrum length has been shown in two species, *Brentus anchorago* and *Claeoderes bivittata*, which both use their rostrum as a weapon during contests with other males, resulting in a large-male reproductive advantage (Johnson 1982, 1983). Small male *C. bivittata* also show some evidence for the evolution of ARTs, as they often hide under females and mate when the guarding males are distracted fighting (Johnson 1983). Disparity in reproductive success and the subsequent evolution of ARTs is not surprising in this family, especially when considering the extreme

intrasexual size variation found in many species, which would have consequences for the relative mating success of males of different sizes.

## 1.6 THE NEW ZEALAND GIRAFFE WEEVIL

The giraffe weevil *Lasiorhnychus barbicornis* Fabricius is endemic to New Zealand, and belongs to the family Brentidae. It is the only representative of the subfamily Brentinae in New Zealand, and is probably most closely related to species found in Sulawesi (Kuschel 2003). Larvae of this species are woodborers of dying or recently dead trees from a range of native host trees (Appendix 1) such as karaka (*Corynocarpus laevigatus*), pigeonwood (*Hedycarya arborea*), and pukatea (*Laurelia novae-zelandiae*) (Meads 1976). *L. barbicornis* are distributed from the top of the North Island of New Zealand, to the north-western regions of the South Island (Kuschel 2003).

*L. barbicornis* display such an extreme level of sexual dimorphism that males and females were originally described by Fabricius as two different species (Kuschel 2003). The most striking aspect of sexual dimorphism is the difference in rostrum morphology, which in males shows extreme elongation such that the rostrum makes up around half of the total body length of the individual. Both sexes also show high levels of adult size variation, with males varying from 16 − 90 mm and females from 13 − 46 mm in total body length (Figure 2) (Kuschel 2003).
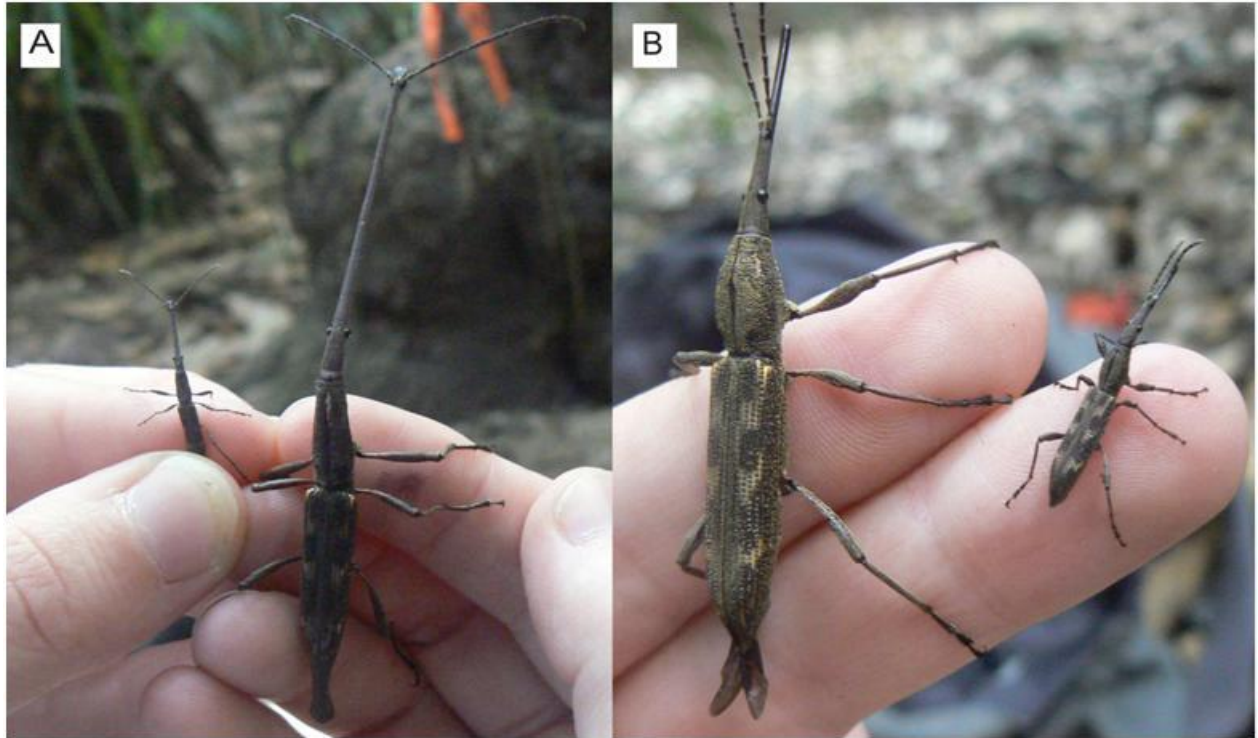
Figure 2. Size variation in adult (A) male and (B) female *L. barbicornis*.

The life history and behaviour of *L. barbicornis* is almost entirely unknown, although Meads

(1976) conducted observations of behaviour in the Orongorongo Valley between 1972 and 1973

that provide the only information, prior to this study, about giraffe weevil ecology. Meads

observed aggregations of adult *L. barbicornis* on a dying karaka tree, and found that females

bore into the wood using their rostrum to prepare a hole for oviposition (Figure 3A, B). While

females drill, males approach and begin copulation (Figure 3A), during which other males will

often approach and fight with the copulating male. During these fights males use their rostrum to

rake across their opponent in an attempt to dislodge them from the tree, which on occasion

occurs when the winning contestant is able to bite onto an opponent's leg with their mandibles,

and lift them up off the tree.
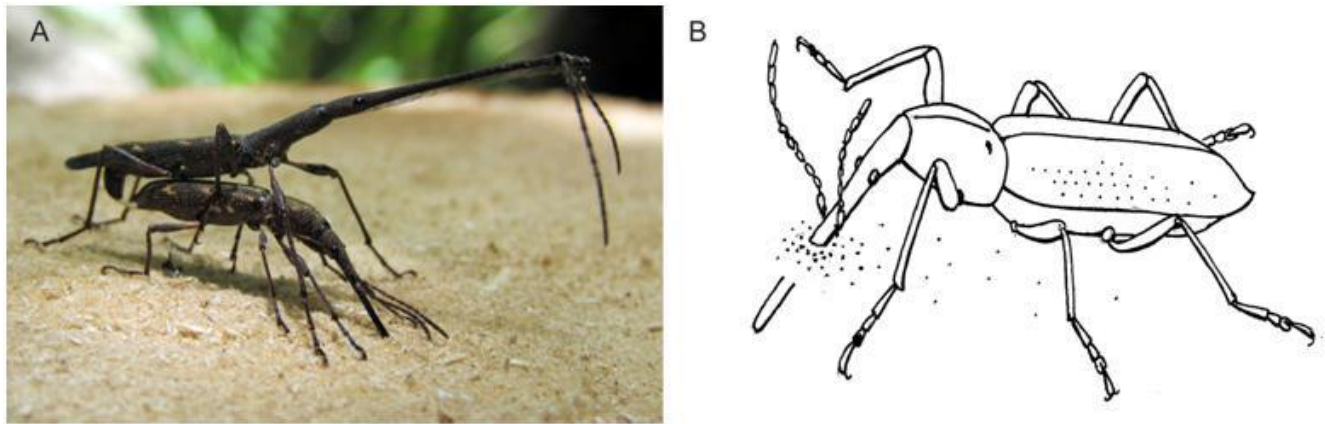
Figure 3. (A) A male *L. barbicornis* (above) mates with a drilling female (below). (B) Line drawing of an adult female *L. barbicornis* with rostrum inserted into wood in order to prepare a hole for egg-laying (Illustration by D. W. Helmore © Landcare Research, used with permission).

Meads (1976) provides several observations that suggest that *L. barbicornis* would be an interesting model for exploring the evolution of weaponry and alternative mating tactics. Firstly, he provides anecdotal evidence that the sexual dimorphism in this species has evolved due to the differences in function of the male and female rostrum, which in males is used during fights with other males, and in females is used as a drill to prepare holes for oviposition. Meads also states that small males do not fight, but instead spend more time hiding and mate only when larger males are distracted by another male. These observations of behaviour suggest that small males may be competitively disadvantaged by their body size, and perhaps suffer lower mating success than larger males due to their inability to defend females. The possible disparity in reproductive success between males of different sizes suggests that this species would be an ideal candidate for the evolution of ARTs, specifically through the use of sneaking behaviour in small males

while larger males rely on fighting behaviour to secure mates. In-depth research to describe the mating system of *L. barbicornis* is required to explore the possibility of ART evolution in this species, and to understand the purpose of the elongated male rostrum and its role in male-male competition and mating success. The large aggregations of *L. barbicornis* on dead and dying trees make them an ideal candidate for a field-based research study, which allows easy observation of behaviour in their natural environment.

## 1.7 THESIS OUTLINE

This thesis aims to investigate the evolution of weaponry and alternative reproductive tactics in the New Zealand giraffe weevil *Lasiorhynchus barbicornis*. This work provides the first detailed study of *L. barbicornis* biology, and aims to highlight this species as a model system for studies of sexual selection and behaviour. I present six studies on *L. barbicornis* behaviour and morphology that are used to support the overall thesis aim.

In Chapter Two I describe the sexual dimorphism, sex ratio and seasonal abundance of *L. barbicornis* over three years for a single population. These findings are discussed in an evolutionary framework, and provide context to the evolution of weaponry and ARTs in this species, which will be used to base findings in subsequent chapters on mating (Chapter 4) and fighting behaviour (Chapter 5). This chapter accepted for publication in *Austral Entomology*.

In Chapter Three I describe the scaling relationship between body size and rostrum length for adult *L. barbicornis*, by comparing six regression models (linear, quadratic, breakpoint, logistic, four- parameter logistic, and Weibull growth curve). Sexually selected traits, such as those used as weapons, commonly show positive allometry, and in this chapter I test for deviation from

isometry for male and female rostrum length. I then determine whether any other body parts

compensate or show trade-offs compared to rostrum length in males. These results are discussed

in the context of resource limitation, physiological constraint, and trait compensation. This

chapter is now published in *PLoS ONE*.

Chapter Four explores the evolution of ART's in *L. barbicornis* males using a series of focal

animal observations. I determine the use of sneaking behaviour by small males, and whether this

is a flexible tactic used in combination with guarding and fighting behaviour. I also determine

whether sneaker-sized males are able to choose the best tactic relative to their opponent's size,

thereby allowing them to optimise their decision-making. Finally I determine the mating success

of males across different body sizes and calculate the strength of selection on male traits. I link

these findings back to the male scaling relationship described in Chapter 3, and discuss evidence

that *L. barbicornis* ARTs are an example of a conditional strategy.

In Chapter Five I use focal male observations to document fighting behaviour and contest

escalation in *L. barbicornis*. I then test between three models of assessment strategy using a

combination of methods (regression analysis and contest structure description) to determine the

likelihood that males use mutual assessment of rivals during contests.

Chapter Six describes geographic variation in body size and weapon allometry for *L.

barbicornis*. Using measurements of males and females from 11 populations spanning the North

Island of New Zealand, I determine geographical variation in body and weapon size in a

latitudinal cline according to Bergmann's rule. While many studies have found evidence that

body size follow latitudinal clines, in this chapter I present the first evidence of a latitudinal cline

in weapon allometry. I also test for the application of Rensch's rule in this species. I then

speculate on possible factors driving geographical variation in *L. barbicornis* weapon and body size.

In Chapter Seven I determine temporal variation in body size and weapon allometry both within (monthly) and between seasons over three years. These results provide further support for the results found in Chapter 6, and I speculate on the possible causes of this temporal variation.

In Chapter Eight I summarise the principal findings from this thesis.

A modified version of Appendix 1 is now in press in the *New Zealand Journal of Zoology.*

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# CHAPTER 2

# Sexual size dimorphism and sex ratio of the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*)

**2.1 ABSTRACT**

Both the sex ratio and the extent of sexual size dimorphism (SSD) can have important implications for the mating system of a species. In particular, the sex that is limited (generally females) is often the subject of intense competition, which can drive changes in behaviour and morphology to increase the competitive ability of the more abundant sex. The giraffe weevil (*Lasiorhynchus barbicornis*) is a large and charismatic species found across much of New Zealand, but very little is known about its natural history. I determined the population structure and ecology of *L. barbicornis* from a single wild population over three years. Giraffe weevils were found to be highly abundant in the warmer months between November and May, but showed a peak in February and March in each year. The sex ratio was consistently male-biased throughout the breeding season (mean sex ratio between seasons $0.59 \pm 0.02$). Univariate and multivariate analyses showed that *L. barbicornis* are highly sexually size dimorphic, mostly due to the extreme elongation of the male rostrum. All male body parts were also more variable in size than females, particularly rostrum length, as shown by higher coefficients of variation. These data are crucial to gain an understanding of giraffe weevil ecology, and of factors driving the mating system in this species. The male-biased sex ratio and SSD are key components of *L. barbicornis* ecology that are likely to have driven the highly competitive, defence-based mating system observed.

## 2.2 INTRODUCTION

The sex ratio of animal populations has been shown to be highly influential on their mating systems (Kvarnemo & Ahnesjö 1996). Fluctuations and bias in the sex ratio can cause changes in male mating tactics and the intensity of competition (Clutton-Brock & Vincent 1991). However, the simple ratio of the number of males to females in a population (adult sex ratio: ASR) can be dramatically different to the ratio of males against receptive, sexually active females (operational sex ratio: OSR). For example, guppy (*Poecilia reticulata*) populations generally consist of a higher number of adult females than males, but females mate infrequently so the scarcity of receptive females results in a male-biased OSR (Jirotkul 1999). The OSR is therefore the most accurate measure of sex ratio bias, and has the greatest effect on mating systems (Emlen & Oring 1977).

 A bias in OSR is important because a limitation of one sex can result in high levels of competition between individuals of the sex in excess (Kvarnemo & Ahnesjö 1996). In male-biased systems, sexual selection is expected to drive behavioural and morphological adaptations such as mate guarding and fighting in males that increase their access to, and monopoly over females. A male-biased OSR can increase levels of competition and aggression between males (Spence & Smith 2005; Weir et al. 2011), influence the use of alternative mating tactics (Zamudio & Chan 2008), and decrease overall male mating success (Arnqvist 1992a). However, in female-biased systems, where males are limited, there will be less competition for access to females, so males are expected to be choosier in mate selection. Furthermore, with fewer competitors it is easier for males to achieve full paternity and they should invest in mating multiple times rather than guarding females (Berger-Tal & Lubin 2011).

The OSR and local population density can fluctuate significantly during a breeding season and vary between populations, which can have important implications for the degree of competition between the more abundant sex (Kvarnemo & Ahnesjö 1996), and can affect overall male copulation duration and mating success (e.g. Alonso-Pimentel & Papaj 1996) . For example, in the sex-role reversed pipefish (*Syngnathus typhle*), the level of competition between females for access to receptive males increases over the season as males become progressively unavailable as a greater proportion becomes 'pregnant' (Vincent et al. 1994).

Population density, independent of sex ratio, has been shown to have an important effect on the intensity of intraspecific competition. When population density is high, males may be unable to defend females resulting in scramble competition, while at low densities large males are successfully able to defend females (Conner 1989; McLain 1992). Alternatively, large males may be able to monopolise females at high densities, excluding smaller males from mating opportunities (Rittschof 2010). Variation in population density can also affect copulation duration and levels of sperm competition in polygamous species (Arnqvist 1992b; Wang et al. 2008). It is therefore important to determine fluctuations in OSR and population abundance and recognise that levels of competition can be dynamic throughout a breeding season.

The spatial and temporal availability of females can also have important consequences on male-male competition. When females aggregate for access to resources (e.g. food, nesting or oviposition sites) and are therefore clumped in space and time, males are expected to evolve guarding behaviour to allow monopolisation and defence of females from other males (Emlen & Oring 1977; Shuster & Wade 2003). Guarding behaviour is commonly accompanied by fighting behaviour to allow males to defend access to females, and in these species sexual selection is likely to favour large male body size and weaponry. Guarding behaviour is also only likely to

evolve in species where females mate multiple times. Here, males must guard females in order to protect their sperm investment from competition with other males post-copulation (Thornhill & Alcock 1983; Alcock 1994). Guarding is potentially costly because the time spent guarding is time that could be spent searching and mating with another female, therefore resulting in a trade-off between mating multiple times, and protecting sperm investment. Because of these costs, guarding is most likely to evolve when females are scarce (such as in a male-biased system) and when there are moderate levels of competition for access to them (Yamamura 1986).

Selective forces acting differentially on male and female size can drive the degree of sexual size dimorphism (SSD) (Andersson 1994). Sexual selection is proposed to be the most important form of selection driving the evolution of SSD, and is thought to act most strongly on the sex that has the lowest level of parental investment (Trivers 1972; Andersson 1994). In mammals and birds, females generally invest much more energy than males into reproduction, particularly during gestation and lactation. In these taxa SSD is generally male biased, driven by sexual selection for large male size to increase success during male-male competition (Andersson 1994). In insects however, females are usually the larger sex, most likely driven by fecundity selection for large female size because larger females have larger egg and/or offspring size or number (Honek 1993). There are many exceptions to this rule, and when males are larger, sexual selection to increase male mating success is thought to be the driver, although counteracting selection on small size in females can also potentially drive this pattern (Stillwell et al. 2010). An alternative hypothesis for SSD is due to ecological niche divergence to reduce intraspecific competition for food; however this theory is not strongly supported because it is difficult to determine whether trophic or morphological divergence evolved first, and does not explain the widespread dimorphism in secondary sexual traits (Fairbairn 1997).

Identifying only one form of selection as being the cause of differences in body size between sexes is an oversimplification. In reality, SSD is caused by the relative strength of all types of sex-specific selection (i.e. viability, fecundity, and sexual selection) (Puniamoorthy et al. 2012). For example, *Sepsis punctum* dung flies show opposite patterns of SSD between two continents, with populations in Europe possessing larger males and populations in North America possessing larger females. It was recently found that the male-biased SSD found in Europe was driven by higher levels of sexual selection on male size relative to the level of fecundity selection for larger female size (Puniamoorthy et al. 2012). Many territorial odonate species are male-biased in SSD, likely driven by sexual selection on male size to increase competitive success against other males (Serrano-Meneses et al. 2008). Male-bias SSD has also been found in some species of coelopid flies and ichneumonid wasps, which are characterised by high levels of female reluctance during mating attempts. Larger males were found to be more likely to achieve successful copulation, and it is therefore suggested that female reluctance is important in driving male-bias SSD in these species (Crean & Gilburn 1998; Crean et al. 2000; Teder 2005).

Before we can understand the evolution of SSD, it is first required that differences between the sexes are quantified to determine the level of SSD that is present in a species (Schulte-Hostedde & Millar 2000). SSD is most commonly defined using univariate measures of body size, as these are easily compared between the sexes, but the estimated SSD is largely dependent on the body trait chosen and can lead to variable conclusions on which is the larger sex (Stillwell et al. 2010). Also, while body mass is a traditional measure of size, particularly for mammals, this can vary in relation to condition, and therefore is not recommended as a suitable measure of overall body size (Stillwell et al. 2010). Multivariate approaches such as discriminate function analysis and principal components analysis are useful for describing an overall difference in body shape

between the sexes, and can eliminate the problem of arbitrarily choosing a single body trait to measure (Schulte-Hostedde & Millar 2000; Stillwell et al. 2010). Using a combination of both univariate and multivariate methods is, however, a balancing act between ease of interpretation and an accurate overall description of SSD.

The straight-snouted weevils (Coleoptera: Brentidae) are an extremely diverse family found worldwide, with more than 1600 species described in 290 genera (Sforzi & Bartolozzi 2004). Many species within the family are highly sexually dimorphic, due often to extreme modifications of traits such as the rostrum and mandibles displayed by males (Sforzi & Bartolozzi 1998). In these species males generally have longer mean body lengths than females, therefore showing reverse-SSD, unusual among Coleoptera. In comparison there are also species within the family which show very little sexual dimorphism, and therefore brentids provide an interesting model for a comparative analysis of the evolution and adaptive significance of sexually selected traits and SSD.

Despite this, very little is known about the behavioural ecology of brentids worldwide. Detailed life history studies have been conducted on three brentid species; *Arrhenodes minutes*, *Brentus achorago*, and *Claeoderes bivittata*. All species were highly sexually dimorphic, with aggressive males and high promiscuity in both sexes (Johnson 1982, 1983; Sanborne 1983; Johnson & Hubbell 1984; Garcia-C 1989). *B. anchorago* and *C. bivittata* also had high levels of intrasexual size variation, and in both species large males had the highest mating success, although small *C. bivittata* males also achieved some mating success and showed evidence of sneaking behaviours (Johnson 1982, 1983).

The New Zealand giraffe weevil *Lasiorhynchus barbicornis* Fabricius is an excellent example of a highly sexually dimorphic species within the Brentidae. Adult males display an extremely elongated rostrum that is equal to the length of the rest of its body, and is used as a weapon during contests with other males while guarding females (Meads 1976). The female rostrum is also modified, but makes up only about a third of the body, and is used to drill holes into tree trunks and branches to lay her eggs. Both sexes are also highly size variable, with adult males previously found to range between 16 – 90 mm in length, and females varying from 13 – 46 mm in length (Kuschel 2003). Little is known about the behavioural ecology of this species, but an earlier observational study of its life-history raised questions about the use of the male rostrum, and how the high size variation in males could have implications for male mating success (Meads 1976).

Importantly, adult *L. barbicornis* aggregate on tree trunks where males copulate with females while they drill holes in preparation to lay their eggs (Meads 1976). Males also guard females prior to and after copulation (C. J. Painting pers. obs.). Clumping of females in space and time has important consequences for the evolution of mate guarding behaviour (Emlen & Oring 1977), and this behaviour in combination with other aspects of *L. barbicornis* will have important consequences on the mating system of this species.

Large, field-based studies of wild animal populations are relatively uncommon due to logistics and time constraints, but *L. barbicornis* provides a unique opportunity to characterise its ecology in its native habitat in order to gain an understanding of what factors drive the evolution of its mating system. The specific aim of this study was to determine the population structure and ecology of *L. barbicornis* using data collected over three years from a single wild population. Firstly, I describe changes in sex ratio and seasonal abundance over three years and then

determine sexual size dimorphism and body size frequency distribution. Because estimates of SSD can be greatly affected by the choice of body measurement, mean body size for six individual body traits are compared to calculate individual measures of SSD, and then multivariate analysis is used to describe SSD in overall body shape. Documenting sex ratio and sexual dimorphism in this species, and increasing the understanding of the ecology and population structure of *L. barbicornis* will have important implications for understanding the mating system of the species. Also, these data form a framework from which to generate questions about the evolutionary ecology and behaviour of the species.

## 2.3 METHODS

### 2.3.1 Study species and site

The giraffe weevil is a wood-boring species endemic to New Zealand, and is found throughout the north island and the northern and western regions of the south island (Kuschel 2003). Larvae are wood-boring in native trees, and emerge as adults during summer months (October-March) where they aggregate on dead or dying trees. *L. barbicornis* utilise numerous tree species as hosts, such as karaka (*Corynocarpus laevigatus*), mahoe (*Melicytus ramiflorus*), and kohekohe (*Dysoxylum spectabile*) (Appendix 1).

Observations were conducted at Matuku Reserve (36° 51.92'S, 174°28.32'E) west of Auckland City, New Zealand (Figure 1). This reserve is privately owned by the Royal Forest and Bird Protection Society and is 120 hectares of predominantly coastal lowland broadleaf forest. The reserve is bordered on one side by an extensive wetland, and also has emerging ridges of

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tanekaha (*Phyllocladus trichomanoides*) and kauri (*Agathis australis*) trees (Forest and Bird 2009). There is also intense mammalian pest control conducted at the reserve (J. E. Staniland, pers. comm.), resulting in lower numbers of rats, cats, stoats and possums which are known invertebrate predators (Miller & Miller 1995; Gillies & Clout 2003). *L. barbicornis* are highly abundant here, found most commonly on dead or dying karaka trees, thus providing an ideal site for conducting observations and collecting data about their population structure.
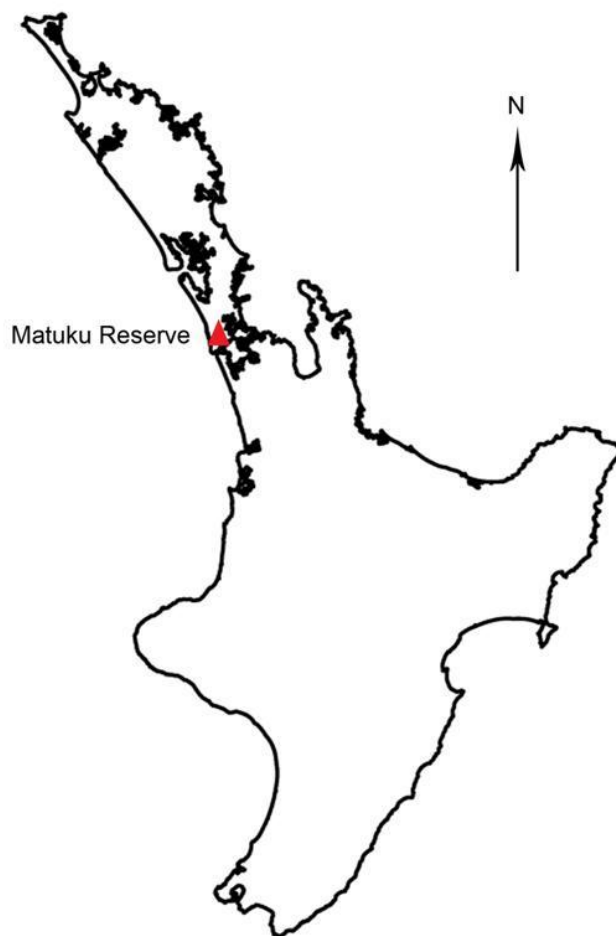


Figure 1. Location of Matuku Reserve in the North Island of New Zealand.

*2.3.2 Field methods*

To describe the sex ratio, abundance and sexual dimorphism of *L. barbicornis* at Matuku

Reserve I conducted regular surveys between November and May over two years (2010/2011,

2011/2012) and between November and April in 2012/2013. Throughout the three year sampling

period, I searched for adult weevils on 19 dying karaka trees that were previously found to host

giraffe weevils, and once a week searched systematically along the trunks and branches until I

was confident all weevils present at that time were located. The trees that were sampled in each

year were not consistent, as after the first year some of the sampling trees were no longer

attracting adult giraffe weevils. However, as the absolute numbers of weevils was not important

for these analyses, and there was no attempt to estimate total population density, this had no

effect on the analyses. Instead it was ensured that the trees used each year were consistent

throughout that sampling period to accurately gauge seasonal patterns in abundance. Although

surveys were conducted on the same day each week where possible, this was not always feasible

due to poor weather, as giraffe weevils tend to hide and become harder to find during heavy rain.

Also, due to logistics, between March and May in each year surveys were conducted less

frequently, but it was still possible compare relative abundance from these data.

Male and female *L. barbicornis* are easily distinguished by the shape of the rostrum and elytra,

and the location of the antenna on their rostrum. The rostrum of the male is greatly elongated

with a dense row of setae on the ventral surface, the antennae are located at the terminal end of

the rostrum directly behind the mandibles (Figure 2), and the elytra is spatulately extended at the

apex (Kuschel 2003). In comparison, the rostrum of the female is shorter, shiny and lacking

setae, the antenna are located further back towards the eyes behind the prorostrum (Figure 2),

and the elytra are tapered at the apex (Kuschel 2003). Six body size measurements were taken

using digital calipers to the nearest 0.01 mm. These were total body length (BL, tip of mandibles

through to end of elytra), rostrum length (RL, total length from anterior end of mandibles to the

base of the head behind the eyes), pronotum width (PW), pronotum length (PL), elytra width

(EW), and elytra length (EL) (Figure 2). I conducted a repeatability test by measuring 20 males

three times, and then calculated the fraction of the summed variance among groups (Whitlock &

Schluter 2009). Repeatability values were 0.99 for each trait measured, and the variation in trait

size between individuals was consistently and significantly higher than within individuals ($F_{1,19}$

$= 257.2 - 487.3$, $p < 0.0001$). All variables were also strongly correlated (Table 1). Weevils were

then marked with a unique colour combination using Queen Bee marking paints (Lega, Italy)

before releasing them back onto their original tree to ensure each weevil was only measured once

(Appendix 1). The maximum recorded lifespan of an adult *L. barbicornis* in the wild is 84 days,

with an average lifespan of approximately two weeks (CJ Painting unpublished data), indicating

that the possibility of recapture in a subsequent sampling year is highly unlikely.

Table 1. Pearson's correlation coefficient matrices comparing six body traits from male and female *L. barbicornis.* Data pooled from specimens measured between 2010 and 2013 at Matuku Reserve.

| *Males* (N = 2922) | | | | | | |
|---|---|---|---|---|---|---|
| | BL | PW | RL | PL | EW | EL |
| BL | | 0.98 | 0.99 | 0.98 | 0.98 | 0.99 |
| PW | | | 0.98 | 0.97 | 0.99 | 0.98 |
| RL | | | | 0.97 | 0.98 | 0.99 |
| PL | | | | | 0.97 | 0.98 |
| EW | | | | | | 0.98 |
| EL | | | | | | |
| *Females* (N = 2116) | | | | | | |
| | BL | PW | RL | PL | EW | EL |
| BL | | 0.98 | 0.99 | 0.97 | 0.99 | 0.99 |
| PW | | | 0.98 | 0.96 | 0.99 | 0.98 |
| RL | | | | 0.97 | 0.98 | 0.98 |
| PL | | | | | 0.97 | 0.97 |
| EW | | | | | | 0.98 |
| EL | | | | | | - |

Table 2. Total abundance of male and female *L. barbicornis* marked and released over three years at Matuku Reserve. Sex ratio was calculated as the number of males divided by the total number of weevils caught. Sampling for 2010/2011 and 2011/2012 was between November and May, but sampling in 2012/2013 was shorter, between November and April.

| | Total no. weevils | No. males | No. females | Sex ratio |
|---|---|---|---|---|
| 2010/2011 | 2916 | 1755 | 1161 | 0.61 |
| 2011/2012 | 2357 | 1467 | 890 | 0.62 |
| 2012/2013 | 1390 | 784 | 606 | 0.56 |

Figure 2. Measurements taken from male and female *L. barbicornis.* Original illustrations by D. W. Helmore, © Landcare Research, used with permission. Modifications by V. Ward and measurements by C. J. Painting. (Removed due to copyright).

*2.3.3 Statistical analysis*

All analyses were conducted in R 2.15 (R Core Team 2013). OSR was calculated as the number of males divided by the total number of males and females combined. For this study OSR and the adult sex ratio were treated as equivalent as all adults present and counted on trees were sexually receptive. All females included in this study were sexually active as they were consistently observed to copulate or be guarded by males and were observed preparing oviposition holes, and males were observed searching for or copulating with females. To test for overall bias in the population, and for seasonal changes in the OSR, the relative percentage of each sex that deviated from a 1:1 ratio was determined using binomial exact tests for goodness of fit.

Coefficients of variation (CV %) were calculated as CV % = [(standard deviation/mean)*100] for each trait measured. We tested whether there was a significant difference in the CV% of males and females for each trait measured using two-tailed *Z*-tests compared to critical values from the *t*-distribution where the degrees of freedom are infinite (Zar 1999, p. 141).

To determine sexual size dimorphism (SSD) the index recommended by Lovich and Gibbons (1992) was used, where SSD = (mean size of larger sex/mean size of smaller sex) – 1. Values were arbitrarily set as positive when the male was larger, and negative if females were larger. Welch's t tests were used to test for differences in mean size between male and female *L. barbicornis* for the six traits measured. To test for significant sexual size dimorphism, a series of pairwise t-tests were conducted for each of the six body size measurements to test if mean trait size differed between the sexes. Univariate analyses such as these are an intuitive and simple way to compare differences between the sexes using linear measurements, but SSD can vary

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significantly in relation to the chosen trait. Multivariate analyses such as principal components analysis (PCA) and discriminate function analysis (DFA) are useful to show an overall index of SSD using a combination of all the traits measured. A PCA was conducted to reduce the dimensions in the data set, and then DFA was used to determine the ability to predict an individual's sex from the calculated PC's.

## 2.4 RESULTS

### 2.4.1 Seasonal changes in abundance and sex ratio

Over the three summer seasons a total of 6663 weevils were marked and measured (Table 2). Due to irregularity in the number of occasions that surveys were conducted in the later months of each season, the mean abundance per month was calculated to look at patterns of overall seasonal abundance. Monthly mean abundance was the total number of weevils found during each survey over the month, divided by the number of surveys during that month. Differences between the years in mean abundance could be due to many factors, including climatic variability and changes in the attractiveness of the chosen sample trees, but because these factors were not measured I can make no conclusions about the factors driving these differences. In all three years total weevil abundance increased steadily from November, showing peak abundance in the month of February, after which abundance decreased (Figure 3).
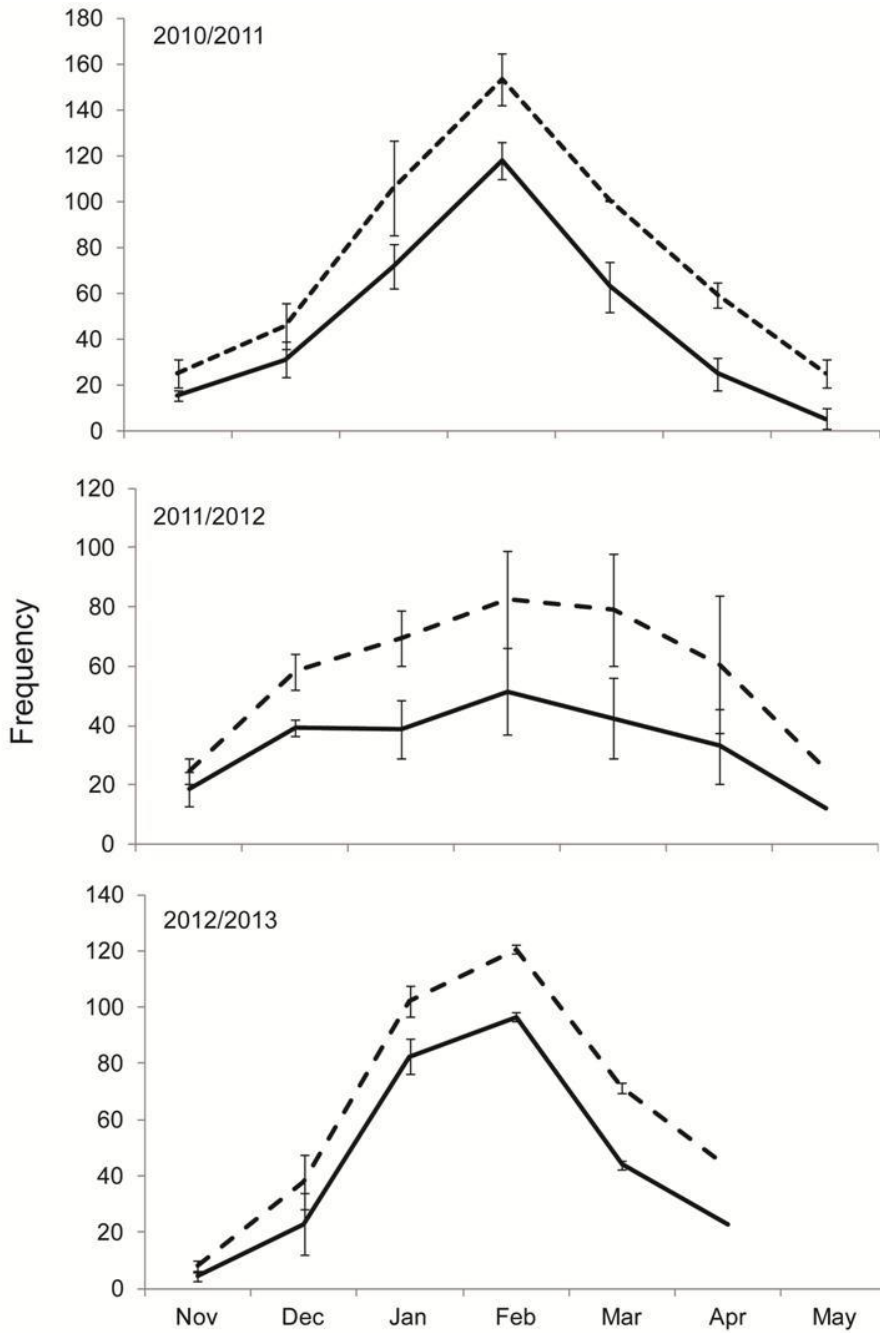
Figure 3. Seasonal variation in mean (±SE) abundance of adult male (dashed line) and female (solid line) L. barbicornis at Matuku Reserve between November and May over three years. Sampling was terminated in April during the 2012-2013 season, hence the lack of data in May for this year. Note also that in May 2012 and April 2013 there was only one sampling event, so standard errors were not presented for these months.

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Both seasons showed an overall male biased sex ratio (Table 2). In the 2010/2011 season there was an overall sex ratio of 1.51 males to every female, in 2011/2012 there were 1.64 males to every female, and in 2012/2013 there were 1.29 males to every female. Binomial exact tests comparing sex ratio against the null hypothesis that the ratios are equal for each month surveyed showed that the sex ratio was always significantly male-biased (Table 3). During all years the sex ratio also fluctuated across the season, such that the most male-biased time periods were at the beginning and end of the season (Table 3, Figure 4). During the peak abundance period around January and February the sex ratio was less male-biased, showing that there were more females available per male during these months. However, in the 2011/2012 season, the percentage of males in the population increased steadily from November to May, showing a different pattern in sex ratio variation than in the other two years (Table 3, Fig. 4).

Table 3. Seasonal variation in sex ratio of *L. barbicornis* over three years. Binomial exact tests assessed deviation from a 1:1 sex ratio. Significant *p* values are highlighted in bold.

| 2010/2011 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nov | Dec | Jan | Feb | Mar | Apr | May |
| Male | 0.62 | 0.63 | 0.6 | 0.57 | 0.61 | 0.81 | 0.82 |
| Female | 0.38 | 0.37 | 0.4 | 0.43 | 0.39 | 0.19 | 0.18 |
| Total N | 204 | 334 | 710 | 1085 | 327 | 147 | 61 |
| *p* | **0.0009** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| *2011/2012* | | | | | | | |
| Male | 0.57 | 0.6 | 0.64 | 0.62 | 0.65 | 0.65 | 0.68 |
| Female | 0.43 | 0.4 | 0.36 | 0.38 | 0.35 | 0.35 | 0.32 |
| Total N | 216 | 390 | 542 | 536 | 243 | 373 | 37 |
| *p* | **0.048** | **0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **0.047** |
| *2012/2013* | | | | | | | |
| Male | 0.65 | 0.62 | 0.55 | 0.55 | 0.62 | 0.65 | - |
| Female | 0.35 | 0.38 | 0.45 | 0.45 | 0.38 | 0.35 | - |
| Total N | 63 | 183 | 923 | 652 | 346 | 66 | |
| *p* | **0.02** | **0.0001** | **0.001** | **0.005** | **<0.0001** | **0.03** | - |
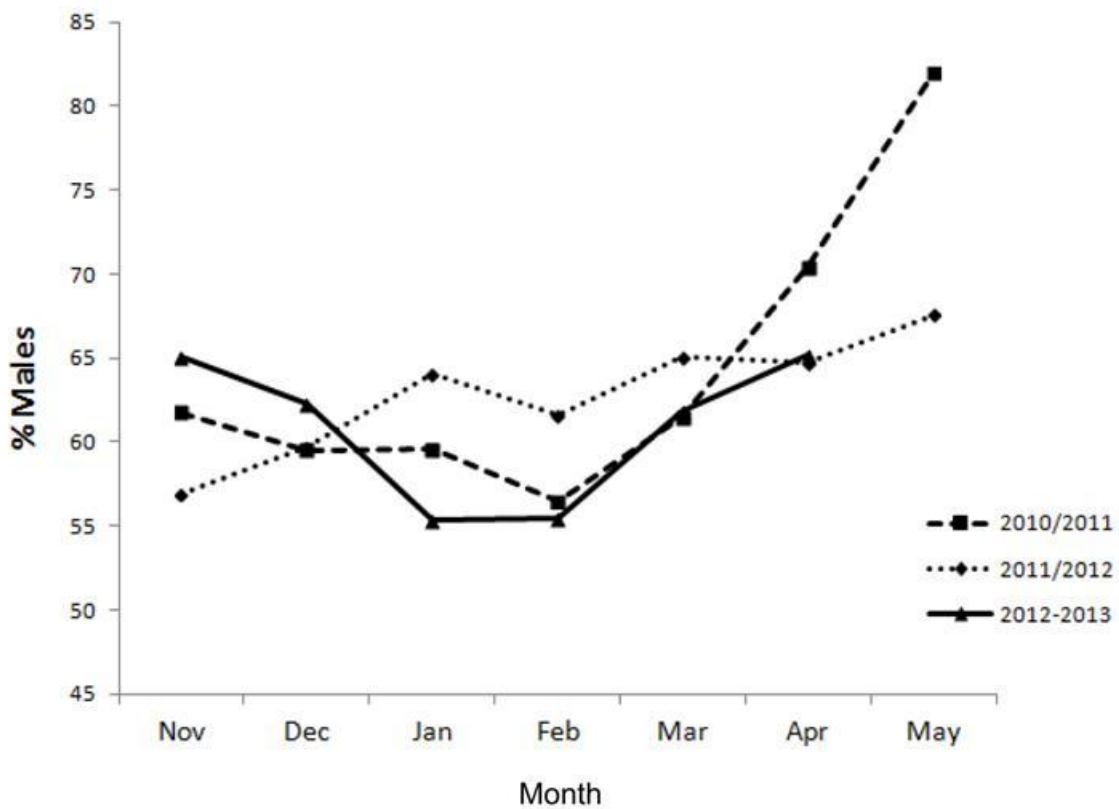
Figure 4. Seasonal variation in sex ratio over three years at Matuku Reserve. Sex ratio is displayed as the percentage of males collected during each month.

Giraffe weevils were found to be highly sexually size dimorphic. Individual t-tests comparing mean size of each trait showed significant differences and are presented in Table 5. Males were on average 32% longer than females, and on average significantly larger for all traits involving length (total body length, rostrum length, pronotum length and elytra length), but mean body width was significantly smaller than for females (pronotum width and elytra width) (Table 5). All male traits were more highly variable than female traits, as shown by their coefficients of variation (Table 5). Rostrum length and body length (which includes rostrum length) had the

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highest CVs for males, while in comparison female CV values showed little variation between traits.

The principal components analysis showed differences in overall shape between the sexes. The factor loadings for the first two PC's are presented, which explained a total cumulative percentage variance of 98.9% (Table 4). All six body size measurements loaded evenly on PC1, such that PC1 was a measure of overall body size. PC2 loaded positively for pronotum and elytra width and was therefore a measure of overall body width. Plotting PC1 against PC2 showed that there was strong grouping with little overlap between the sexes (Figure 5). However, there was less difference in body proportions between smaller males and females, although they are still easily identifiable by the shape of the rostrum and elytra (see Methods). A discriminant function analysis (DFA) using PC1 and PC2 correctly assigned 99.4% of individuals to the correct sex (5008 individuals from 5038), of which 99.7% of females and 99.2% of males were correctly assigned. Much of the size difference between males and females is due to the extreme elongation of the rostrum in males (Figure 6).

Table 4. Factor loadings from the Principal Components analysis

| Body size characters | PC1 | PC2 |
|---|---|---|
| Total body length | **-0.419** | -0.336 |
| Rostrum length | **-0.39** | -0.519 |
| Pronotum width | **-0.373** | **0.597** |
| Pronotum length | **-0.433** | -0.104 |
| Elytra width | **-0.394** | **0.496** |
| Elytra length | **-0.437** | -0.068 |
| **Proportion variance explained** | 0.862 | 0.128 |
| **Cumulative variance explained** | 0.862 | 0.989 |

Table 5. Mean, maximum, minimum, and variation in trait size for male and female *L. barbicornis* for three years of combined data

| Trait | Males (n =2922) | | | | Females (n=2116) | | | | Z-value | SSD index | t |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean size (SE) | Max size | Min size | CV (%) | Mean size (SE) | Max size | Min size | CV (%) | | | |
| Total body length | 36.80 (0.23) | 89.75 | 14.7 | 33.81 | 25.05 (0.20) | 50.44 | 12.47 | 21.90 | 18.96** | 0.47 | 45.30*** |
| Pronotum width | 2.26 (0.01) | 4.78 | 1.13 | 25.17 | 2.54 (0.01) | 5.84 | 1.23 | 22.96 | 5.75** | -0.13 | 17.27*** |
| Rostrum length | 17.46 (0.13) | 46.44 | 3.09 | 39.98 | 8.92 (0.04) | 17.4 | 4.52 | 21.59 | 25.69** | 0.96 | 135.21*** |
| Pronotum length | 5.60 (0.03) | 12.26 | 1.85 | 28.19 | 4.62 (0.02) | 9.06 | 2.4 | 21.20 | 12.91** | 0.21 | 27.15*** |
| Elytra width | 2.76 (0.01) | 5.9 | 1.27 | 25.18 | 2.96 (0.02) | 6.81 | 1.27 | 23.51 | 3.19* | -0.07 | 9.68*** |
| Elytra length | 13.87 (0.07) | 31.36 | 6.19 | 28.73 | 11.49 (0.06) | 23.94 | 3.74 | 22.87 | 10.36** | 0.21 | 188.17*** |

Notes: Z-values were calculated and compared to critical values in a *t*-distribution to test for a significant difference in the coefficient of variation (CV) between males and females for each trait. Welch t tests were used to test for significant difference in mean size between the sexes for each trait. * = p <0.01, ** = p <0.001, *** = p<0.0001. Data from all seasons (Nov-March: 2010/2011, 2011/2012, 2012/2013) were pooled. Positive SSD values indicate male bias; negative SSD values indicate female bias.
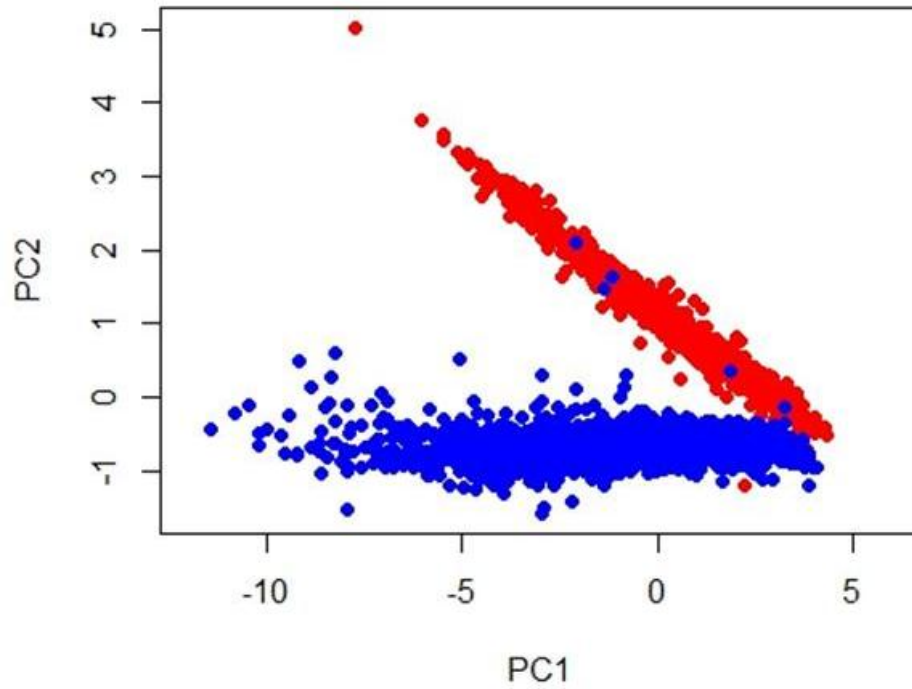
Figure 5. Relation between PC1 and PC2 showing the dissimilarity between males (●) and females (●) in overall body size
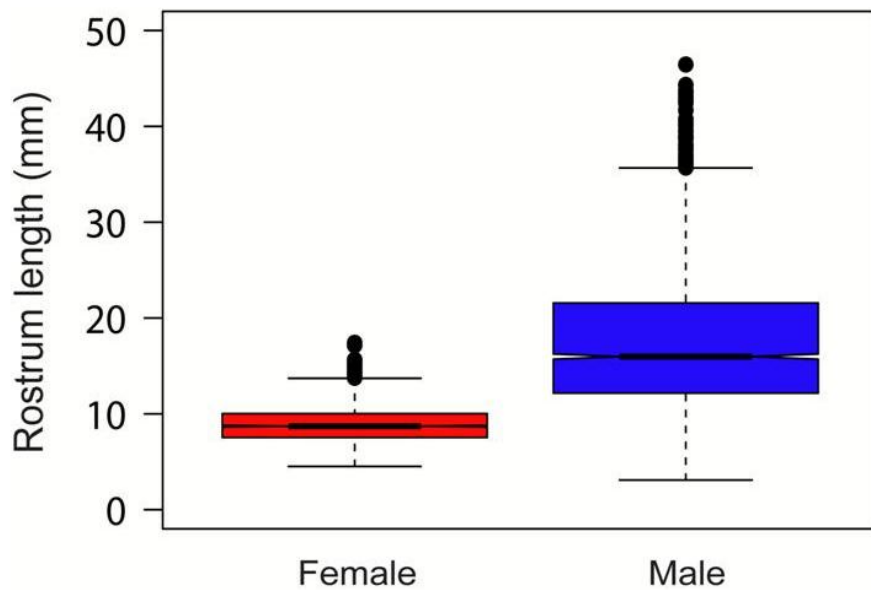


Figure 6. Rostrum length variation between female and male *L. barbicornis* (N = 2186 males, 1533 females).

The frequency distribution for body size for males and females both show a right-skew, suggesting that the largest individuals are relatively uncommon (Figure 7). The right skew was particularly apparent for males, which had a larger range of possible body lengths than females, with the largest male 6.1 times larger than the smallest male. The largest female was 4 times larger than the smallest female, and body length showed a unimodal, normal distribution (Table 4, Figure 7). Pronotum width, a trait commonly measured as a proxy for overall body size in insects, showed high overlap between males and females.
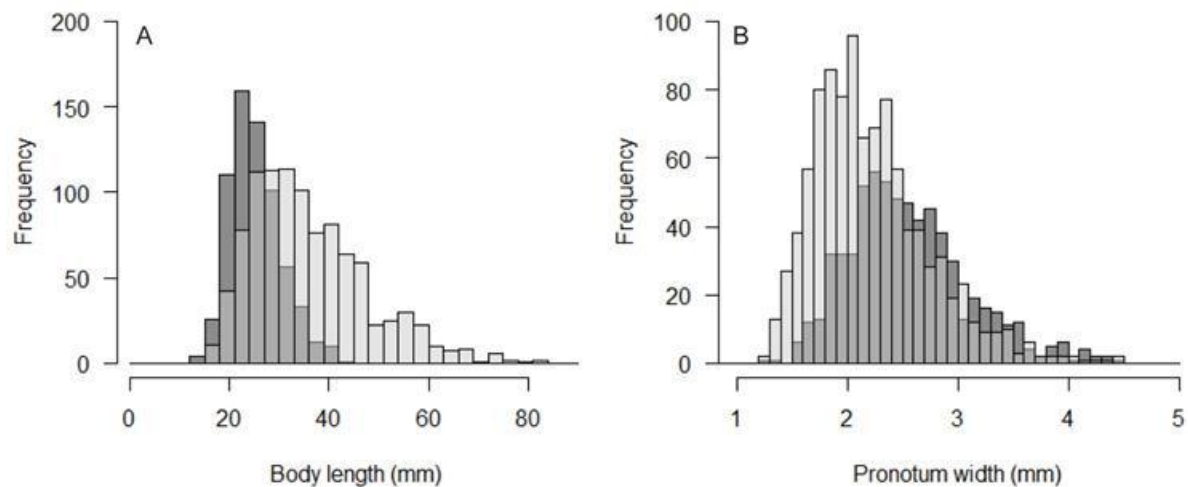


Figure 7. Frequency distribution of (A) body length and (B) pronotum width of females (dark grey; N = 653) and males (pale grey; N = 987) of *Lasiorhynchus barbicornis* collected at Matuku Reserve between November 2011 and March 2012. Mid-grey regions of the histogram are where male and female distributions overlap.

## 2.5 DISCUSSION

The data collected on sex ratio, abundance and sexual dimorphism in this study is crucial for describing the mating system of *L. barbicornis*. This study shows that multiple components of giraffe weevil ecology have likely led to the highly competitive, defence-based mating system that is observed.

The sex ratio of *L. barbicornis* at Matuku Reserve was consistently male-biased throughout each of the three documented breeding seasons. Both the sex ratio and the local population density can have a significant effect on a species mating system, particularly by influencing the relative levels of intrasexual competition. Importantly, in a polygamous species such as *L. barbicornis*, sex ratio and population density can affect the probability and intensity of sperm competition (Gage 1995; Wang et al. 2008). The male-biased OSR and the highly aggregated female distribution has likely had a large effect on other important aspects of *L. barbicornis* biology, such as the extreme investment in rostrum length, and guarding and fighting behaviour. During the peak of the season up to 41 males and 32 females were present in a single aggregation on the survey trees, with females clumped together at optimal sites for drilling and oviposition (C. J. Painting pers. obs.). The aggregation behaviour of females, and the relative scarcity of them in comparison to males, has likely led to male guarding behaviour as an important strategy to maximise reproductive success, particularly because females mate multiple times (Chapter 4) and there is therefore a high risk of sperm competition. When females are a limited resource, particularly when both sexes mate multiple times, the most successful males will be those that can monopolise females to increase their mating success and prevent other males from gaining

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access to the female (Shuster & Wade 2003). This high level of competition between males has likely driven the directional selection for increased rostrum investment and guarding and fighting behaviour to increase mating success.

During each season the sex ratio fluctuated, with females becoming less limited during the peak of the breeding season in February, and becoming more limited towards the end of the season in April and May. It is not currently known what causes the changes in sex ratio throughout the breeding season, but it could be due to fewer female late-season emergences or shorter overall lifespan for females. Regardless of the cause, the increasing male-bias must result in higher levels of competition between males for access to females in these later months. The mating success of males with different phenotypes could change over the season in relation to sex ratio and changes in overall abundance, such that, for example, large males could experience relatively higher mating success as females become rarer. Both sex ratio and population density have been shown to have important effects on overall male mating success and copulation duration, on the relative success of males of different sizes, and on the investment in alternative mating tactics (e.g. Conner 1989; Wang et al. 2008; Wang et al. 2009; Rittschof 2010).

In general, most insect species show female-biased sexual size dimorphism, which is in contrast to most mammals and birds which are generally male-biased in size (Teder & Tammaru 2005; Stillwell et al. 2010). A review of SSD in insects found that 72% of the 69 Coleopteran species measured were female-biased in size, and the only group that was generally male-biased in size were the Odonata (Stillwell et al. 2010). Large male size in damselflies is likely to be driven by strong sexual selection on males to guard territories (Serrano-Meneses et al. 2008). In contrast, the mating systems of Coleoptera are more variable, ranging from scramble competition (e.g. seed beetles; Moya-Laraño et al. 2007), to resource-based defence (e.g.stag beetles on sap flows;

Hosoya & Araya 2005),  female or harem defence (e.g. bark and dung beetles; Kirkendall 1983; Emlen et al. 2005; Latty et al. 2012), lekking (e.g. fireflies; Cicero 1983), and monogamy with parental care (e.g. burying beetles; Trumbo 1992; Eggert & Sakaluk 1995). Mating systems are often tightly correlated with the direction and magnitude of SSD (Dunn et al. 2001; Shuster & Wade 2003; Bro-Jorgensen 2007; Dale et al. 2007). In particular, species with mate guarding, polygynous or lekking mating systems generally have higher levels of male-biased SSD than monogamous species, and high levels of sperm competition are also positively related to the level of SSD (Pyron 1996; Dunn et al. 2001). Male-biased SSD is therefore likely to be the result of directional sexual selection for large body size to increase competitive ability or to be more attractive to females.

Giraffe weevils were found to be strongly male-biased in body and rostrum length, although females were on average larger in body width. Multivariate analysis using an overall measure of body size further supported the individual measures of SSD, and an individual could be assigned to the correct sex with 99.4% certainty. *L. barbicornis* are an exception in comparison to most other Coleoptera that generally show female-biased SSD, although the trait used as the measure of body size is extremely important. Pronotum or thorax width is the most common character used as a measure of overall body size in beetles (e.g. Eberhard & Gutierrez 1991; Tomkins et al. 2005; McCullough et al. 2012) which could explain why for some species females are found to be the larger sex. Despite this, SSD in giraffe weevils was very pronounced, mostly due to the extreme elongation of the rostrum in males.

Rostrum length also showed higher levels of variation (CV %) than rostrum length in females, or compared to any other body parts measured. Unfortunately, we do not have information about the relative roles of genes, the quality of larval food sources, or the number of larval instars

required for *L. barbicornis* larvae to mature in relation to the variation in body size observed. For example, variation in the number of instars that an individual undergoes before maturity has been shown to effect variation in adult body and weapon size, sexual size dimorphism, and mating tactics in tree wēta (Kelly 2005). However, the nutritional quality of larval food sources has also been shown to be important in determining the final body and weapon size of insects, such as the *Onthophagus* dung beetles (Emlen 1994; Moczek & Emlen 1999). Sexually selected traits are expected to show higher phenotypic and genetic variation than non-sexual traits, because they are highly condition-dependent and under direct sexual selection which drives variation (Pomiankowski & Moller 1995; Cotton et al. 2004a). A key assumption of the handicap hypothesis is that exaggerated traits are honest signals of genetic quality, and therefore high variation in these traits are assumed to relate directly to the ability of males to acquire and allocate resources (Zahavi 1975; Cotton et al. 2004b). For *L. barbicornis*, the higher level of variation in male rostrum length suggests that this trait is likely to be condition-dependent and under sexual selection.

*L. barbicornis* males have previously been documented to use their rostrum during fights with other males over access to females (Meads 1976), and several other brentids also use their rostrum as a weapon and so they are expected to have evolved under sexual selection (Johnson 1982, 1983). As male-biased SSD is often the result of strong sexual selection for male size, and as the data presented here shows that the sex ratio of *L. barbicornis* is similarly male-biased, it is likely that the ecology and mating system of this species has led to the extreme SSD in this species. The mating system of *L. barbicornis*, and the importance of the rostrum and overall large body size for reproductive success will be explored in subsequent chapters (Chapter 4 & 5).

The range of total body lengths measured at Matuku Reserve closely matches those previously compiled by Kuschel (2003) from nation-wide museum records. However, weevils found at Matuku Reserve did extend the known body length range for females, as female body length showed a range of 12 – 50 mm compared to previous records of 18 – 47 mm (Kuschel 2003).

Very little research has been published on other brentid species, although Johnson (1982) previously reported *Brentus anchorago* to be the most size variable species in the family, with large males up to 5.2 times longer than the smallest males. However, *L. barbicornis* were found to be more size variable than *B. anchorago*, with males varying up to 6.1 fold in body length, and this species is to my knowledge the longest weevil in the world (C. J. Painting, unpublished obs.). The magnitude of SSD between *L. barbicornis* and *B. anchorago* was the same, with an SSD index for total body length 0.47 for both species (calculated from mean body lengths presented in Johnson 1982). Males of *Claeoderes bivittata*, another brentid species, were also found to have larger mean body lengths than females, but were less variable with a SSD of 0.23 (calculated from mean body lengths presented in Johnson 1983) and mean body lengths of 25.91 mm and 20.97 mm for male and females, respectively. *Cylas formicarius* (sweet potato weevil) on the other hand, is considered to be a monomorphic species, with only minor differences in antennae, eye, and hind wing size between the sexes (Starr et al. 1997). Although *C. formicarius* males mate multiple times during their lifetime, females generally mate only once (Sugimoto et al. 1996), and males possibly invest more into mate location than defence. Although few Brentids have had mean body size or SSD quantified, the three species with clear male-bias (*L. barbicornis*, *B. anchorago, C. bivittata*) are all characterised by high levels of male-male competition and the presence of elongated rostrums in males used as weapons. Future work comparing the magnitude and direction of SSD throughout a diversity of brentid species is

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required to more comprehensively understand patterns of SSD in this family in relation to secondary sexual trait evolution and mating systems. Given that many brentids are characterised by strong male-biased SSD, unusual in Coleoptera, this family would make an exceptional group for comparative analysis.

Overall, this study has shown that the ecology of this species has likely driven intense male-male competition and resulted in the evolution of extreme sexual size dimorphism to increase male mating success. Subsequent studies (Chapter 4 & 5) focusing on male mating and fighting behaviour will further highlight the importance of the male-biased sex ratio and sexual size dimorphism in this species.

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# CHAPTER 3

# Exaggerated trait allometry, compensation and trade-offs in the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*)

(Figure removed due to copyright)

## 3.1 ABSTRACT

Sexual selection has driven the evolution of exaggerated traits among diverse animal taxa. The production of exaggerated traits can come at a cost to other traits through trade-offs when resources allocated to trait development are limited. Alternatively some traits can be selected for in parallel to support or compensate for the cost of bearing the exaggerated trait. Male giraffe weevils (*Lasiorhynchus barbicornis*) display an extremely elongated rostrum used as a weapon during contests for mates. Here I characterise the scaling relationship between rostrum and body size and show that males have a steep positive allometry, but that the slope is non-linear due to a relative reduction in rostrum length for the largest males, suggesting a limitation in resource allocation. I also measured testes, wings, antennae, fore- and hind-tibia size and found no evidence of a trade-off between these traits and rostrum length when comparing phenotypic correlations. However, the relative size of wings, antennae, fore- and hind-tibia all increased with relative rostrum length suggesting these traits are under correlational selection. Increased investment in wing and leg length is therefore likely to compensate for the costs of flying with, and wielding the exaggerated rostrum of larger male giraffe weevils.

## 3.2 INTRODUCTION

Competition between males for mates has led to the evolution of exaggerated traits through sexual selection (Darwin 1871; Andersson 1994). Exaggerated traits can be used as either weapons to increase success in direct conflict with other males, or as ornaments subject to female choice. Males possessing the largest or most elaborate traits are often demonstrated to benefit from higher mating success than their counterparts (reviewed in Andersson 1994).

In many species sexual selection for an exaggerated trait has resulted in a steep positive allometric relationship with body size, such that the trait gets disproportionately larger with increasing body size (Kodric-Brown et al. 2006; but see Bonduriansky 2007). Exaggerated trait scaling relationships are often best described by a straight line after log transformation of body and weapon size, but in many species the relationship is non-linear and is better described as being curved, sigmoidal or discontinuous (Knell 2009). Many species exhibit dimorphism in male weapon size as a discontinuity in the scaling relationship between body and weapon size. Weapon dimorphism has been traditionally associated with a developmental threshold, such that only males above a certain body size are reprogrammed to display the trait, and below the threshold the trait will be rudimentary or absent (Emlen & Nijhout 2000). However, recent analyses have shown that these thresholds are not always straightforward, and that some model species such as the scarabaeid beetle *Onthophagus taurus* show a continuous relationship between weapon and body size and do not show evidence for a reprogramming event between small hornless (minor) and large horned (major) males, supporting instead a non-linear model of exponential horn growth followed by a growth constraint due to competition between body parts during resource allocation (Tomkins et al. 2005a). Furthermore, the weapon dimorphisms of some species are not highlighted by a bimodal frequency distribution, and are only detected by

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analyzing scaling relationships using nonlinear techniques (Knell 2009). In many cases the resulting dimorphism between small and large males is coupled with important differences in behaviour between the two morphs, such as alternative mating tactics (Gross 1996; Oliveira et al. 2008). These tactics are generally divided at a threshold between minor and major males, and in many cases minors adopt sneaking tactics while majors rely on aggressive mate guarding tactics (Eberhard 1982; Emlen 1997; Moczek & Emlen 2000).

The development of exaggerated secondary sexual traits such as horns and enlarged mandibles can come at the cost of investment in other characters due to the trade-off for resources prior to development into adults (Emlen 2001). The demand placed on the development of the adult by the exaggerated trait can result in an overall reduction in body size, or smaller individual traits such as wings and eyes.  Alternatively some adult body structures may be under genetic correlational selection for their role in supporting the exaggerated trait, such that the resulting phenotype of the body part correlates with the phenotype of the trait in question (Tomkins et al. 2005b). Compensation can also occur where the potential reduction in performance (e.g. locomotion) due to bearing a large exaggerated trait results in the correlated increase in another trait (e.g. wing size). These correlated traits may physically support the growth of the exaggerated trait, play a role in its use, or compensate for any negative impact on another aspect of the individuals performance. For example, the exaggerated eye-span of large male stalk-eyed flies should cause a major disadvantage in flight performance due to the cost of bearing this trait; however studies of these species have shown a surprising lack of male disadvantage in flight, likely due to correlational selection on increased wing size in relation to the increased investment in eye span (Swallow et al. 2000; Ribak & Swallow 2007; Husak & Swallow 2011). Overall, individuals which have large exaggerated traits are expected to possess larger supporting or

compensatory structures which are selected alongside the exaggerated trait, while also being subject to trade-offs with other body parts.

Resource investment into the development of each trait is particularly important in holometabolous insect species because development takes place in a closed system (Tomkins et al. 2005b).  During larval development, clusters of cells form to make up imaginal structures (or imaginal discs) that will become adult appendages after metamorphosis. Each group of cells is separate such that, for example, the cluster that will form the left leg is separate from that which will form the right leg (Emlen & Allen 2003). Although these cells are set aside early in larval growth during embryogenesis, most of the growth of imaginal discs occurs after the larva has stopped feeding, prior to metamorphosis into the pupal stage (Nijhout & Wheeler 1996; Nijhout & Emlen 1998). This has implications for development because each imaginal disc must share resources that have been previously acquired. The final size of each imaginal disc is a function of body size, complicated by the fact that they grow in a closed system where disc growth comes at the expense of other parts of the body and overall size (Nijhout & Emlen 1998). This can result in morphological trade-offs where the development of an exaggerated trait (e.g. horns) can result in other structures (e.g. eyes, genitalia) growing disproportionately smaller (Emlen 2001).

In addition to trade-offs and compensation between organs, the expression of exaggerated traits can also be limited such that after a certain size relative to the body, further exaggeration of the trait would result in problems with the development of crucial parts of the rest of the body (Knell et al. 2004). This is observed as a curvature or asymptote in the slope when plotting weapon size against body size, and shows that the largest males in the species actually decrease their relative investment in weapon size (Huxley 1932). Although this decreasing slope at the upper size limit of males has been described in some studies, it has been relatively overlooked until recently

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when Knell et al. (2004) conducted a comparative analysis using lucanid beetles to show that the largest males of a species had decreasing investment in weapon size, and that across the family species with the largest mandibles had the greatest decrease of slope for large males. The closed system for development of holometabolous species as described above may explain these decreasing allometries because resources must be shared between traits, thereby resulting in competition between traits during growth and development. Individuals that invest heavily into an exaggerated trait could therefore deplete any available resources for growth resulting in a physiological constraint in trait size (Knell et al. 2004).



Figure 1. A large male giraffe weevil guards a drilling female, highlighting the dimorphism between the sexes mostly due to the elongation of the male rostrum.

The giraffe weevil *Lasiorhynchus barbicornis* (Fabricius) (Coleoptera: Brentidae) is the longest weevil in the world, mostly owing to the extremely elongated rostrum (extension of head) displayed by adult males (Figure 1). Although both sexes show high size variation, females are generally the smaller sex (12 – 50 mm in length), with large males varying 6-fold in size from 15 – 90 mm in length (Kuschel 2003; Chapter 2). Giraffe weevils are an endemic wood-boring species in New Zealand, found in native forest in association with dead or dying trees. Adult weevils emerge and aggregate in summer months on tree trunks or branches where females will drill the tree surface to prepare a hole suitable for laying an egg (Meads 1976). During this time males will compete fiercely for access to females for copulation, using their greatly elongated rostrum and enlarged mandibles to push, bite, pull and grapple other males from the female, occasionally throwing their opponent off the tree (Meads 1976; Chapter 5). The use of the rostrum by males during these contests highlights its importance as a weapon, and is likely to be under direct sexual selection, however the scaling relationship between rostrum and body size is currently undescribed.

This study (1) characterises the shape and slope of the scaling relationship between rostrum length and body size of male and female giraffe weevils, (2) determines the presence of curvature in weapon allometry at the upper end of male size, and (3) investigates the potential for trade-offs and/or compensation between weapon size and that of testis, wing, antennae, and leg size by comparing phenotypic correlations. A description of the scaling relationship of rostrum size for this species is important as a morphological framework from which to test the importance of this trait as a weapon in males. Characterising the shape of the scaling relationship gives us clues to the likelihood of alternative mating tactics in conjunction with a morphological switchpoint, and a decreasing allometry for the largest males can suggest an increasing cost in

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weapon production due to a limit in resource allocation. Given the extreme length of large males and the heavy investment into rostrum length, any tradeoff or compensation in the relative size of other traits can help us to understand the cost of bearing such a greatly exaggerated trait. A previous study found that the strongest phenotypic trade-offs were between the weapon and the nearest developing trait (e.g. eyes, wings or antenna), which is hypothesised to be due to local competition between traits for a limited pool of resources (Emlen 2001). The antennae of *L. barbicornis* grow directly from the rostrum, and we therefore predict that antenna size will show a phenotypic trade-off with rostrum size. Furthermore, we predict a trade-off between testis size and rostrum length because animals are expected to have a limited amount of resources for reproduction, which should be divided between the ability to gain access to females (e.g. increased weapon size) and fertilisation through increased sperm production (testis size) (Simmons & Emlen 2006). It is assumed that there is a biomechanical cost to bearing weaponry, and that therefore wings and legs will be increased in size to assist with flying ability and wielding of the weapon.

## 3.3 METHODS

*3.3.1 Field sampling and morphological measurements*

Measurements of 987 male and 653 female adult *L. barbicornis* were taken to characterise the scaling relationship between body and weapon size from a wild population at Matuku Reserve (36°51'55.205"S, 174° 28'19.262"E) in West Auckland, New Zealand. I conducted weekly trips to the reserve between 4 November 2011 and 26 March 2012 to take regular snapshots of the giraffe weevil population. Weevils were found by searching on 16 dying or partially dying,

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standing karaka (*Corynocarpus laevigatus*) trees which we had previously determined to host aggregations of adult *L. barbicornis*. Searching was conducted systematically along the trunk and branches of each tree until all weevils present were located. The weevils were then removed, measurements taken, and then marked with a unique colour combination (Queen Bee Marking Paints, Lega, Italy) to ensure they were only ever measured once during the season, and then released back onto the trees. Weapon size was measured as the total length of the rostrum from the tip of the mandibles to the base of the head (RL). Body size measurements were total body length (BL, tip of mandibles through to end of elytra), pronotum width (PW), pronotum length (PL), elytra width (EW), and left elytra length (EL). Pronotum width was used in the following analyses as an overall measure of body size because this is the most common character used in the analysis of scaling relationships in other beetles (e.g. Eberhard & Gutierrez 1991; Tomkins et al. 2005a; McCullough et al. 2012). Pronotum width was also highly correlated with all other body size measurements (Pearson's correlation coefficients compared to PW: Males BL = 0.988, PL = 0.983, EW = 0.991, EL = 0.987; Females BL = 0.986, PL = 0.969, EW = 0.990, EL = 0.982). I also previously determined that both rostrum length and pronotum width had high levels of repeatability (0.99), and the variation between individuals was significantly higher than within individuals (RL: $F_{1,18} = 257.2$, $p < 0.0001$; PW: $F_{1,18} = 323.5$ , $p < 0.0001$; Chapter 2). Given that body size and scaling relationships for some species can vary significantly during a season (Hardersen 2010; Hardersen et al. 2011; Wong-Muñoz et al. 2011) taking representative measurements from the whole season provides a baseline description of the scaling relationship from which to compare in the future.

*3.3.2 Trait trade-offs and compensation*

To investigate the possibility of tradeoffs and compensation between rostrum length and other traits, I collected 49 males Matuku Reserve in March 2010. I attempted to collect representatives of all possible male body sizes, ranging from 18.5 to 82.1 mm in total body length (compared to a possible range of 15 to 90 mm). Each individual was frozen, and after measurement were subsequently stored in 70% Ethanol. I took body (PW) and weapon size (RL) measurements as above and also measured the total length of the left antenna, and the length of the left fore- and hind-tibia. The left hind wing was then dissected from each specimen and each wing mounted on a slide with a drop of mounting agent (Microscopy Aquatex, Merck) and a cover slip. Wing size was measured under the microscope using an ocular micrometer (Leica M216 microscope). I took four different measurements of the width and length of each wing (Figure 2), and all measurements of wing size were very highly correlated (all correlation coefficients at least 0.99). For a subset of 15 males I also determined that wing area was highly correlated with wing length (0.98, $p < 0.0001$). Although relative rostrum size was compared to each wing measurement, there was no difference in the relationship so only the results for overall wing length are presented here.
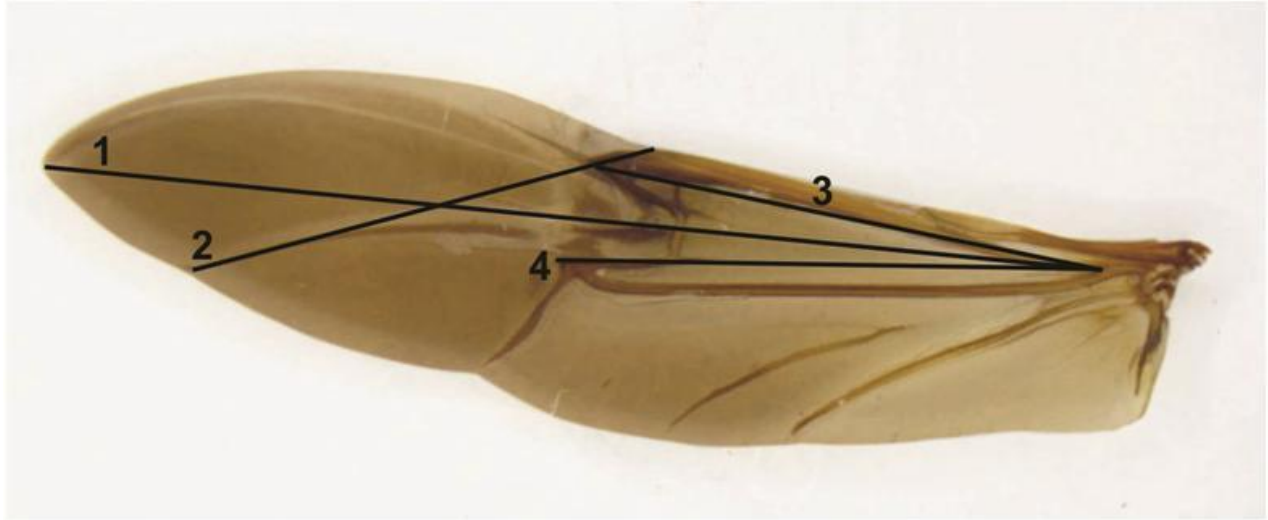
Figure 2. Wing measurements taken from *L. barbicornis*. As all measurements were highly correlated we used measurement 1 (wing length) as a proxy for overall wing size.

Testes were carefully dissected from each individual and immediately measured on a slide using a microscale (Bioquip) under a dissecting microscope. Giraffe weevils have bilobed testes, so to simplify the analysis only the width and length of the left lobe of the left testis was used. The average of the width and length of this lobe was taken as a measure of overall testis size. A paired t-test showed no difference in the mean size of the right and left lobe (t = 1.68, df = 47, p = 0.10). As a proxy for measurement accuracy the Pearson's correlation coefficient was calculated between mean size of left and right testis lobe (r = 0.93). I also ran the analyses using the width and length of the left lobe separately to check for any differences, but found the same result as using the mean; hence only data from the mean is presented here.

*3.3.3 Statistical analysis*

All statistical analyses were conducted in R 2.15 (R Core Team 2013). I first characterised the shape and slope of the scaling relationship for male and female weevils using individuals measured at Matuku Reserve. To analyse the shape of the scaling relationship between rostrum length and pronotum width the steps outlined in Knell (2009) were followed. Six models were compared: (a) simple linear regression, (b) quadratic regression, (c) non-linear regression (logistic, four-parameter logistic, and Weibull growth function), and (d) breakpoint models using Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC) to determine the model which best described the data. After selecting the best model, AIC was used to compare models with different variance structures to check and account for heteroskedasticity. The breakpoint model, analysed using the *segmented* package in R (Muggeo 2003, 2008), is useful when the relationship between trait and body size is best described by two straight lines rather than a single straight line or a curve (Eberhard & Gutierrez 1991; Knell 2009). Since Huxley (1932) allometric relationships have been described using log-transformed data to enable easier fitting of an exponential model using linear regression. However, several recent publications have highlighted important issues with transforming raw data in these analyses, and modern techniques using nonlinear regression makes log transformation no longer necessary (Packard 2009, 2011, 2012). To accurately describe the scaling relationship of *L. barbicornis* raw data was used, but the same models were also conducted using natural log transformation of both variables and it was found that very similar model selection applied (Appendix 2). For a more detailed discussion of the log-transformation debate see Appendix 3.

The difference in the scaling relationship of rostrum length and body size (pronotum width) between males and females was analysed using the R package *smatr* Version 3.2.3 (Warton et al. 2006; Warton et al. 2012). This package was developed specifically for analyzing allometries, and uses type II regression techniques such as standardised major axis regression (SMA) to test whether a slope deviates from isometry ($b <$ or $> 1$), or whether two slopes are significantly different from each other. This is a more appropriate technique than linear regression when the goal is to estimate the line of best fit from a bivariate data set rather than predicting the value of one variable from another because linear regression underestimates the line of best fit and could therefore lead to an incorrect decision as to whether two variables are isometric (Warton et al. 2006). Although I have pointed out that issues are currently debated regarding the use of log transformation in allometry (Kerkhoff & Enquist 2009; Packard 2009, 2011, 2012), there are currently no type II regression techniques available using raw data to test for isometry. Therefore to enable comparison between the sexes data was log-transformed to test for positive allometry.

The relative size of testes, wings, antennae, and legs were compared to weapon size (rostrum length) using residual analysis (Emlen 2001). Firstly relative rostrum size was calculated by taking the residuals from a Weibull growth function analysis of rostrum length against body size (pronotum width) using untransformed data. Then, the relative size of each subsequent trait was determined by calculating the residuals from a linear regression of that trait against pronotum width. Linear regression models were then conducted using relative trait size as the response variable, and relative rostrum length as the explanatory variable. A significant negative relationship indicates that males with longer rostrums are trading off against other traits such as testes and wings. A positive relationship suggests that males are compensating for the increasing cost of bearing the weapon by increasing the size of the other trait. The residual analysis used

untransformed data, however I also ran these analyses using log-transformed data and found the results were almost identical so are not presented here.

The use of residual analysis has been criticised because correlations between variables can lead to biased parameter estimates (Freckleton 2002; García-Berthou 2001). I therefore also conducted multiple regression analyses for each trait (testes, wing, antennae, fore and hind tibia) using log pronotum width and log rostrum length as explanatory variables, all of which found the same results as for the residual analyses[1].

## 3.4 RESULTS

### 3.4.1 Shape and slope of scaling relationship

To analyse the scaling relationship of female *L. barbicornis* a scatterplot of rostrum length against pronotum width was made to visually inspect the relationship (Figure 3). There was a clear linear relationship between these two traits, with no obvious deviation from a straight line. As expected, a simple linear regression model showed a very strong relationship between female rostrum length and body size ($R^2 = 0.96$, rostrum length = 0.69 + 3.24* pronotum width, p <0.001). Following the pragmatic approach of Knell (2009) it was decided that it was not necessary to go further with this analysis, and it was concluded that the shape of the female scaling relationship is best described by a continuous, straight line. This conclusion is also

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[1] The results of the multiple regressions were included in an earlier draft of the manuscript that has since been published based on this chapter (Painting & Holwell 2013). However, the multiple regression analyses were removed after a reviewer raised concerns with collinearity between the explanatory variables. These were subsequently replaced with the residual analyses.

supported by the extremely tight 95% confidence intervals for the slope estimate (Figure 3, 95%

CI = 3.19 − 3.3).
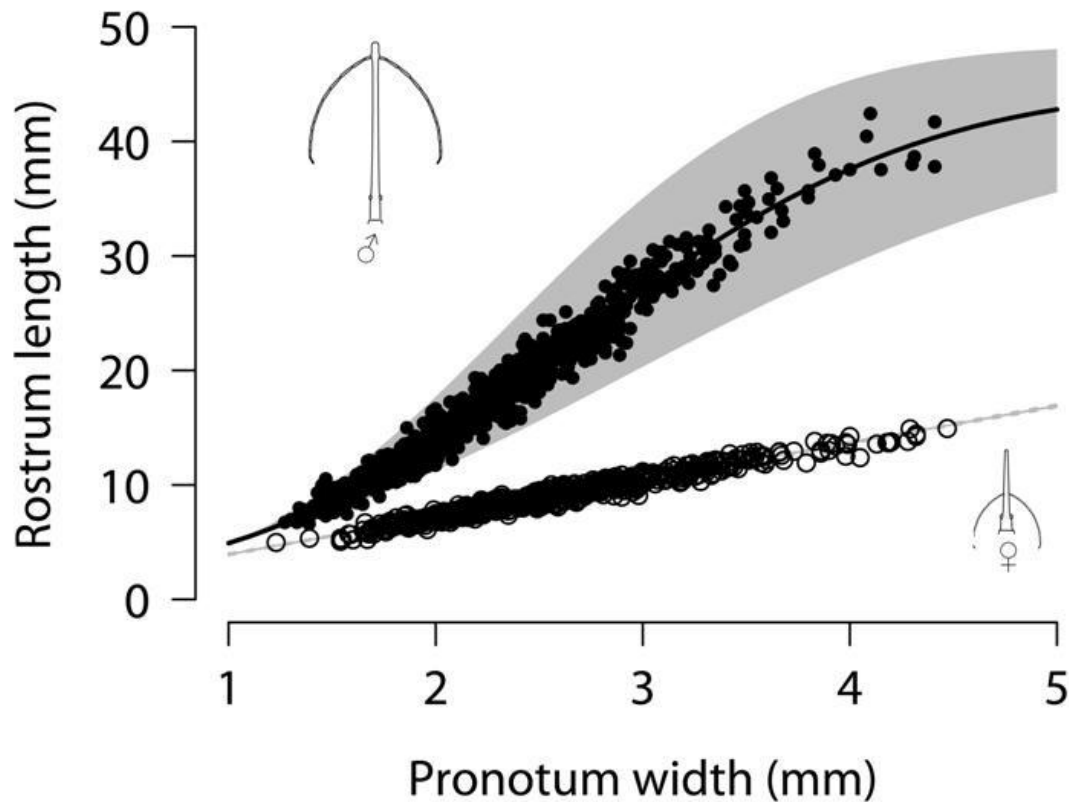


Figure 3. The scaling relationship between body size (pronotum width) and rostrum length of *L. barbicornis* males (●) and females (○) at Matuku Reserve. The line of best fit for each sex is fitted on the data, showing a Weibull growth curve for males, and a simple linear regression line for females. The grey shaded area around each set of points is the 95% confidence intervals for each model (seen as a grey line for females due to CI's being very tight). Inset drawings show the sexual dimorphism in rostrum morphology (drawings by Vivian Ward).

An initial inspection of a scatterplot of untransformed rostrum length (weapon size) against pronotum width (body size) for male *L. barbicornis* showed a continuous relationship between the two variables, but there appeared to be a decrease in relative rostrum length for the largest males (Figure 3). The slope appears to decrease at around pronotum width of 3.8 mm. The scaling relationship was characterised by comparing a range of linear and non-linear models using AIC calculated as [2x (-log likelihood) + 2x (no. parameters in the model)] to compare models. Models with the lowest AIC values best describe the relationship between the variables in question, and models with a difference in AIC scores of more than two are considered to be different (Akaike 1973). Changes in BIC (Bayesian Information Criterion) were also used to compare models as this approach takes into account sample size with the equation [-2x ln (likelihood) + no. parameters in the model * log (sample size)] (Schwarz 1978). BIC therefore penalizes more complicated models when sample size is large, and in this case can be used to ensure that the choice of a non-linear model to describe scaling relationship is really a better fit than a simple linear model.

The allometric relationship was best described by the Weibull growth function model, indicating a sigmoidal relationship between rostrum length and pronotum width (Table 1, Figure 3). Although the asymptote in the Weibull model is due to the decrease in relative rostrum size of a few very large males, and subsequently the deviation from linear is slight, the fit of this model was much better than a simple linear model ($\Delta$AIC = 98.9, $\Delta$BIC = 89.1). The subtle differences in $R^2$ between the models further emphasise that the deviation from linearity is slight and due to a small number of very large males. The frequency distribution of rostrum length for both males and females shows a unimodal distribution, but is highly right-skewed for males (Figure 4).

Table 1. Models fitted to describe the scaling relationship of untransformed rostrum length and pronotum width of male *Lasiorhynchus barbicornis.* The best model is highlighted in bold, and models are arranged by increasing values of AIC and BIC.

| Model | AIC | ΔAIC | BIC | ΔBIC | $R^2$ | Model Parameters |
|---|---|---|---|---|---|---|
| **Weibull growth function** | **3019.6** | **0** | **3044.1** | **0** | **0.971** | **a (asymptote) = 44.63** |
| | | | | | | **drop (asymptote - y intercept) = 41.94** |
| | | | | | | **lrc (ln rate constant) = -2.91** |
| | | | | | | **power (power x is raised to) = 2.51** |
| Four-parameter logistic | 3020.4 | 0.8 | 3044.8 | 0.7 | 0.971 | a (lower asymptote) = -2.72 |
| | | | | | | b (upper asymptote) = 46.61 |
| | | | | | | c (scale) = 2.62 |
| | | | | | | d (x value for inflection point) = 0.92 |
| Logistic model | 3044.8 | 25.2 | 3064.4 | 20.3 | 0.970 | a (asymptote) = 74.11 |
| | | | | | | b (mid value of x when y is a/2) = 1.36 |
| | | | | | | c (scale) = 0.46 |
| Breakpoint model | 3067.7 | 48.1 | 3092.2 | 48.1 | 0.969 | intercept = -10.56 |
| | | | | | | slope left = 12.38 |
| | | | | | | breakpoint = 4.08 |
| | | | | | | slope right = -15.58 |
| Linear model | 3118.5 | 98.9 | 3133.2 | 89.1 | 0.968 | a (intercept) = -10.26 |
| | | | | | | b (slope) = 12.23 |
| Quadratic model | 3120.2 | 100.6 | 3139.8 | 95.7 | 0.968 | a (intercept) = -10.54 |
| | | | | | | b1 (first slope) = 12.47 |
| | | | | | | b2 (second slope) = -0.05 |
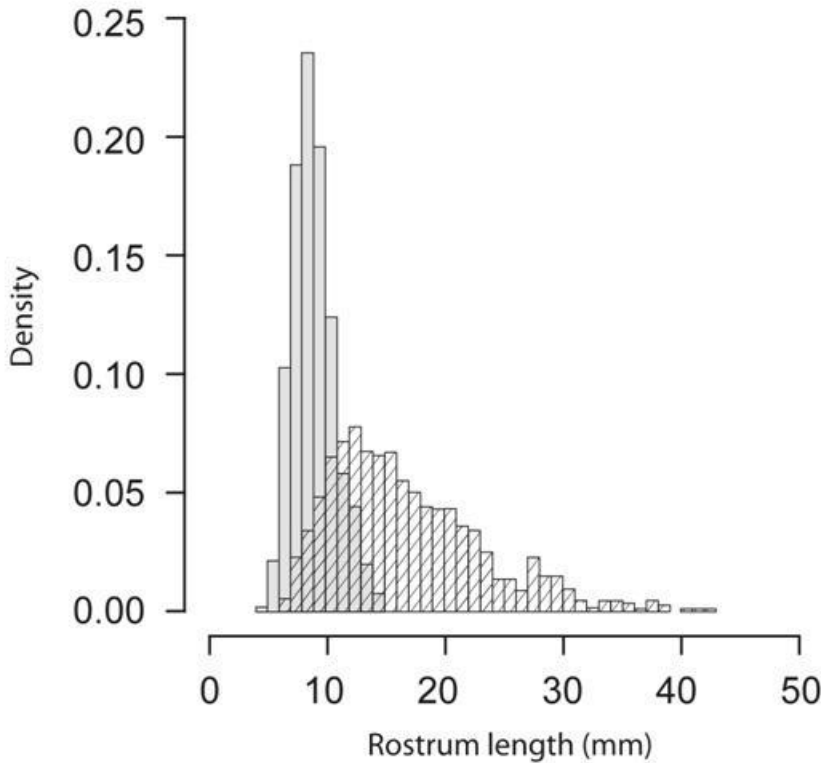
Figure 4. Frequency distribution of rostrum length for males (▨, n = 987) and females (▧, n= 653). Frequencies are plotted as probability densities to allow for comparison between the two uneven sample sizes.

To enable the comparison of the scaling relationship between the sexes and to test for deviation from isometry the slopes for each sex were treated as a straight line using log-transformed data. First, the allometric slope for males was calculated using all specimens, but to determine the effect of the decreasing slope for the largest males the slope was also calculated without these largest males. As the Weibull growth curve described earlier predicted an asymptote at a greater rostrum length than we observed (44.63 mm, max. observed was 42.45 mm), the allometric slope was calculated for males that had a pronotum width smaller than 4.08 mm, which was the predicted change in slope in the breakpoint model. Only six males (from 987) were above the

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predicted breakpoint, so the slope for these largest males was not calculated.  The relationship

between rostrum length and body size was steeply positively allometric for males, when

including all males and when excluding males beyond the breakpoint (Table 2). The allometric

slope is analogous to the power function in the Weibull growth curves (Table 1), however

because data was logged for the SMA analysis the two values are not directly compared (but see

Appendix 2). Females showed weak negative allometry. The slopes between males and females

were compared using methodology in *smatr* analogous to ANCOVA, but using the slopes

estimated by SMA rather than least squares regression. Using a likelihood ratio test compared to

a chi-squared distribution it was found that the male and female slopes were significantly

different from each other (Table 2).

Table 2. Scaling relationship between body and rostrum size for males and females at Matuku Reserve.
Males above the predicted breakpoint were removed and the slope reanalysed without the largest males.
The allometric slope is the standardised major axis slope (±95% confidence interval) of log rostrum
length against log pronotum width. Slopes (*b*) significantly greater than 1 indicate a positive allometry,
and *b* less than 1 indicates negative allometry. The r statistic tests for deviation from isometry. The
likelihood ratio ($\chi^2$) compares whether male and female slopes significantly differ from each other. ***
indicates p <0.0001.

|  | n | Slope (*b*) | lower CI | upper CI | r | $\chi^2$ |
|---|---|---|---|---|---|---|
| Males (all) | 987 | 1.65 | 1.63 | 1.67 | 0.94*** | 1362*** |
| Males (ex. large males) | 981 | 1.66 | 1.65 | 1.68 | 0.95*** | 1368*** |
| Females | 653 | 0.95 | 0.93 | 0.97 | -0.21*** |  |
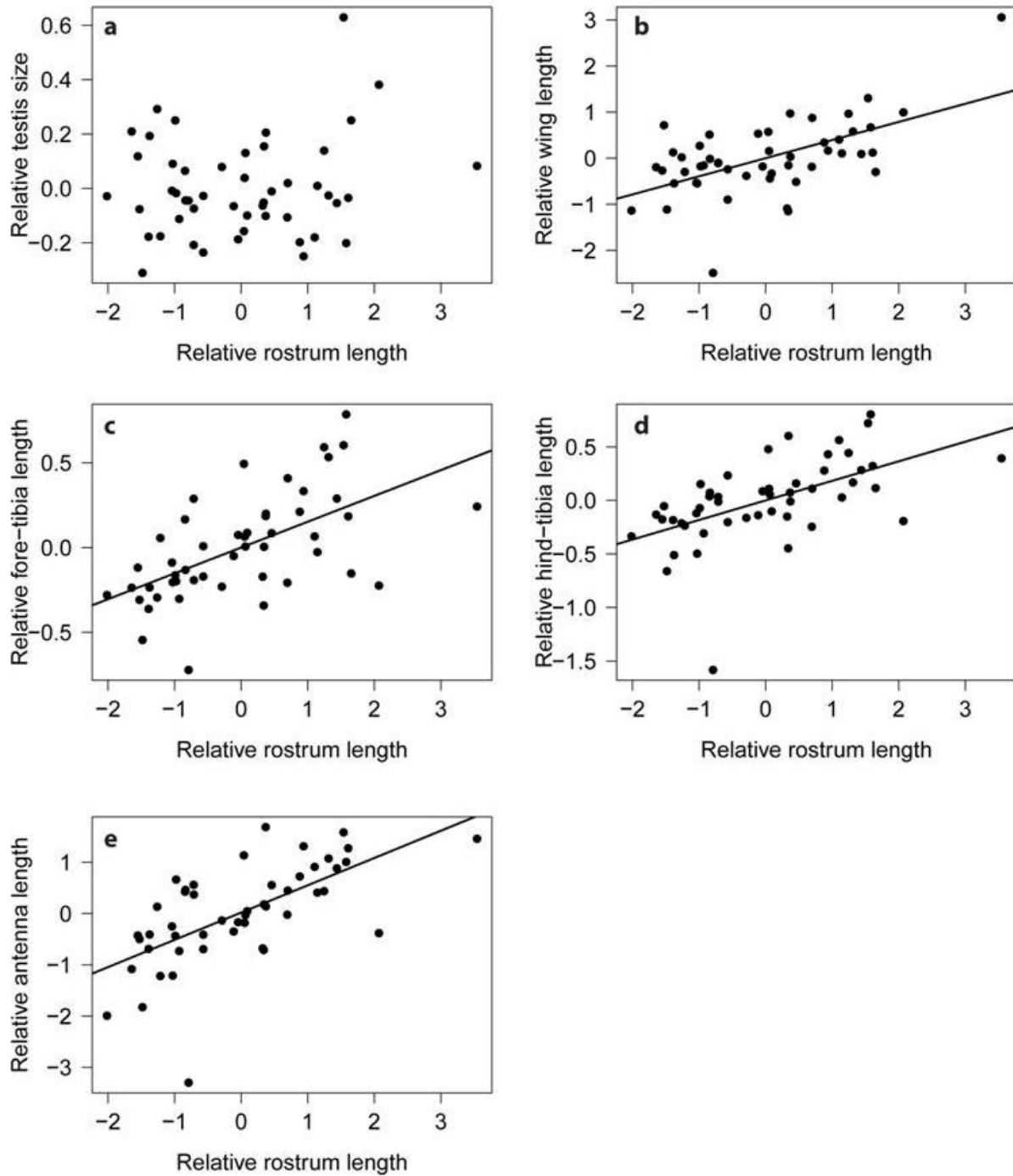
Figure 5. Relative investment into (A) testis, (B) wing, (C) fore-tibia, (D) hind-tibia, and (E) antenna size by male *L. barbicornis* in relation to rostrum length. Linear regression line plotted for significant ($p < 0.05$) correlations only.

*3.4.2 Trade-offs and compensation*

The residual analysis showed that there was no relationship between relative testes size and relative rostrum length, suggesting that there is no tradeoff or compensation between testes and rostrum size (Figure 5A; $b = 0.024$, df = 47, p =0.28, $R^2 = 0.03$). Relative wing size showed a positive relationship with relative rostrum length, indicating that males with the largest rostrums invest disproportionately more into wing size than males with smaller rostrums (Figure 5B; $b = 0.393$, df = 47, p <0.0001, $R^2 = 0.33$). Similarly, fore-tibia (Figure 5C; $b = 0.152$, df =47, $R^2 = 0.35$), hind-tibia (Figure 5D; $b = 0.183$, df =47, $R^2 = 0.31$), and antennae length (Figure 5E; $b = 0.532$, df =47, $R^2 = 0.41$) showed positive relationships with relative rostrum length.

**3.5 DISCUSSION**

*3.5.1 Shape of scaling relationship*

The analysis of the scaling relationship between rostrum and body size showed a high level of dimorphism in the shape and steepness of the allometric slope between the sexes. The steep positive allometry displayed by males indicates that larger males are putting a disproportionate amount of resources into rostrum length than smaller males. The presence of a positive allometry when studying an exaggerated trait in males (particularly when coupled with behavioural observations) is generally used as evidence that the trait is under sexual selection, and therefore demonstrates the importance of the trait as a weapon or ornament used during mate acquisition (Kodric-Brown et al. 2006). However, a recent review highlighted that not all sexually selected traits show positive allometries when scaled against body size, and not all traits showing positive

allometries are under sexual selection (Bonduriansky 2007). An historic fascination with spectacularly exaggerated traits has led to the incorrect conclusion that all sexually selected traits exhibit positive allometry, while in fact this is not a universal trend across all taxa. When positive allometries are observed for an exaggerated trait, this suggests that (1) there is an overall selective advantage for larger males that leads to an increase in relative trait size (i.e. increasing trait size increasing mating success more for larger males than small males), and (2) that there is corresponding selection for small males to invest relatively less in weapon size (i.e. small males cannot wield weapons effectively so invest in alternative mating tactics) (Bonduriansky & Day 2003; Bonduriansky 2007). Caution must therefore be taken, and it is important to couple these morphological data with behavioural observations that determine the significant role of the rostrum in male-male competition. Behavioural experiments determining whether males with relatively long rostrums have a competitive advantage during contests with other males will enable us to have a clearer understanding of how sexual selection might have shaped rostrum evolution in *L. barbicornis* (Chapter 5).

Female rostrum length showed a linear relationship with body size, whereas males showed a clear tapering of the slope for the largest males, best explained by the Weibull growth function model showing a sigmoidal relationship between weapon and body size. Male dimorphism is particularly common in highly size variable species that display weaponry, and although dimorphisms can be difficult to identify, modern techniques such as those outlined in Knell (2009) that were followed in this study, have allowed these analyses to become easier. Despite this there was no evidence of dimorphism in weapon expression, rather rostrum length scaled steeply with body size until the curve starts to asymptote for the very largest males. This relationship suggests a physiological constraint on weapon size due to resource limitation in a

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similar pattern seen in other armed insects (Knell et al. 2004; Tomkins et al. 2005a; Zatz et al. 2011). Furthermore, a frequency plot of rostrum length showed no evidence of bimodality. In *L. barbicornis* males of all sizes, despite extensive size variation, possess elongated rostra, and future studies will show how these are used across the spectrum of male sizes (Chapter 4 & 5).

Asymptotes in weapon size in larger males are likely in species that have a relatively high investment into weapon size relative to body size (Knell et al. 2004). For example, within the lucanid beetles, those species that have steep positive scaling relationships between mandible and body size show a higher level of constraint for the largest males than in those species that do not invest substantially into mandible size (Knell et al. 2004). The declining investment into weaponry for the largest males in holometabolous insects such as beetles reflects the environment that these structures develop in, where most of the growth of the discs that will become adult appendages occurs after feeding has ceased, therefore reflecting the closed system in which they develop (Nijhout & Wheeler 1996; Nijhout & Emlen 1998). The growth of large exaggerated structures such as the rostrum in the giraffe weevil is therefore expected to be limited by available resources, which at the same time must be shared between all other developing adult structures. However, because these data are correlational we cannot rule out other possible reasons for a decrease in relative rostrum length such as limitations due to increasing energy requirements for wielding increasingly large weapons (Knell et al. 2004). While the Weibull growth curve had the best fit to the data, I recognize that the deviation from linearity in the model is subtle, and that a limitation in rostrum length is only occurring for the very largest males which are a scarce but important component of the population. Consequently, the scaling relationship for male giraffe weevils is a continuous and mostly linear relationship, with a decrease in slope for the very largest males.

*3.5.2 Trade-offs and compensation*

There was no evidence of a trade-off demonstrated by negative phenotypic correlation between rostrum size and any of the traits measured in males of the giraffe weevil. Among other species, previous studies investigating the potential for trade-offs between secondary sexual traits and other appendages have found mixed results. Some species show evidence of a trade-off, and others do not, demonstrating that there is no universal pattern across those taxa that display exaggerated traits. In the past, studies on trade-offs mostly focused on traits that were developing in close proximity to the exaggerated trait, and have found a tight link between the ecology of the species and the presence and location of these traits (Emlen 2001; Emlen et al. 2005). Trade-offs between adjacent developing traits are expected because the developing tissues mostly use a local pool of resources available for growth (Emlen 2001). However many correlational (Simmons et al. 1999; Simmons et al. 2007; Pizzo et al. 2012) and experimental studies manipulating weapon size have identified tradeoffs between disparate traits such as testes (Moczek & Nijhout 2004; Simmons & Emlen 2006; Okada & Miyatake 2009; Yamane et al. 2010). These patterns are not universal, possibly because testes and genitalia are not always sensitive to changes in the concentration of circulating hormonal signals (Simmons & Emlen 2006; Emlen et al. 2012), or because trade-offs of this nature are only apparent when environmental conditions are stressful (i.e. during food shortages) (Messina & Fry 2003; Simmons & Emlen 2006). Furthermore, by limiting the analysis to phenotypic correlations, it is not possible to determine whether underlying tradeoffs are obscured by variation in individual quality due to genetic differences in the ability of individuals to acquire resources (van Noordwijk & de Jong 1986; Metcalfe & Monaghan 2003).

There was no correlation between relative testis size and rostrum length, indicating that males invest proportionately into testis size relative to their own overall body size. The relative investment in weapon and testis size reflects an overall trade-off in traits that increase the likelihood of acquiring mates vs. a trait that increases fertilization success (Simmons & Emlen 2006). Males with the highest sperm competition risk are expected to be under selection for adaptations that reduce this risk, such as by increasing testis or ejaculate size (Parker 1990). In some species (e.g. Atlantic salmon, dung beetles, etc.) small males have been found to increase the relative investment into testis, presumably due to selection to compensate for an increase in sperm competition and a decrease in mating opportunities (Stockley & Purvis 1993; Gage et al. 1995; Taborsky 1998; Simmons et al. 1999; Simmons et al. 2000). However, not all species with a small male morph increase relative testis size, perhaps due to variation in the cost of gaining mates in comparison to other species (e.g. Munguia-Steyer et al. 2012). One further possibility is that rather than increase testis size, small males instead have larger ejaculates per copulation because of a perceived disadvantage in mating opportunities (Kelly 2008).

The relative size of wings, antennae, and legs showed positive phenotypic correlations with relative rostrum length, indicating these traits increase in size as rostrum increases. This can be interpreted as males compensating for increased rostrum length (and possibly the increased cost of locomotion and fighting) by increasing the relative length of their hind wings, antennae, and legs. The development of exaggerated traits is repeatedly claimed to be costly, and can negatively affect the performance of other aspects of the individual not directly related to mate acquisition. If the production of an exaggerated trait negatively affects the performance of the individual (e.g. reduces flight ability), natural selection can operate to allow compensation of other physical or behavioural traits. Individuals that can reduce the cost of bearing the

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exaggerated trait will be at a selective advantage (Husak & Swallow 2011). Compensation through integration of trait development can make it difficult to assess the cost of exaggerated trait production, especially if the individual is able to offset any reduction in performance, therefore complicating the ability to simply measure performance to imply the costliness of the trait (McCullough et al. 2012). I do not have data on flight in *L. barbicornis*, but intuitively one would expect that large males would have reduced flight capability due to the hindrance of bearing such long rostrums and additional weight. However, it is possible that by increasing the relative size of their hind wings, males have compensated for the cost of bearing a larger rostrum, and suffer little or no disadvantage in flight. This has been found to be the case in other species carrying large ornamentation, and highlights the need to assess the possibility of compensatory traits when studying the cost of sexually selected traits (Oufiero & Garland Jr. 2007; Husak & Swallow 2011; McCullough et al. 2012). Compensation in wing size could be further analysed in the giraffe weevil by measuring flight behaviour between males across the size spectrum, and by using geometric morphometrics to assess variation in wing shape and its allometry in addition to wing length.

Interpreting positive correlations as evidence of compensation and a lack of trade-offs in a study relying on evidence from phenotypic correlations alone must be done with caution. Positive phenotypic correlations between traits can be observed even when an underlying negative genetic correlation due to a trade-off in resource allocation is expected (van Noordwijk & de Jong 1986). These models have been better developed in the field of life history trade-offs, and have shown that there can be high levels of genetic variation in the ability of individuals to acquire resources from the environment, which can lead to differences in resource allocation (Robinson & Beckerman 2013). Particularly when there is a large variation in how individuals

acquire resources, a positive correlation between the phenotype of two size traits could simply be due to the ability of larger individuals to acquire resources, therefore having more available to allocate to both traits (van Noordwijk and de Jong 1986). In a large-bodied species such as *L. barbicornis* which has a larval stage that lasts several years (C. J. Painting unpublished data), breeding experiments to test the relative genetic variability in resource acquisition and allocation are prohibitive, and in this respect *L. barbicornis* may not make an ideal model to determine the genetic co-variance of these traits. While the lack of predicted trade-offs between traits, and the subsequent discovery of phenotypic correlation between traits could be interpreted as evidence for genetic correlation through developmental integration, it is also recognized that these correlations are a complex reflection of differences in resource acquisition and allocation between individuals.

Species with exaggerated traits that develop in a closed system, such as in beetles, are most likely to undergo competition with other developing traits, but are also likely to be under selective pressure to increase the size of traits that enable the exaggerated trait to be physically supported (Klingenberg & Nijhout 1998; Nijhout & Emlen 1998; Emlen 2001; Moczek & Nijhout 2004; Tomkins et al. 2005b). Genetic correlation due to developmental integration offers a possible explanation for the observation of increased investment in both fore- and hind-tibia size in relation to rostrum length. Males fight fiercely for access to females, using their rostrum and mandibles to push, pull and grapple with competing males (Meads 1976; Chapter 5). The first part of this study showed that as overall body size increases, so does the relative investment into rostrum length, and therefore if larger, stronger legs are required to support the individual during fights it is not surprising that a correlated increased investment in leg size occurs through developmental integration (Klingenberg 2004). Similar results have been found in other species,

for example the fore-tibia in large, major males of *Onthophagus taurus* have been shown to be relatively larger than minor male legs, probably to compensate for the hindrance of bearing horns during digging in tunnels, and to support them during contests with rivals (Tomkins et al. 2005b).

The observed increase in relative antenna length in relation to rostrum size is less likely to be explained by correlated selection to increase the success of large males. Instead it is more likely that the positive correlation between these two traits is due to direct interactions between cells during development because antennae grow directly from the surface of the rostrum. If the rostrum and antenna develop from the same module (i.e. same imaginal disc) any factor that causes variation in the growth of that module will be reflected in a positive correlation between the final adult traits (Klingenberg 2004). Alternatively, positive correlations can occur even if the development of these traits is separate as signals between pathways can also generate covariation if the variation in one set of cells results in the communication of a chemical signal that affects the other cells in a similar way (Klingenberg 2004). It is not known if the source of variation in body size (and rostrum length) in *L. barbicornis* is due to genetic or environmental factors, but any variation that affects the size of one trait can manifest in covariation in another trait(s). The positive correlation between rostrum and antenna size in *L. barbicornis* reflects different developmental processes occurring compared to the production of horns in *Onthophagus* beetles, where horns and antennae share resources available for growth, but develop from different imaginal discs, resulting in trade-offs between the traits (Emlen 2001).

 A principle tenet in sexual selection theory is that exaggerated traits are costly to produce and bear (Andersson 1994). Giraffe weevil males can grow up to 90 mm long, and are the longest weevil in the world, with their rostrum making up half of their total body length. With such a

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huge investment into rostrum length, it seems sensible to assume that this comes at the cost of other body parts. However, I did not find evidence that the production of the rostrum in this species inflicts costs on the relative size of any of the traits that we measured; rather large males actually increased their investment in wing, leg, and antenna size, presumably to compensate for the increased load during flight and fights, and in the case of antennae, perhaps due to developmental integration with rostrum size. Overall this study has shown that male giraffe weevils invest heavily into rostrum size and shows a steep positive relationship with body size, with the largest males under weapon-size constraint. This will therefore provide an important baseline for future studies on variation in body and weapon size investment.

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# CHAPTER 4

# Alternative reproductive tactics in the New Zealand giraffe weevil *Lasiorhynchus barbicornis*

## 4.1 ABSTRACT

Alternative reproductive tactics (ARTs) occur in many species where there is intense competition for mates and substantial variation in reproductive success between males of different size, age, or condition. The majority of ARTs are defined as being a conditional strategy, where males employ the tactic that will return the highest fitness in association with its phenotype. Males of the giraffe weevil *Lasiorhynchus barbicornis* display an elongated rostrum used as a weapon during contests with other males. In addition, both sexes are extremely variable in body size, such that there is a 6-fold difference in length between the smallest and largest individuals. It was predicted that such size variation in this species coupled with intense competition for access to females would have driven the evolution of ARTs to allow small males to compete with their larger counterparts. Larger males were found to rely only on aggressive behaviour to secure females, but smaller males exhibited greater plasticity in their reproductive tactics. Males less than 40 mm in body length frequently employed sneaking strategies but would also readily fight with smaller or equally matched opponents. There was no difference in mating success between males greater or less than 40 mm long, with neither total copulation duration or frequency of copulation correlating with body size. There was also no relationship between mating success and body size or rostrum length, and therefore no evidence of sexual selection operating on rostrum length in this species. Giraffe weevils are a unique example of a species that has evolved ARTs characterised by highly context-dependent behaviour in which sneaking and aggressive behaviours are both used by small males dependent on opponent body size.

## 4.2 INTRODUCTION

Alternative Reproductive Tactics (ARTs) commonly arise in species where sexual selection and competition for mates is particularly intense, and when there is great disparity in reproductive success between males (Gadgil 1972; Shuster & Wade 2003). ARTs are discrete phenotypes that are characterised by differences in age, size, condition, colour, polymorphism in trait expression, or behaviour, and are used to maximise fertilisation success (Taborsky et al. 2008b). ARTs in males are commonly divided between dominant and subordinate individuals, where dominant (often larger) males adopt an aggressive, territorial or guarding strategy, while subordinate (usually smaller) males use sneaking or satellite tactics to achieve mating success (Oliveira et al. 2008). Some ARTs are determined by genetic polymorphisms (e.g. Ryan & Causey 1989; Shuster & Wade 1991; Lank et al. 1995), but more commonly the ART is a conditional strategy driven by an individual's status and the environmental conditions in which they live (Gross 1996; reviewed in Oliveira et al. 2008). For example, in *Onthophagus taurus* beetles larval diet has been shown to be important in determining body size, which in association with a threshold determines the presence or absence of a horn used as a weapon, and consequently the reproductive tactic used by adult males (Moczek 1998). An individual 'chooses' the ART, in association with its phenotype, that will return the highest fitness, leading to differences between males in the behaviours they employ because it will not always be beneficial for all males to use the same tactic to achieve reproductive success. Under Dawkins (1980) theory of inferior males "making the best of a bad job", and Gross' (1996) concept of status-dependent selection, ARTs under a conditional strategy do not have to be driven by underlying genetic differences between morphs, nor do the morphs require equal fitness to persist in the population .

ARTs can be performed simultaneously or sequentially by individuals, change throughout

ontogenetic stages, or can be fixed during its lifetime (Taborsky 1998). Highly unpredictable

conditions can result in selection for simultaneous ARTs, so that individuals can switch tactic

depending on momentary conditions such as the number of competitors present, relative size,

body condition, or prior residence (Taborsky 1998; Taborsky et al. 2008b). Alternatively, if

conditions do not change during the individual's lifetime, fixed ARTs are more likely to be

selected for.

ARTs are particularly common in arthropod species where males possess exaggerated secondary

sexual traits used as weapons during contests with other males (e.g. Goldsmith 1987; Forsyth &

Alcock 1990; Danforth 1991; Emlen 1997; Moczek & Emlen 2000; Hongo 2007; Okada et al.

2007; Okada et al. 2008; Buzatto et al. 2011; Zatz et al. 2011). Many of these species have been

shown to be dimorphic in weapon expression, such that males below a certain threshold size will

express weapons at a much smaller size proportionate to body size than large males, while other

species show continuous variation in weapon size and the ARTs are not associated with a

dimorphism. ARTs in dimorphic species are discrete behaviours performed according to the

male morph. For example, in *Onthophagus* dung beetles large males wield horns used to

compete with other males and guard tunnels leading to female brood chambers. However, small

males with corresponding small or rudimentary horns adopt sneaking tactics as an alternative to

aggressive fighting that relies on the use of weaponry (Emlen 1997; Moczek & Emlen 2000).

ARTs are also present in species that show intrasexual size variation and/or dimorphism, but lack

weaponry.  For example, dung flies (*Scathophaga stercoraria*) and Dawson's burrowing bees

(*Amegilla dawsoni*) exhibit size-related ARTs in the absence of weaponry (Alcock 1996; Pitnick

et al. 2009), and among fish there are many examples of ART's where male behaviour is often

divided between sneaking, satellite, female mimicry, or parental investment/female defence strategies (Gross & Charnov 1980; Gross 1984; Taborsky 1994; Vladić 2001; Taborsky et al. 2008a). ARTs in fish are highly diverse in form but are often conditional in relation to age and body size, and have been shown to be due to genetic polymorphism between male phenotypes in some species (Taborsky 1994; Taborsky 1998; Taborsky 2001; Taborsky et al. 2008a).

Beetles of the weevil family Brentidae are a diverse group with 1690 species currently described worldwide (Sforzi & Bartolozzi 2004). A great majority of the species within this family are characterised by marked sexual dimorphism, often due to an elongation of the rostrum in males. Little is known about the biology and ecology of brentids, although studies of three species have shown high levels of intrasexual size variation and that males will readily fight to gain or defend access to females for reproduction (Johnson 1982, 1983; Sandborne 1983; Johnson & Hubbell 1984). In addition, although Johnson (1983) did not discuss ARTs specifically, it appears that small *Claeoderes bivittata* males may exhibit size-related sneaking tactics because Johnson refers to small males hiding under females and copulating while large males stand guard. Brentids are therefore useful candidates to explore the evolution of ARTs in species with high size variation and intense male-male competition.

The giraffe weevil (*Lasiorhynchus barbicornis* Fabricius) is an endemic brentid in New Zealand, distributed throughout the North Island and across the north of the South Island. Larvae of the species are wood-borers of a range of native hard-wood trees. From October to April adults emerge and aggregate on the trunks of host trees where copulation and oviposition occurs (Meads 1976). Adults of both sexes show remarkable body length variation (males 15 to 90 mm, females 12 to 50 mm, Chapter 2; Kuschel 2003) and possess an elongated but sexually dimorphic rostrum. The female rostrum contributes approximately 35% of their total body length, and is

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used to drill holes in wood prior to oviposition. The male rostrum however is so greatly

elongated that it comprises half the total body length of the weevil and is used as a weapon

during male-male contests (Meads 1976). Although males of all sizes possess an elongated

rostrum, rostrum length shows steep positive allometry when scaled against body size, indicating

the potential for sexual selection to have driven the evolution of this male trait (Chapter 3;

Kodric-Brown et al. 2006), but it is important to test this assumption with behavioural

observations (Bonduriansky 2007). During the summer, aggregations of adults can be readily

observed in the wild, making this species an ideal candidate for extensive field observations of

behaviour.

Due to the extreme body size variation and existence of an exaggerated trait in males, it was

predicted that small males would use alternatives such as sneaking or satellite behaviours to

compensate for their size disadvantage during interactions with larger males. While many model

systems are restricted to laboratory studies of behaviour, this study consisted of a large-scale

series of field observations on a population of wild *L. barbicornis* to determine the existence of

conditional alternative mating tactics in association with status (body size). From these

observations I predicted that the tactic chosen by males during male-male interactions relates to

body size. Specifically, I predicted that large males used fighting tactics while small males used

sneaking behaviour. I also predicted that the decision to sneak by small males was based on the

relative size of the opponent male. Lastly these data were used to determine whether there was

any difference in the mating success of males across different sizes. Under a conditional strategy

of ARTs, Gross (1996) predicted that there will be unequal fitness between tactics, and so I

predicted that that large males with the dominant tactic would copulate more often and for longer

than small males using sneaking tactics. A positive relationship between mating success and

body size would also offer direct support for sexual selection operating on large size and rostrum length in this species.

## 4.3 METHODS

### 4.3.1 Study site

Observations were conducted at Matuku Reserve (36° 51.92'S, 174°28.32'E) west of Auckland, New Zealand. This 120 hectare reserve is privately owned by the Royal Forest and Bird Protection Society and consists predominantly of coastal lowland broadleaf forest. Searches were conducted in October and November 2010 to locate host trees on which adult giraffe weevils were aggregated. At Matuku Reserve *L. barbicornis* were usually found on karaka (*Corynocarpus laevigatus*) (n=26), but they were also found adults on single stems of rangiora (*Brachyglottis repanda*), mahoe (*Melicytus ramiflorus*), and tarata (*Pittosporum eugenioides*). For this study we observed only those weevils found on *C. laevigatus* and chose ten suitable trees for observation by locating erect stems where weevils were mostly aggregated from zero to three metres above ground level.

### 4.3.2 Observational experiments

Before observations began, all weevils were initially removed from the host tree and morphological measurements made using Rok digital callipers to the nearest 0.01 millimetres. Total body length was measured from the base of the elytra to the tip of the rostrum at the mandibles. All weevils were given an individual number and marked accordingly with a unique colour combination on the pronotum and elytra with non-toxic Queen bee marking paints (Lega SRL, Italy). After marking, weevils were returned to the tree and on subsequent observation days

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any unmarked weevils were also measured and marked before observations began. After marking at least one hour was allowed before observations commenced.

Observations were conducted using a focal animal technique (Altmann 1974) to observe individual adult male giraffe weevils for a one hour period between 11th January and 18th February 2011 (n=79). Observations were made between 0800 and 1800 hours, avoiding days of heavy rain during which giraffe weevils left the trees to hide. On arrival at a tree a male was randomly chosen and all behaviours were recorded including mating frequency (number of copulations during the hour), mating duration (total amount of time in copulation during the hour), and total time spent walking, solitary, and in interactions with other weevils such as guarding females and fighting with other males. Males from 18.29 – 89.75 mm in total body length were observed, in an attempt to cover the full-size range of animals present in aggregations. In addition, in January 2012 female giraffe weevils were observed for one hour periods to describe general behaviour and time spent drilling and copulating (n= 31).

*4.3.3 Statistical analysis*

There were 385 interactions between the focal male and an opponent, of which 220 interactions were able to be classified as either sneaking (52 observations, defined below) or fighting (168). These 220 interactions were used in a generalised linear mixed effects model (GLMM) in the R package *lme4* (Bates et al. 2012) to determine the probability of sneaking or fighting in relation to body size. All incidences of sneaking and fighting throughout the focal animal observations were used in this analysis, and this behaviour was treated as the response variable with binomial errors and a logit link. Non-independence in the data due to repeated observations on the same study tree and interactions between the same individuals during our hour-long observation

periods were accounted for by including the focal weevil and observation tree as random effects. However, to calculate the mean body length of sneaker vs. fighter males all repeat observations of behaviour were removed.

In addition to discrete sneaking behaviour, I also determined if smaller, sneaking sized males allocated their time differently to large males. Sneaking sized males were determined to be males less than 40 mm in body length, as 92% of sneaking observations were for males less than this length (see Results). Only three males greater than 40 mm in length were observed using sneaking behaviour. The mean percentage of time spent walking, resting or hiding, guarding, copulating, and interacting with other males were compared between the two size groups of males. As the residuals for the percentage data were highly right skewed and not able to be transformed by standard procedures, non-parametric Kruskal-Wallis tests were used for each activity.

To determine if the decision to sneak or fight was conditional on opponent size, a subset of the data was made to include only those observations where the focal male was less than 40 mm long (likely "sneaker" size). There were 255 observations in this subset, but all instances where the focal male retreated during an interaction rather than attempted to sneak or challenge the opponent were excluded, resulting in a final data set of 129 observations. Opponent relative size was calculated by subtracting the focal weevil length from the opponent body length. A GLMM was used with behaviour type (0=fight, 1= sneak) as the binary response variable, the size difference between the focal and opponent as the explanatory variable, and the identity of the focal weevil and observation tree as random effects to account for repeated observations for the same individuals.

To determine whether mating frequency (number of copulations per male in hour-long observation) correlated with body size for each focal male (n=79) a generalised linear model (GLM) with a Poisson distribution was used. The data were overdispersed so the model was refitted using quasi-Poisson errors. The correlation between total copulation duration and body size was determined by removing all zeros and fitting an ordinary least squares (OLS) regression model with log transformed total time spent copulating during hour-long observations (seconds) as the response variable, and log transformed body length as the explanatory variable (n=50). For these analyses, observations of focal weevils were removed if during the one hour observation period no females were sighted on the tree.

To estimate the influence of body and rostrum size on the mating success of male giraffe weevils, I used the regression-based approach outlined in Lande & Arnold (1983). I estimated directional and stabilising selection using standardised male traits (mean = 0, sd = 1) and relative fitness (mean subtracted from each value). The number of unique copulations with different females during the hour –long observations was used as a measure of fitness (mating success). Body length (excluding rostrum length), pronotum width and rostrum length were all highly correlated (correlation coefficients between 0.98-0.99); therefore it was not possible to conduct a multiple regression analysis using all traits. I instead conducted separate analyses for each trait. All statistical analyses were conducted in R 2.15 (R Core Team 2013). Akaike's Information Criteria (AIC) using the equation [2x (-log likelihood) + 2x (no. parameters in the model)] was used to compare each model to an intercept only model to validate the model choice (Akaike 1973).

**4.4 RESULTS**

*4.4.1 General mating behaviour*

The activity budget of females and males (divided into small and large males as discussed in Methods) is summarised in Table 1. My observations showed that males and females will aggregate on the trunks of dying or dead trees where both mating and oviposition occurs. Females walk up and down the trunks, tapping their rostrum and antennae on the tree as they walk, searching for a suitable site to drill a hole in which to oviposit their egg. After the egg is laid the female rocks back and forth between 7-34 times (mean $\pm$ SE = 14.6 $\pm$ 3.01), scraping bark to plug into the hole.

Copulation usually occurs while the female is drilling (60.5% of copulation events), but males will also copulate with females while they search for new drill sites (23.3%), or when the female is motionless and not drilling following oviposition (16.3%). Males do not exhibit courtship behaviour prior to copulation; rather they will approach females and immediately mount and attempt to copulate or guard the female while she is drilling. Males of all sizes will guard females before or after copulation, and will remain with the female until disturbed by another approaching male or until the female oviposits. Males guard females by standing directly over them (as if they were copulating, but without genital contact), or by standing perpendicular to the drilling female with their fore-legs resting on top of the female. Challenging males will approach the pair and attempt to usurp the guarding male by using their elongated rostrum and mandibles to rake and bite at the guarding male, sometimes dislodging the male from the female. Guarding males react to approaching male competitors by first kicking their legs, and if this does not result in the departure of the opponent, they will also attempt to rake and bite at the other male using

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their rostrum and mandibles. Unresolved conflicts between similar sized males will at this point

escalate to grappling, where males meet together side by side and then attempt to dislodge the

opponent completely from the tree. The winner is successful when the loser either retreats or

when the winner is able to grab hold of a leg and use their rostrum as a lever to flick the loser

from the tree. It was found that representative males from all sizes employed fighting behaviour,

regardless of body size (see Chapter 5 for more detail on fighting behaviour).

Table 1. Mean percentage of time spent on each activity event for female, and small and large male *L. barbicornis* during hour-long observations.

| Behaviour | Females | All males | Kruskall Wallis Test | | Small males (<40 mm) | Large males (> 40 mm) | Kruskall Wallis Test | |
|---|---|---|---|---|---|---|---|---|
| *Both sexes* | | | $H_1$ | *p* | | | $H_1$ | *p* |
| Copulating | 29.89 (4.74) | 16.94 (2.27) | 7.51 | **0.006** | 15.48 (2.97) | 18.51 (3.50) | 0.38 | 0.54 |
| Walking | 17.60 (2.84) | 5.45 (0.78) | 9.23 | **0.002** | 5.22 (1.21) | 5.71 (0.99) | 0.28 | 0.60 |
| Resting/Hiding | 22.28 (4.15) | 56.71 (3.85) | 21.04 | **<0.0001** | 60.32 (5.33) | 52.82 (5.56) | 1.22 | 0.27 |
| *Males only* | | | | | | | | |
| Guarding | - | - | - | - | 15.41 (3.38) | 20.72 (3.71) | 1.97 | 0.16 |
| Male-male interaction | - | - | - | - | 3.1 (0.59) | 4.54 (1.22) | 0.02 | 0.89 |
| *Females only* | | | | | | | | |
| Drilling | 40.89 (5.88) | - | - | - | - | - | - | - |
| Ovipositing | 5.75 (1.62) | -- | - | - | - | - | - | - |

Standard error is in parentheses. Kruskall-Wallis tests were used to test for differences in activity budgets between males and females, and between size classes of males. Significant differences are highlighted in bold.

Both males and females were highly promiscuous, copulating multiple times during the one hour observations. Focal females (mean ±SE = 3.13 ± 0.54) had a higher mean copulation frequency than focal males (mean ±SE = 1.61 ±0.19) (Figure 1A; Mann-Whitney U test: W = 1646, $p$ =0.004), and also spent a greater amount of time copulating during the hourly observations (mean ±SE females: 1076.03 s ± 170.75; males: 601.83 s ± 81.12) (Figure 1B; Mann-Whitney U test: W = 1644, $p$ = 0.004).
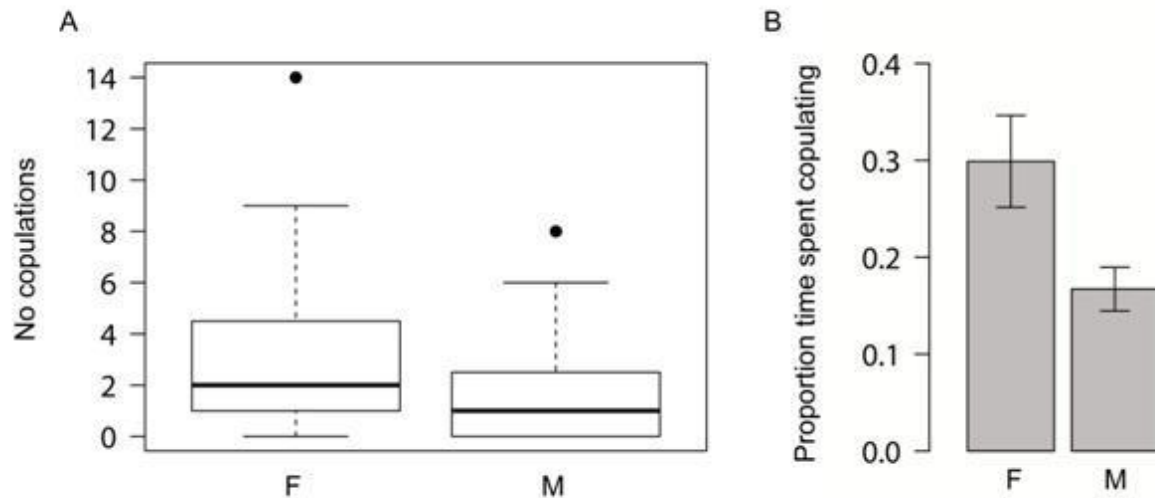


Figure 1. (A) Copulation frequency of female and male *L. barbicornis* during one hour focal observations, and (B) Proportion time spent copulating during one hour focal observations for females (F) and males (M). N= 31 Females, 79 males.

*4.4.2 Alternative Reproductive Tactics*

There were 52 examples of sneaking by 21 individual focal males observed. Sneaking was defined as (1) when a small male hid under a female that was being guarded or copulated with by

another male (Figure 2A), (2), when a small male guarded or copulated with a female while being guarded by another male (Figure 2B), (3) or when a male tucked his rostrum around the side of the female or flattened himself against the female to avoid detection (Figure 2C). Males that hid under or beside females while another male copulated with her usually waited for the mating male to be distracted by a third rival before attempting copulation (Video 1; see attached CD) or slowly wedged himself between the pair to attempt copulation (Video 2; see attached CD).
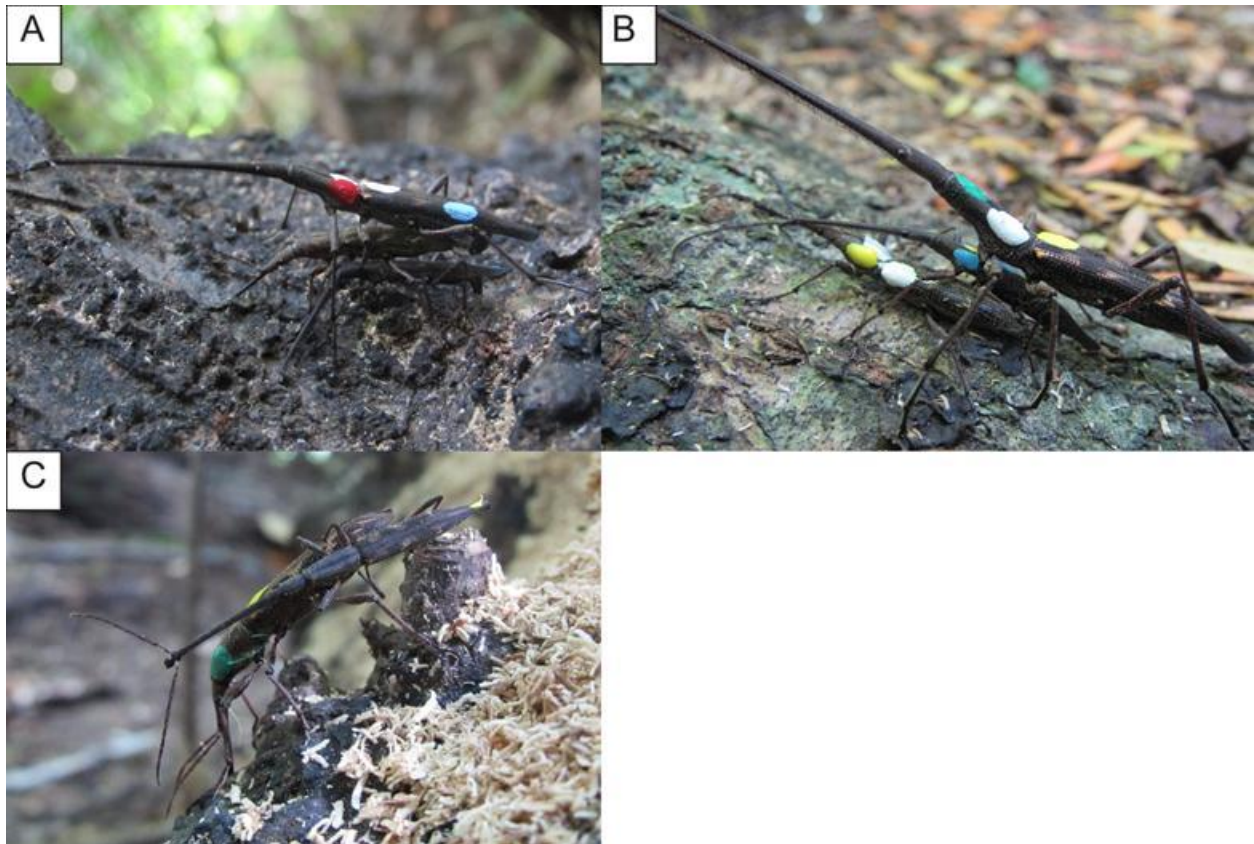


Figure 2. (A) Sneaker male hides under mating pair waiting for an opportunity to copulate, (B) A large male giraffe weevil guards a sneaker male and a female, (C) Sneaker male tucks around side of female to avoid detection from other approaching males.

The maximum body size for a male that exhibited sneaking behaviour was 47.5 mm long; however 48 from 52 (92%) observations were by males less than 40 mm long. Males that used sneaking behaviour had a mean body size (± SE) of 25.68 mm (±1.59), while fighters had a mean body size of 44.13 mm (±1.84). The GLMM supported this result, and showed that small focal males were more likely to sneak than fight when guarding a female or approaching another male (Table 2, Figure 3). However, small males less than 40 mm long also frequently challenged their opponents (35%, 45 from 129 interactions), and therefore absolute size of small focal weevils was clearly not the only important factor determining mating tactics. Despite this, sneaking behaviour was solely a small male tactic, and most males larger than 40 mm, and all males larger than 47.5 mm relied only on fighting behaviour to gain access to or defend females from other males. In addition, sneaker-sized males (< 40 mm long) did not differ in the mean percentage of time allocated to each observed activity when compared to larger males (Table 1). These data indicate that small males do not spend a greater proportion of time walking around searching for available females (possible satellite behaviour), and that small males spend as much time guarding females as larger males.

Table 2. Model estimates for GLMM of focal male decision to sneak or fight in relation to body size

|  | Estimate | Standard Error | Z | $p$ |
| --- | --- | --- | --- | --- |
| Intercept | 6.243 | 1.541 | 4.051 | <0.0001 |
| Focal Length | -0.233 | 0.050 | -4.633 | <0.0001 |

The explanatory variable for this model is the total body length of the focal male. Response variable was the decision to fight or sneak. The AIC for this model was 124.51 with a log-likelihood of -58.26. In comparison the null model (intercept + random effects only) had an AIC of 155.94 (ΔAIC of 31.42) and log-likelihood of -74.97.
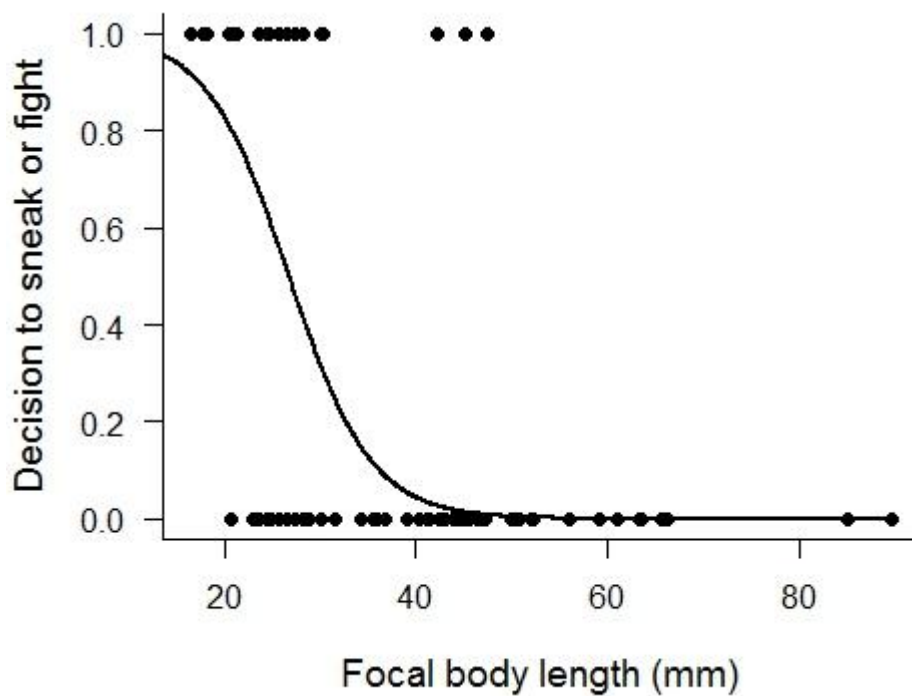
Figure 3. Focal body size (length mm) was a significant predictor of the probability of sneaking (y = 1) or challenging (y = 0) during interactions with another male.

*4.4.3 Small male decision making*

Males of all sizes were observed guarding females, and using their rostrum to fight opponent males, indicating that small males are not restricted to sneaking behaviour to gain access to females. The mixed effects model determined that the size difference between the focal and opponent males was a significant predictor of whether the focal male chose to sneak or fight (Table 3). The inflection point predicted by the GLMM where there is a 50% probability of small males sneaking or fighting is at a body length difference of approximately 8-10 mm (Figure 4).

Small males were more likely to attempt to challenge their opponent if the opponent was smaller

or approximately equal in size, but would employ sneaking tactics if their opponent was larger.

Table 3. Model estimates for GLMM of absolute size difference between focal and opponent males in

relation to decision to sneak or fight

| | Estimate | Standard Error | Z | $p$ |
|---|---|---|---|---|
| Intercept | -1.570 | 0.545 | -2.88 | 0.0039 |
| Size difference | 0.166 | 0.035 | 4.735 | <0.0001 |

Only focal males <40 mm long were included in this analysis (N = 46 males, 129 observations). The explanatory variable is the absolute difference in size between the focal male and the opponent male. The AIC for this model is 101.3, with a log-likelihood of -46.65. In comparison the null model (intercept + random effects only) had an AIC of 148.7 ($\Delta$AIC = 47.3), with a log-likelihood of -71.37.
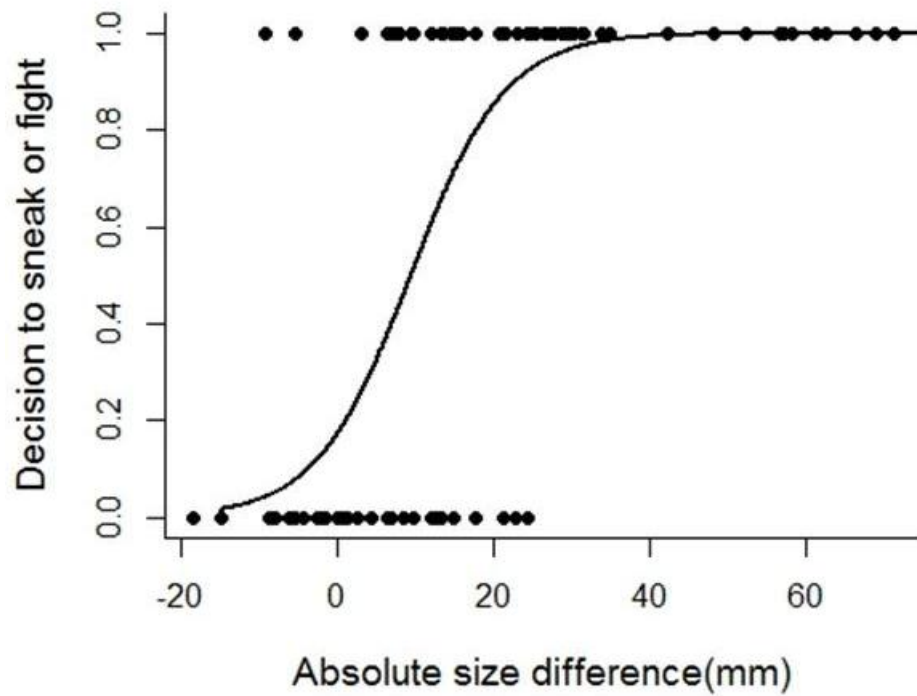
Figure 4. Decision making of small males (< 40 mm body length) when interacting with an opponent. Size values greater than zero indicate that the opponent was larger than the focal weevil. Relative size was a significant predictor of the decision to sneak (y = 1) or challenge (y = 0) the opponent, using a GLMM with a binomial distribution.

### 4.4.4. Mating success across body sizes

Contrary to the prediction no correlation between body size and mating frequency was found, indicating a lack of evidence for any size advantage for large males (Table 4). Similarly, mating duration (the total number of seconds spent copulating during hour-long observations) showed no correlation to body size (Table 4).

Table 4. Relationship between body size and mating frequency and duration

| Mating frequency (n=79) | | | | |
| --- | --- | --- | --- | --- |
| | Estimate | SE | t | p |
| Intercept | 0.15 | 1.13 | 0.13 | 0.89 |
| Log body length | 0.09 | 0.31 | 0.29 | 0.77 |
| Mating duration (n=50) | | | | |
| | Estimate | SE | t | p |
| Intercept | 6.17 | 1.39 | 4.4 | <0.001 |
| Log body length | 0.03 | 0.38 | 0.07 | 0.94 |

*4.4.5 Evidence for sexual selection*

There was no evidence for directional or stabilizing sexual selection on any of the traits measured, with standard errors exceeding the estimated selection gradients in most cases (Table 5).

Table 5. Estimates of selection gradients from simple linear ($\beta$) and second-order polynomial ($\gamma$) regression of relative mating success on male phenotypic traits

| Trait | $\beta \pm SE$ | $\gamma \pm SE$ |
| --- | --- | --- |
| Body length ex. rostrum | 0.02 ± 0.1 | 0.51 ± 0.48 |
| Pronotum width | 0.02 ± 0.1 | 0.67 ± 0.53 |
| Rostrum length | 0.03 ± 0.1 | 0.42 ± 0.42 |

Gradients are calculated from separate regression analyses of relative mating success on standaradised male traits because of the extremely high correlation between body size measurements. None of the selection gradients were significant at the $\alpha = 0.05$ level.

## 4.5 DISCUSSION

This study provides the first evidence that small male *L. barbicornis* employ sneaking behaviour as an alternative mating tactic to gain access to females for copulation. Although body size was a significant predicator of the mating tactic used (fighting vs. sneaking), it was also found that small males in addition to sneaking would readily guard females and engage in aggressive combat with other males. This is a fascinating and rare example of the use of highly context-dependent ARTs, where small males can readily switch between fighting and sneaking behaviour. The decision to sneak or fight was found to depend on the presence and size of a second male, with small males likely to guard females and challenge opponents if they are of similar or smaller size, but will employ sneaking tactics if the opponent is larger. The tactics presented here point to an example of ARTs set in a conditional strategy framework, with the decision to choose a tactic associated with status (in this case body size) (Gross 1996). Secondly, the observation that males are readily able to switch between tactics indicates that the use of a tactic is not determined by genotype (Gross 1996).

The use of ARTs by male *L. barbicornis* are less fixed by phenotype than in many other species, and shows that males are highly behaviourally plastic such that they can perform ARTs in a context-dependent manner in relation to momentary conditions. This ability to readily switch between tactics is rarely reported in animals, but has been found in several other species displaying exaggerated traits. Males of the staphylinid beetle *Leistorophus versicolor,* possess enlarged mandibles used as weapons, but some males (usually smaller) can also use female mimicry to avoid aggression (Forsyth & Alcock 1990). Also, small hornless male *Onthophagus taurus* and *Trypoxylus dichotomus septentrionalis* beetles will fight with other hornless males in

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addition to sneaking behaviour, although small *O. taurus* were never seen to defend territories (Moczek & Emlen 2000; Hongo 2003). The green tree frog (*Hyla cinerea*) has also been shown to be able to readily switch between calling and satellite tactics within a single night (Perrill et al. 1978) in association with body condition rather than size (Humfeld 2013). For *L. barbicornis* it was identified that the relative size of the opponent is a critical variable influencing the decision to sneak or fight, but there is likely to be other factors such as the local intensity of intrasexual competition and body condition that affect tactic choice (Taborsky 1998, Taborsky et al 2008). Although it is not known how *L. barbicornis* decide which tactic to use, the very strong correlation between choice of tactic and the relative size of the opponent suggests they directly assess rivals and can compare opponent size to their own.

The evolution of ARTs in *L. barbicornis* is not surprising when put into context with the intense competition between males in this species. I previously found that the operational sex ratio of the population is consistently male-biased throughout the breeding season (Chapter 2). Both male and female *L. barbicornis* were found to be highly promiscuous, mating multiple times with different individuals during hour long observations. Importantly, females were observed to copulate with multiple males while drilling, prior to oviposition, which suggests high levels of sperm competition in this species, as well as generally high levels of competition between males for access to females. When male-male competition for females is particularly intense, and especially in species where there is potential for a group of individuals to have a lower reproductive success than others, alternative reproductive tactics are expected to evolve (Gadgil

1972; Shuster & Wade 2003).
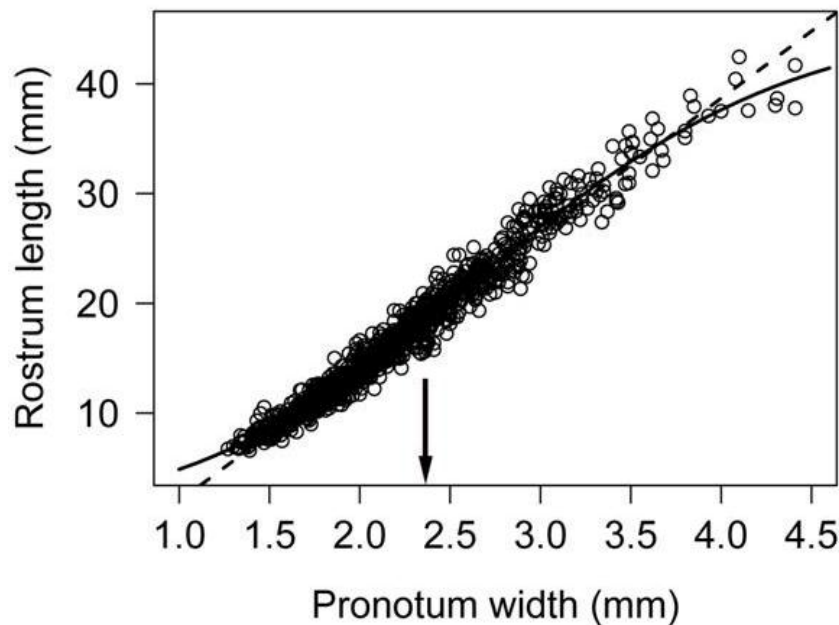


Figure 7. Scaling relationship of weapon (rostrum length) and body size (pronotum width) for male *L. barbicornis*. The solid line shows the Weibull growth curve function that best describes the scaling relationship (Chapter 3). The dashed line is the linear regression line of best fit. The arrow corresponds with an approximate body length of 40 mm (pronotum width 2.39mm), which is the point at which sneaking behaviour ceases to occur. The data is from 987 males measured at Matuku Reserve between November 2011 and March 2012.

I previously found no dimorphism in rostrum size for male *L. barbicornis*; instead the scaling relationship was best described by a non-linear Weibull growth function curve with an asymptote showing physiological constraint in rostrum size for the very largest males (Chapter 3, Figure 7). While many other species have been shown to employ ARTs in association with a dimorphism (Goldsmith 1987; Danforth 1991; Emlen 1997; Moczek & Emlen 2000; Hongo 2007; Okada et al. 2007; e.g. Buzatto et al. 2011; Zatz et al. 2011), this is not the case for giraffe weevils. However, the current study suggests a size limit of around 40 mm when males cease using

sneaking behaviour and which does not correspond with any change in rostrum investment (Figure 7). It is unclear what determines this shift in behavioural tactic, but sneaking behaviour could be simply limited to males that are able to easily fit under larger opponents without detection. Furthermore, the largest females found have been up to 50 mm in length (Chapter 2), which roughly corresponds to the largest males using sneaking behaviour. Therefore sneaking could be limited to males able to fit under guarding males during sneaking attempts, such that they go unnoticed or are mistaken for females by the defenders. Female mimicry is not particularly common as an alternative mating tactic in insects, but it has been reported in several species (Thornhill 1979; Wendelken & Barth 1985; Peschke 1987; Forsyth & Alcock 1990), and is well-known in other taxa, such as fish (Taborsky 1994), cuttlefish (Norman et al. 1999), marine isopods (Shuster 1987; Shuster & Wade 1991), and birds (Slagsvold & Saetre 1991). Male *L. barbicornis* were commonly seen to guard or attempt copulation with other males during our observations, and this was particularly common when large males guarded a female already copulating with another male (C. J. Painting pers. observations). This further supports the possibility that sneakers are often mistaken to be females.

The lack of dimorphism associated with ARTs in the giraffe weevil could be due to the type of weaponry used during aggressive encounters. The rostrum is an extension of the head, with mouthparts located at the anterior end of long rostrum. While the rostrum and mandibles are important as weapons, the mouthparts are also important for feeding, with both males and females regularly observed to bury their mouthparts into sap dripping out of injured trees (Appendix 1). The use of the rostrum for feeding should therefore limit the potential for rostrum development to be lost entirely from small males, although a shorter, non-elongated rostrum should be possible if the presence of rostra in small males was due to feeding benefits alone. The

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lack of dimorphism in the giraffe weevils therefore suggests that (1) bearing an elongated rostrum does not present a selective disadvantage for sneaking males (i.e. it does not make them more detectible to guarding males, therefore decreasing sneaking success), and (2) that it can be a useful and successful weapon used in fighting behaviour by even small males in some contexts (i.e. when small males fight with other small males).  Rostrum evolution in *L. barbicornis* can be compared to other armed insect groups such as the *Onthophagus* dung beetles, which have evolved horns used in male-male contests. Horns in these beetles are extensions from the head or thorax that are not imperative structures used for other aspects of survival, potentially allowing them to be more easily selected upon under sexual selection than a giraffe weevil rostrum. Furthermore, male dimorphism in these species has likely evolved because ARTs favour opposite phenotypes for small and large males (Moczek & Emlen 2000). In *Onthophagus taurus* and *O. nigriventris*, horns in large males are advantageous during fights, but the lack of horns in small males is advantageous in this group because long horns would limit movement during sneaking behaviour in dung tunnels (Moczek & Emlen 2000; Madewell & Moczek 2006). If intermediate sized-males performed poorly at both behaviours, selection against genotypes that express intermediate phenotypes would occur, such that eventually intermediate-sized weaponry is lost, and a dimorphism in weapon size remains.

The relative reproductive fitness of males using ARTs has been rarely determined for most species where ARTs have been identified (Schradin & Lindholm 2011). This is surprising given its critical importance for explaining how ARTs evolve and are maintained in populations. Under the model of status-dependent selection (Gross 1996) the male with the inferior phenotype is said to have a lower mating success than the dominant phenotype, and because all males are genetically identical in how they express the conditional strategy, equal fitness is not required to

maintain the alternative strategies in the population. Under the status-dependent model sneaking male giraffe weevils are expected to have a lower mating success than large males. However, Shuster and Wade (2003) claim that previous studies measuring mating success of male phenotypes possibly overestimated the success of the dominant phenotype. Instead they hypothesise that a second group of males (β-type) can invade a population when there is high variation in mating success due to a small number of males dominating the majority of females. In this situation β-type males must have higher fitness than the average original (α-type) male, which is possible when there are many α-type males unable to gain access to females. Overall, the fitness of the invading alternative strategy is not inferior to the original dominant strategy, but it can be lower than the fitness of the most successful males from the dominant strategy. However, problems with Shuster and Wade's (2003) critique of status-dependent selection have been raised, particularly because they model alternative strategies where the phenotypes are genetically rather than conditionally determined (Tomkins & Hazel 2007), and these issues currently remain unresolved. Despite these complications in fitness predictions, the mating success of *L. barbicornis* did not vary between males of different sizes employing different behavioural strategies. Both mating frequency and total time spent copulating during one hour observations showed no correlation to body size, suggesting that at least in terms of mating success, small males are just as successful as large males.

There was no directional or stabilizing sexual selection on overall body size or rostrum length in male giraffe weevils. This is surprising given the high potential for sexual selection on rostrum length to increase reproductive success in this highly polygamous and male-biased species with extreme sexual dimorphism. There are several possible reasons for the lack of evidence for sexual selection on male traits in this species. Firstly, the use of mating success as a proxy for

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fitness differences between male sizes must be used cautiously, because measuring copulation frequency and total copulation duration alone can underestimate the reproductive success of some males if they are employing cryptic adaptations such as increased ejaculate load or enhanced sperm quality (Simmons et al. 2004). If mating success is not a true measure of reproductive success, and therefore a suitable index of fitness, then this could be a plausible explanation for the lack of evidence for sexual selection on body size or rostrum length for *L. barbicornis.* To fully understand size-related reproductive success in *L. barbicornis* future studies should determine the influence of sperm competition and sperm precedence, assess evidence for cryptic female choice, and incorporate paternity analyses.  Relative reproductive success of males across the size spectrum could also be density-dependent (Conner 1989; Rittschof 2010), or frequency-dependent on the proportion of small sneaker males in the population (Gross 1985; Parker 1990; Repka & Gross 1995). As Schradin and Lindholm (2011) recently showed in African striped mice (*Rhabdomys pumilio*), population density and sex ratio can have a significant effect on the relative fitness of males using different tactics, highlighting the importance of environmental conditions in determining male success. Furthermore, if adult life-span varies between males due to size-biased predation, parasitism, or mortality then life-time reproductive success could be higher in the male phenotype with the longer life-span (Tsubaki & Ono 1987; Tsubaki et al. 1997; Setsuda et al. 1999; Tsubaki & Hooper 2004).

Further evidence to support the importance of using a more comprehensive approach to measuring the average fitness of male morphs was demonstrated in *Onthophagus* beetles. Mating and reproductive success have rarely been determined in studies of alternative tactics under a conditional strategy, possibly because it is an often difficult task to accurately measure the average success of each male phenotype. However, the relative fitness of major and minor

morphs of *Onthophagus taurus* beetles has been determined in several studies (Tomkins & Simmons 2000; Hunt & Simmons 2001; Hunt & Simmons 2002; Simmons et al. 2004). When given equal access to females there is no difference in the competitive fertilization ability of minor and major males, and sperm competition between minor and major males is therefore equal and conforms to random mixing (Tomkins & Simmons 2000). However, when taking mating success into account in addition to fertilisation success, Hunt & Simmons (2002) were able to show that major males have higher fitness than minor males, because they are able to engage in retaliatory copulations with females after discovering sneaks in their tunnels which subsequently protects their paternity. Giraffe weevil males are easily observed in the wild, and the approach of choosing random focal males and watching them for a full hour (even when some individuals spent most of their time hiding) ensured an accurate measure of mating success for all males, not just those that were seen mating. However, this does not necessarily mean that total mating success is a good measure of reproductive success if large males are able to perform retaliatory copulations after encountering sneakers, therefore increasing their chance of paternity.

In species where there are both guarding and sneaker morphs, the sneakers are expected to suffer higher levels of sperm competition than guarding males, although this is dependent on the proportion of sneaks in the population (Parker 1990). This increased risk of sperm competition has resulted in sneakers or small males of some species evolving larger ejaculates or sperm of higher fertilization success (Simmons et al. 1999; Fu et al. 2001; Vladić 2001; Kelly 2008). In a previous study I found no evidence that small *L. barbicornis* males increase relative testis size to compensate for any hypothetical reduction in reproductive success (Chapter 3), and this result coupled with the current finding that small males mate just as often and for as long as large males suggests that sneaking behaviour and the ability for small males to be flexible in their

mating tactics has allowed them to be as successful as their larger counterparts. These flexible

tactics could therefore weaken sexual selection on body size and rostrum length by reducing the

importance of these traits in determining an individual's fitness (Sousa & Westneat 2013).

There is also the possibility that the strength of sexual selection fluctuates over time, such that

we measured fitness during a year of weak selection. Variation in the strength of selection on

male traits over time has been identified in several species of birds, suggesting that sexual

dimorphism in traits can persist for long periods of time after strong sexual selection has reduced

(Westneat 2006; Chain & Lyon 2008; Sousa & Westneat 2013). This could be a possibility for *L.

barbicornis*, although another experiment that measured mating success of males across a

subsequent breeding season found similar results with no evidence of variation in mating success

in relation to body or rostrum size (C. J. Painting unpublished data).

Finally, it is important to follow up this study with multivariate non-linear selection analyses

using surface response methodology (for examples see Phillips & Arnold 1989; Blows & Brooks

2003; Blows et al. 2003; Hall et al. 2008; Wojcieszek & Simmons 2011; Wheeler et al. 2012).

Frequently, the use of standard regression techniques in selection analyses can underestimate the

presence and strength of selection on traits (Blows & Brooks 2003). For example, Wojcieszek &

Simmons (2011) did not find evidence of selection on the genital morphology of millipedes

using standard multivariate regression analyses. However, by using a canonical analysis of the

matrices of nonlinear selection, which increases the ability to detect nonlinear selection on a set

of traits, they were able to find significant stabilizing and disruptive selection acting on genital

morphology. A multivariate non-linear selection analysis is an important next step in

determining sexual selection on rostrum length in male giraffe weevils. These analyses could

help to identify whether the strength and direction of selection varies in relation to the

importance of relative rostrum length at different body sizes. For example, there may be negative selection on rostrum length in small males to avoid detection during sneaking, but positive selection for increased rostrum length in large males where a small increase in this trait could increase fighting ability.

This study, and several previous studies (Johnson 1982, 1983; Johnson & Hubbell 1984), show that for those brentid species that have received any attention, sexual selection acts on males to drive the evolution of elaborate weaponry and complex behaviour to cope with intense competition for mates. *L. barbicornis* displays parallels in sneaking behaviour to other brentids, particularly *Claeoderes bivittata* where small males also use tucking and hiding behaviour to avoid aggression by larger males, and enjoy similar mating success to large males (Johnson 1983). In comparison, sneaking behaviour was not alluded to in studies of *Brentus anchorago*, and in this species large males were more likely to be found copulating than small males (Johnson 1982; Johnson & Hubbell 1984). Comparison of these three species (*L. barbicornis, C. bivittata, B. anchorago*) and their relative mating success across size classes suggests that sneaking behaviour has allowed small males to increase mating success and avoid any disadvantage in the rate of access to females. Brentids are therefore an important group to explore questions about the evolution and maintenance of weaponry and ARTs, particularly as they are easily observed in the wild in high abundance. This study of the New Zealand giraffe weevil provides the first detailed insight into the mating behaviour of this highly size variable species with an exaggerated secondary sexual trait, and could be used in the future as part of a comparative analysis of weapon and ART evolution among the Brentidae.

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# CHAPTER 5

# Fighting behaviour and the competitive assessment strategy of male giraffe weevils

**5.1 ABSTRACT**

Fighting between males is a widespread behaviour among animals, used as a means to defend access to resources or females, and subsequently increase reproductive success. During contests, males may mutually assess the relative differences between their own and their opponent's resource holding potential (RHP). Alternatively, males may simply persist in a contest according to an individual threshold corresponding to their own RHP (self-assessment). Game theory models such as the sequential assessment model (SAM), energetic war of attrition (eWoA), and the cumulative assessment model (CAM) have been developed to put assessment strategy in an evolutionary framework. Distinguishing between these models is best done by documenting the contest structure and by testing predictions of the relationship between the size of the contestants and contest duration. This study determined the escalating fighting behaviour and competitive assessment strategy of the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*). Aggressive fighting occurred frequently between males of all sizes, and both weapon size (rostrum length) and body size were important predictors of contest outcome. Males were found to most likely use mutual assessment under the sequential assessment model during contests because males of all sizes escalated contests to the highest degree and there was no relationship between grappling duration and mean paired rival size, although intense fights only occurred between the most closely matched individuals. Contests were also found to escalate, but not de-escalate, behavioural matching did not occur during all phases, and there was a negative relationship between contest duration and the size of the larger male in the contest. Overall this study shows the importance of the male rostrum as a weapon, and that it has likely evolved under direct sexual selection to increase male reproductive success.

**5.2 INTRODUCTION**

Fighting among males when competing for access to females is common across diverse animal taxa, and in many species high levels of competition has driven the evolution of weaponry through sexual selection. Contests between males are usually won by the individual that has the highest resource-holding potential, which is a measure of the ability of an animal to win a fight (RHP, Parker 1974), most commonly defined by body or weapon size. The ability of an individual to win a contest can however, also be determined by age (Tsubaki & Ono 1987; Kemp 2003), ownership (Kemp & Wicklund 2001; Kasumovic et al. 2011), previous experience (Kemp & Wicklund 2004; Stuart-Fox & Johnston 2005; Hsu et al. 2006), or physiological state (e.g. warmer *Pararge aegeria* butterflies are more likely to win contests; Stutt & Willmer 1998).

The ability of an individual to resolve a contest quickly based on an assessment of one's own RHP compared to their opponent is important to prevent unnecessarily long and costly contests, particularly between distantly matched individuals, and is the basis of 'war of attrition' (WoA; Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Hammerstein & Parker 1982) and 'sequential assessment' models (SAM; Enquist & Leimar 1983; Leimar & Enquist 1984; Enquist et al. 1990). These models assume that initially, an individual has limited information about its opponent's ability, but during the contest gains more information about the opponent's ability and adjusts their strategy accordingly. Opponents are therefore assumed to use mutual assessment to determine any asymmetry in RHP, and if an interaction escalates to physical combat, they are expected to fight until one contestant is able to assess that it cannot win and will terminate the interaction (Enquist & Leimar 1983; Leimar & Enquist 1984; Enquist et al. 1990). More recently however, game theory models have been developed to account for species where persistence during contests is determined by an individual's own RHP only, described by

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'energetic WoA' (eWOA; Mesterton-Gibbons et al. 1996; Payne & Pagel 1996, 1997), and 'cumulative assessment' (CAM; Payne 1998) models. Rivals under these models may lack the sensory ability or experience to accurately assess rivals, or assessment may be too costly (Arnott & Elwood 2008). Consequently under self-assessment, contest duration is determined by individual thresholds rather than comparative assessment of the rival, where the weaker individual will reach its limit first, ending the fight (Mesterton-Gibbons et al. 1996; Payne & Pagel 1996; Arnott & Elwood 2009). Although both the energetic war of assessment and cumulative assessment models are based on self-assessment, under the cumulative assessment model the individual's persistence is affected by their rival because stronger rivals inflict higher costs (including injury) during physical contact (Payne 1998).Recently it has also been shown that some species are able to switch assessment strategies throughout different stages in a contest (e.g. Morrell et al. 2005; Hsu et al. 2008; Yasuda et al. 2012)

Assessment strategies were traditionally investigated by determining the relationship between RHP difference and contest duration (or another measure of cost), and a negative relationship between these factors was interpreted as support for mutual assessment between rivals. However, Taylor and Elwood (2003) presented evidence to show that focussing only on RHP difference does not necessarily provide evidence towards mutual assessment. Instead, distinguishing between self and mutual assessment strategies requires the comparison of linear regression models looking at the direction of the relationship between contest duration and the size of the loser (or smaller rival if contest outcome is determined by size; Table 1). Specifically, under self-assessment, duration should correlate positively with both the smaller and larger male size, although the slope for larger male size will be weaker. Under mutual assessment correlation coefficients should be positive for the relationship between contest duration and the size of the

smaller male, but negative with approximately the same magnitude for the size of the larger male.

Studies published since Taylor and Elwood (2003), have had mixed success in distinguishing between self and mutual assessment, with some studies showing no or only weak evidence for correlation between the size of the smaller male and contest duration (e.g. Jennings et al. 2004; Reichert & Gerhardt 2011; Peixoto & Benson 2012). Furthermore, using contest duration in relation to smaller and larger male size does not distinguish between sequential assessment models and cumulative assessment models because both predict the same correlations despite rivals using different decision-making processes during contests (Table 1; Briffa & Elwood 2009). It is therefore useful when determining assessment strategy to look at both the relationship between contest duration and rival size, and other predictions about each model such as the contest structure and intensity (Table 1; Briffa & Elwood 2009). Specifically, the sequential assessment model predicts that contest escalation is only possible between phases and that contest intensity remains constant within phases. The cumulative assessment model, however, allows for the possibility of escalation and de-escalation of intensity within contest phases (Briffa & Elwood 2009). Furthermore, when contests are staged between size-matched opponents it is expected that if persistence is related only to an individual's own RHP (self-assessment) then contests should be longer between strong individuals (Table 1). However, there should be no relationship between contest duration and the mean RHP of matched individuals if mutual assessment is used (Table 1; Taylor & Elwood 2003; Arnott & Elwood 2008).

Table 1. Characteristics and predictions of three models describing contest dynamics in animals*

| Prediction of model | Energetic war of attrition (eWOA) | Cumulative assessment model (CAM) | Sequential assessment model (SAM) |
|---|---|---|---|
| Assessment type | Self | Self | Mutual |
| Decision based on: | Own strength and ability, and costs from own actions | Own strength and ability, and costs inflicted by rival | Information about the rival in relation to self |
| Contest pattern | Escalation with de-escalation possible within phases | Escalation with de-escalation possible within phases | Escalation only between phases |
| Behavioural matching? | Yes | No | No |
| Relation of contest duration & smaller (loser) male | Strong positive | Positive | Positive |
| Relation of contest duration & larger (winner) male | Weak positive | Negative | Negative |
| Relationship between contest duration & average size of matched opponents | Positive | Positive | No relation |

* modified from Arnott & Elwood (2008) and Briffa and Elwood (2009)

Brentid weevils (Coleoptera) are a diverse beetle family, known to engage in aggressive contests. Some species display high levels of sexual dimorphism, mostly owing to the evolution of exaggerated secondary sexual traits in males (Sforzi & Bartolozzi 2004). In particular, males of many species have evolved an elongated rostrum and/or enlarged mandibles, both of which have been shown to be important traits used in contest resolution (Johnson 1982, 1983; Sanborne 1983). The giraffe weevil *Lasiorhynchus barbicornis* is an endemic brentid weevil from New

Zealand, and exhibits both pronounced sexual dimorphism, and extreme size variation in both sexes (males range from 15 - 90 mm in body length; Kuschel 2003; Chapter 2). Sexual dimorphism is characterised by striking differences in rostrum morphology. The female rostrum is relatively short, straight and narrow. The male rostrum, by comparison is extremely elongated to make up around half its total body length, and the mandibles on the end of the rostrum are enlarged in comparison to females (Kuschel 2003). During warmer months large aggregations of adult giraffe weevils can be found on dead or dying trees in forested areas, where females drill in wood to excavate holes for oviposition (Meads 1976). While females drill, males copulate with or guard them, and aggressive fights occur when intruding males attempt to compete for access to the female. Males use their elongated rostrum and mandibles as a weapon during fights, by attempting to dislodge an opponent through raking their rostrum along their opponents body, and biting them. To date there has been no in-depth analysis of fighting behaviour or the importance of the elongated rostrum in this species, despite its likely significance in determining the relative reproductive success of males.

Often, the observation of fighting and assessment behaviour of insects has been conducted in controlled conditions using staged encounters between two individuals. While these studies have been essential in shaping our current understanding of fighting and assessment, it is also important to conduct observations in the wild where possible (for examples see: Kemp 2003; Pratt et al. 2003; Jennings et al. 2004; Jennings et al. 2005; Morrell et al. 2005; Stapley & Whiting 2006; Jennings et al. 2012; Peixoto & Benson 2012; Umbers et al. 2012), especially to document natural behaviours for a previously unstudied organism. Giraffe weevils are easily observed in large numbers in their natural environment, and therefore provide a rare chance to observe fighting behaviour in a wild insect population.

In this study I test a combination of predictions from game theory models to determine how males make decisions that influence contest duration and outcome. First, I describe the types of fighting behaviour used by male *L. barbicornis* and then document fight frequency and duration. I test whether differences in RHP (body and weapon size) and ownership (guarding) influence fighting success. The type of assessment strategy employed by males is assessed by first testing whether the probability of a fight escalating correlated with the size of the focal male and the size difference between the focal and opponent weevils. I then determine the relationship between contest duration and RHP difference and the absolute size of the smaller and larger rivals following predictions from Taylor and Elwood (2003). Under mutual assessment contest duration should be positively correlated with smaller male size, but negatively with larger male size and RHP difference. Alternatively if males use a self-assessment strategy, both smaller and larger male size should positively correlate with duration, with the slope for largest males in each interaction less steep than for the smaller males.

## 5.3 METHODS

### 5.3.1 Study site and population

Field observations were conducted at Matuku Reserve (36° 51.92'S, 174°28.32'E), west of Auckland in New Zealand. The reserve is privately owned by the Royal Forest and Bird Protection Society, and is 120 hectares of predominantly coastal broadleaf and podocarp forest. Giraffe weevils are particularly abundant at Matuku Reserve, therefore making it an ideal site for a field-based observational study. Adult *L. barbicornis* are found from October to May, but are most abundant from January to March (Chapter 2). During these summer months adults aggregate on dead or dying trees where females select sites to drill and lay eggs while males

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copulate and stand guard. Nine dying but standing karaka trees (*Corynocarpus laevigatus*) that hosted aggregations of adult giraffe weevils were identified. Trees were chosen when the majority of weevils were aggregated from ground to eye height to ensure weevil behaviour could continuously and accurately be observed.

*5.3.2 Field observations*

The agonistic behavioural repertoire of male *L. barbicornis* was first determined by conducting pilot observations at Matuku Reserve and integrating these behaviours into an ethogram which was used in the subsequent observations (Table 2). Between 11th January and 18th February 2011 focal observations of 79 male giraffe weevils were conducted. Observations were made between 0800 and 1800 on the nine chosen trees, avoiding days of heavy rain when giraffe weevils hide. A focal male was randomly chosen and all interactions and fighting behaviour with other males were recorded for a one hour period. To accurately gauge fighting behaviour across the full size range of males present on trees, we observed animals from 18.29 – 89.75 mm in total body length. An interaction was timed from when one male physically contacted another male, and the interaction was deemed finished when one male retreated to more than 2 cm from the opponent and did not approach again within 10 seconds. The loser was recorded as the male that retreated away from its opponent, or was dislodged from the tree. If both retreated or there was no obvious winner this interaction was excluded from the analyses. As all behaviours were being recorded at the time of observation, precise timing of very brief aggressive interactions was difficult. If a male simply approached an opponent and was immediately pushed away this was counted as a two-second interaction and this was consistent throughout the analyses. All other longer interactions were timed to the nearest second.

To ensure each weevil present during the observations could be identified, all weevils were marked with Queen Bee Marking Paint (Lega, Italy) using a unique combination of colours that coded to a number (Appendix 1). Before observations begun any unmarked weevils were removed, body size traits measured, marked, and then released back to their host tree. After release no observations were conducted on that tree for at least one hour, after which it was found that most newly marked individuals would return to normal activity (walking, mating, fighting, etc). Measurements were: total body length minus rostrum length (BL), rostrum length (RL; total length from base of head to tip of mandibles) and pronotom width (PW; widest section of pronotum).

*5.3.3 Statistical analysis*

All analyses were conducted in R 2.15 (R Core Team 2013). Generalised linear mixed effects models (GLMM) were employed using the *lme4* package in R (Bates et al. 2012). Where relevant, Akaike's information criteria and model selection theory (Akaike 1973; Burnham & Anderson 2002) was used to select a single model that best explained the variance in each response variable throughout the analyses. The fit of models were tested with different combinations of explanatory variables (no interaction terms fitted), and the best model was selected according to the smallest AIC value. AIC is calculated as [2x (-log likelihood) + 2x (no. parameters in the model)].

I first tested whether the number of aggressive interactions that each focal male was involved in during hour-long observations was correlated with body size and the abundance of weevils on the tree by fitting a generalised linear model (GLM) with a Poisson distribution. The number of fights was the response variable and focal body length and the total number of weevils present on

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the tree were explanatory variables. The model was refitted to correct the standard errors using a quasi-GLM model due to overdispersion of the data, where the variance is given by the dispersion parameter ($\varphi = 3.48$) multiplied by the mean ($\mu$) (Zuur et al. 2009).

To analyse the effect of body and weapon size on fight outcome, a GLMM with focal weevil and observation tree as random effects was used. The mixed model takes into account the non-independence in the data because multiple male-male interactions were recorded during the hour-long focal observations, and observations of weevils were conducted multiple times on the same tree. The three body size measures were highly correlated (Pearson's correlation coefficients: BL-RL = 0.99, BL-PW= 0.99, RL-PW= 0.99), so to avoid problems in the models with collinearity models were run separately for each body size measure. The explanatory variables in the models were therefore a measure of difference in size between focal and opponent ($BL_{F-O}$, $RL_{F-O}$, $PW_{F-O}$) and the intruder status of the focal weevil (I = intruder, D = defender), while the response variable was a binomial measure of whether the focal weevil won or lost the fight.  AIC was then used to select a single model that best explained fight outcome in relation to size difference and focal intruder status.

To analyse the assessment strategy used by males during contests I first determined the probability of fights escalating from stage one/two aggression (touching/pushing/raking) to stage three aggression (grappling) in relation to focal body size and the absolute size difference between the two rivals. This was a similar approach as above, using GLMM to account for multiple fights conducted by the focal weevil, with fight escalation as a binary variable (0 = stage 1&2 aggression only, 1 = escalates to grappling).

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I then attempted to determine the relationship between contest duration and male size using linear regression following procedures outlined in Taylor & Elwood (2003). However, the data were severely inflated by an overabundance of very brief two second bouts (Figure 1), and no standard transformations normalised the data residuals. Standard linear regression normally used to test assessment theory was therefore inappropriate for our data. Instead all the two second interactions were first removed and linear regression models were conducted with log transformed duration in relation to the absolute body length difference between males, smaller male body length, and larger male body length. I then conducted a further analysis treating contest duration as a binomial response variable with all two-second fights in one group (0) and all longer durations as another group (1). I ran GLMMs with focal weevil identity and observation tree as random effects to investigate assessment strategy. The explanatory variable for each model was (1) absolute size difference between opponents, (2) the size of the smallest male in the contest, and (3) the size of the larger male.

Finally, to further determine between self and mutual assessment models I performed a linear regression model to determine the relationship between the average size of each pair of fighting weevils and the duration of the grappling stage of the contest. Self-assessment models such as the cumulative assessment and energetic war of attrition models predict that strong, evenly matched individuals should fight for longer than weak, RHP-matched individuals (Arnott & Elwood 2008). If individuals fight using self-assessment it is expected that there should be a positive relationship between fight duration and the average size of matched individuals, but no relationship between these variables under mutual assessment models.

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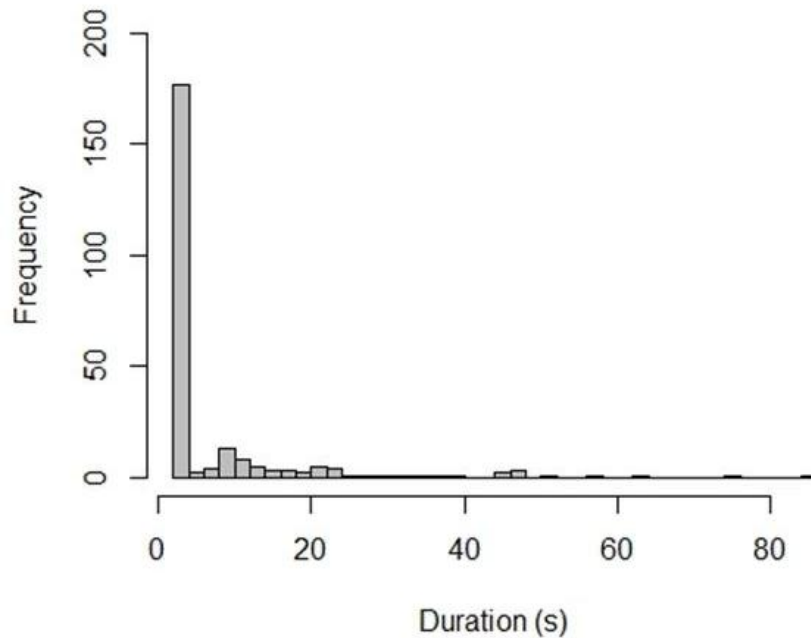
Figure 1. Frequency distribution of contest duration. Of the 244 contests, 174 (71%) were two-second bouts.

## 5.4 RESULTS

### 5.4.1 Description of fighting behaviour

Fights between males usually occurred when a female was present and represented an attempt by an intruder male to usurp a guarding male. Three escalating stages of conflict were identified between males (Table 2, Figure 2). The lowest level of conflict (stage one) occurred when an intruder male first approached a guarding male and female. When the intruder touched the guarding male with his rostrum the guarding male waggled his antennae and kicked his legs (Figure 3A). Although some interactions were resolved at the touching stage, conflict was most commonly resolved when one or both of the rivals attempted to usurp the other by using their elongated rostrum and mandibles to bite and rake at the other male (stage two), in an attempt to

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dislodge the male from the female (Figure 2&3B, Video 1, see attached CD). Unresolved

conflicts at this point escalated to grappling, where males met together side by side and then

attempted to dislodge the opponent completely from the tree (stage three), although we did not

witness any males being thrown from trees during our focal observations (Figure 2&3C, Video 2,

see attached CD). Grappling also occurred immediately after an intruder approached and touched

a resident male without undertaking the raking stage, although I did not witness any de-

escalation of conflict where males would scale back to pushing and raking after a grapple bout

(Figure 2).

Table 2. Ethogram of aggressive behaviours observed between male *L. barbicornis*

| Behaviour | Description |
|---|---|
| Approach opponent | Intruder male approaches opponent |
| **Stage One Aggression** | |
| Physical contact | Intruder touches guarding male with rostrum and mandibles |
| Waggling & kicking | Defending male waggles antennae and kicks legs out at challenger. If not facing the intruder the defending male will also turn head side to side as if to look at approaching male |
| **Stage Two Aggression** | |
| Pushing & raking | Either intruder or resident male will use rostrum like a lever or rake to pull and remove opponent. While raking, biting of a leg or antennae often occurs, also as a means to dislodge opponent. |
| **Stage Three Aggression** | |
| Grappling | Both intruder and resident enter conflict equally by meeting side-to-side. Each male's rostrum is wedged under opponent's legs. The grapple ensues as each combatant attempts to free their rostrum, bite onto opponent's legs, antennae, or elytra tip. |
| Pick up/throw | Grapples are either terminated by loser retreating by choice, or being thrown off tree by winner. Loser is picked up in mandibles by winner and thrown off tree. |
| **Conflict resolution** | |
| Retreat | One male walks away from opponent |
| Conflict terminates | Loser is at least 2 cm away from opponent and has not approached opponent within 10 seconds |
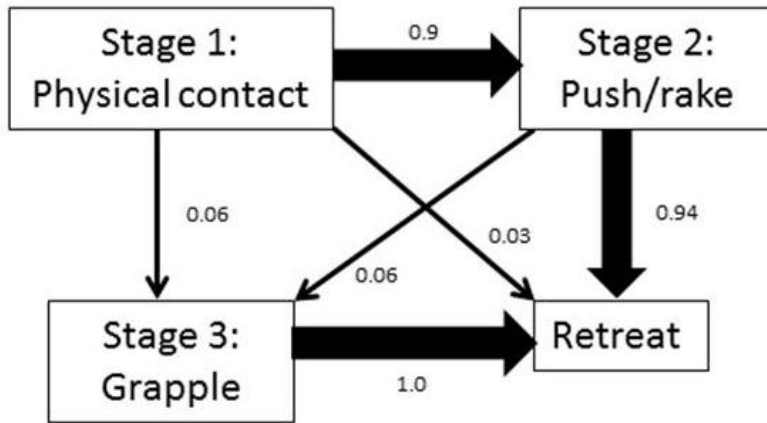
Figure 2. Kinematic ethogram showing structure of fighting behaviour. The arrow thickness denotes the probability that one behaviour follows another as calculated from a transition matrix.



Figure 3. (A) Challenger male is kicked with back legs by guarding male (female drilling), (B) Male on left attempts to pull middle male away from log by using his rostrum as a rake and biting onto the opponent's legs, (C) two males locked in grapple position.

*5.4.2 Fight duration and intensity*

Out of the 79 focal males observed, 52 (66%) males were involved in aggressive encounters during the one hour period. Males across the full size spectrum were observed to challenge opponents aggressively, with the smallest aggressor 18.29 mm in length, and the largest 89.75 mm. The mean ± SE number of aggressive interactions per one hour observation was 3.34 (±0.53) fights, with a range of 0 - 31 fights per male. Of the 244 aggressive encounters that were observed (where a winner and loser could be determined), the most frequent type of interaction was raking or biting (206 observations, 84.4%) followed by grappling (27 observations, 11.1%). A small percentage of male interactions were concluded after one male made physical contact only (9 observations, 3.7%). The total mean duration ± SE of agonistic interactions between males was 7.54 s (± 0.82).

The number of aggressive interactions that each focal male participated in was significantly correlated with total abundance of weevils present on the tree during observations and focal male body size. However, the removal of an extreme outlier that had significant leverage in the model resulted in body size no longer being a significant predictor of fight frequency. The minimal adequate model therefore included only total abundance as a significant explanatory variable, excluding the outlier (Figure 4, $\beta = 0.04$, $p < 0.0001$). Total abundance explained 17.24% of the variation in the number of fights, which was the explained deviance in the model.
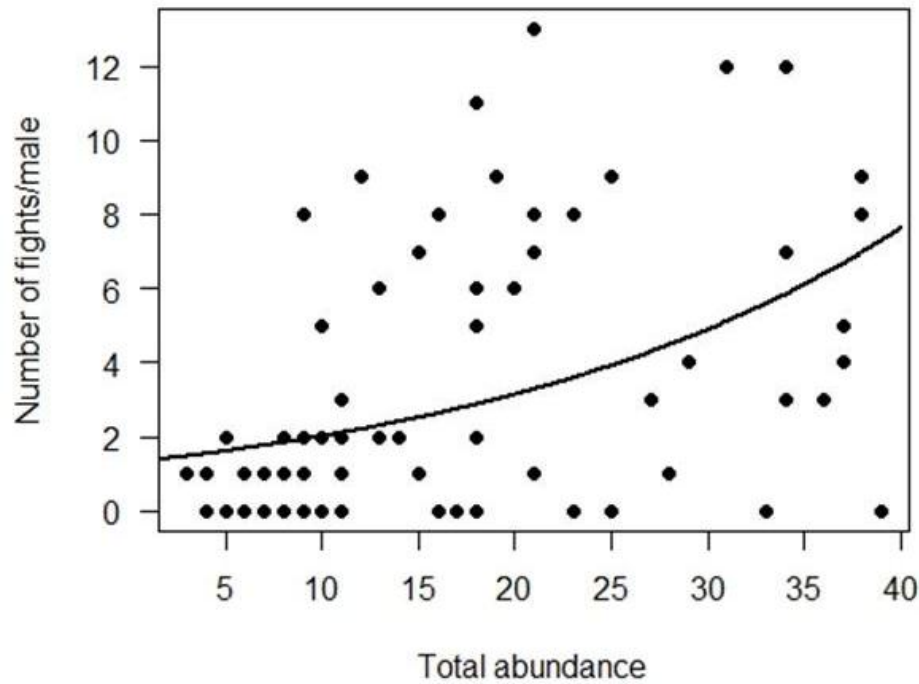
Figure 4. The number of fights per male increases with the total number of individuals present on the tree during the observation period. An extreme outlier was removed due to high leverage on the model estimates, and is not presented in the figure here (N = 78).

### 5.4.3 Size-related fighting success

Size asymmetry was an important factor determining fight outcome in contests, but focal intruder status (ownership) did not add any explanatory power to the models (Table 3). From 240 agonistic interactions, 90% of fights were won by the larger male. Using AIC, the best model to explain fight outcome included only the difference in rostrum length between the focal and opponent weevils (Table 4, Figure 5). The inflection point at which winning and losing had a probability of 50% centred at a point where the rostrum length of opponents was equal, indicating that the larger opponent was most likely to win the fight. Although the focal intruder status was not an important factor affecting fight outcome when included in a model with size

asymmetry, resident males won 62.8% (103 wins) of contests, significantly more wins than intruders (binomial test: N = 164, $p$ =0.001). The mean size of the focal male when it was a challenger was significantly smaller than focal males that were defenders (mean body length intruder ± SE = 40.65 ± 2.01; mean body length defender ± SE = 48.71 ± 1.9, Wilcoxon rank-sum test: W= 5854, $p$ = 0.02).

Table 3. Multi-model selection using Akaike's information criterion (AIC) of most important factors determining fight outcome

| Model | k | AICc | ΔAICc |
|---|---|---|---|
| $RL_{F-O}$ | 4 | 145.29 | 0 |
| $RL_{F-O}$ + Focal role$_{I/D}$ | 5 | 147.27 | 1.98 |
| $PW_{F-O}$ | 4 | 147.58 | 2.29 |
| $PW_{F-O}$ + Focal role$_{I/D}$ | 5 | 149.35 | 4.07 |
| $BL_{F-O}$ | 4 | 150.78 | 5.49 |
| $BL_{F-O}$ + Focal role$_{I/D}$ | 5 | 152.70 | 7.41 |

Explanatory variables were differences in RL (rostrum length), PW (pronotum width), and BL (body length minus rostrum) between focal and opponent weevils, and focal intruder status (Intruder or Defender).

Table 4. Estimates for the best GLMM model to explain fight outcome in *L. barbicornis*. $RL_{F-O}$ was the difference in rostrum length between the focal and opponent.

| | Estimate | SE | Z | $p$ |
|---|---|---|---|---|
| Intercept | -0.49 | 0.45 | -1.08 | 0.28 |
| $RL_{F-O}$ | 0.33 | 0.05 | 6.89 | <0.0001 |
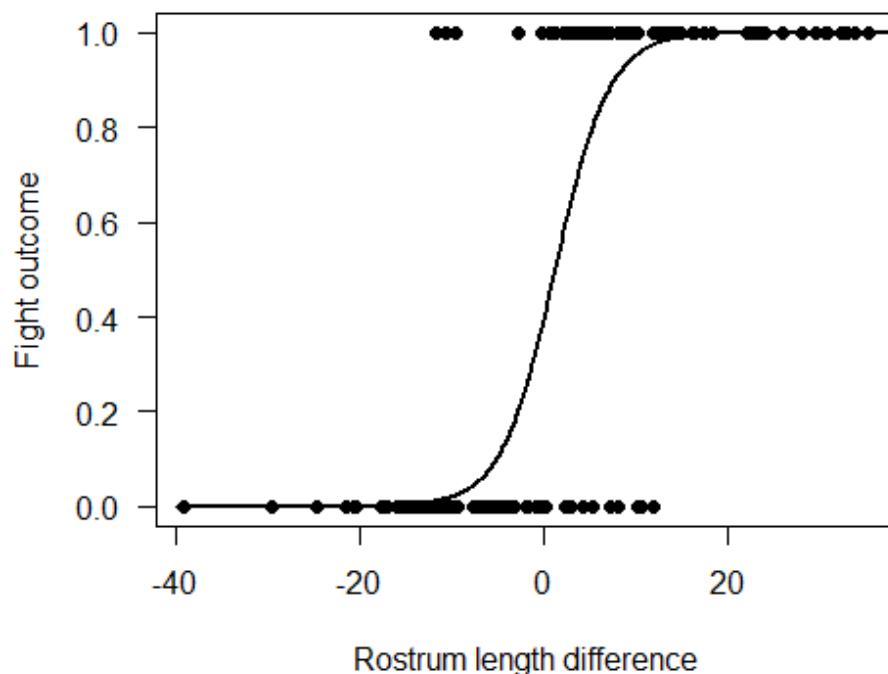
Figure 5. Fight outcome in relation to the difference in rostrum length between opponent weevils ($RL_{F-O}$). Positive values in rostrum size difference indicate that the focal male had a longer rostrum than the opponent; negative values indicate that the focal male had a smaller rostrum than the opponent. A fight outcome of 1 = focal male wins, 0 = focal male lost.

### 5.4.4 Assessment strategy

The models used to determine whether the probability of fight escalation was related to focal size (BL, RL, PW) or the difference in size between the males ($BL_{F-O}$, $RL_{F-O}$, $PW_{F-O}$) were compared using AIC (Table 5). Fights were more likely to escalate to grappling when males similar in size interacted, and all three models including only body size differences had very similar AIC values. Including the size of the focal male did not add explanatory power to the models, and the models where only focal male size was included ranked lowest in the comparison table (Table 5). Importantly these results show that males across the size spectrum will escalate fights to

grappling, such that these fights do not only occur between large males (Figure 6A). Here only

the estimates from the model with pronotum width difference are presented, as although the three

body traits had a very similar AIC, $PW_{F-O}$ was marginally lower than the other models (Table 6,

Figure 6B).

Table 5. Multi-model selection using Akaike's information criterion (AIC) of most important factors

determining whether fights escalate between male *L. barbicornis*.

| Model | k | AICc | ΔAICc |
|---|---|---|---|
| $PW_{F-O}$ | 4 | 163.74 | 0 |
| $RL_{F-O}$ | 4 | 164.00 | 0.26 |
| $BL_{F-O}$ | 4 | 165.06 | 1.32 |
| $PW_{F-O}$ + Focal PW | 5 | 165.14 | 1.40 |
| $RL_{F-O}$ + Focal RL | 5 | 165.55 | 1.81 |
| $BL_{F-O}$ + Focal PW | 5 | 166.10 | 2.36 |
| Focal RL | 4 | 172.62 | 8.88 |
| Focal BL | 4 | 172.68 | 8.93 |
| Focal PW | 4 | 172.70 | 8.96 |
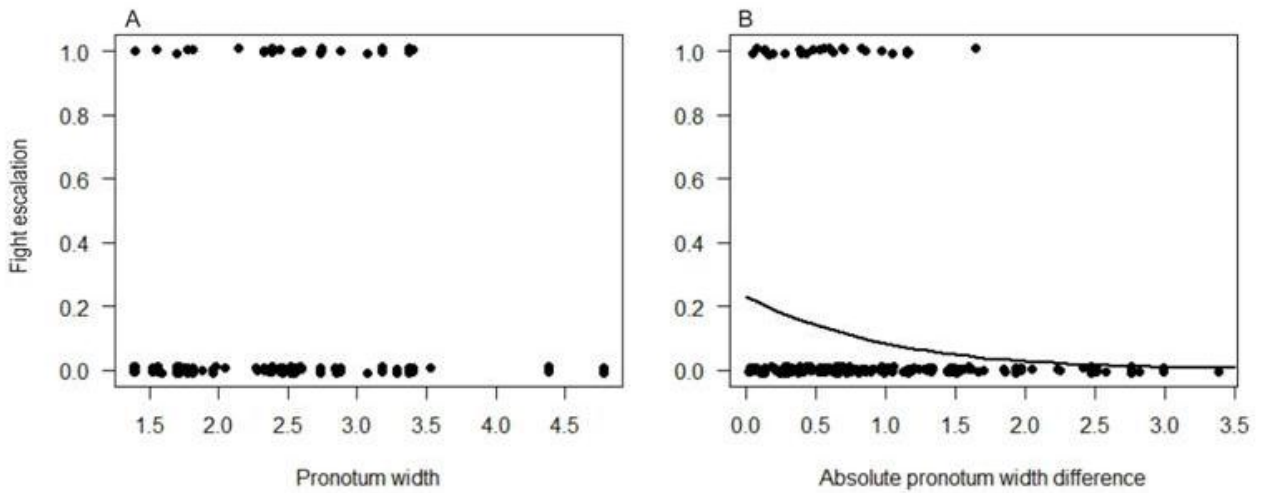
Figure 6. (A) Fight escalation in relation to body size (pronotum width) of focal male. (B) Fight escalation in relation to absolute difference in size between males. 0 = stage one and two aggression, 1 = stage three aggression (see Table 2). Points have been offset to show density of points at each locus.

Table 6. Estimates for the best model to explain the escalation of fights in *L. barbicornis*

|           | Estimate | SE   | Z     | *p*   |
|-----------|----------|------|-------|-------|
| Intercept | -1.221   | 0.45 | -2.74 | 0.006 |
| PW$_{F-O}$ | -1.18    | 0.47 | -2.54 | 0.01  |

The second part of the analysis of assessment strategy determined the relation between contest duration and size difference or smaller/larger male size to test predictions outlined by Taylor and Elwood (2003). However, due to severe inflation of the data due to an overabundance of brief interactions it was not possible to use simple regression models as previously explained (see

Methods). There was no relationship between contest duration and body length difference, or body lengths of the smaller and larger rivals using a subset of fights longer than two seconds. However, the scatterplot of fight duration for all contests in relation to body size difference shows that although there are a large number of brief fights between similar sized individuals, distantly matched individuals never fought for longer than two seconds (Figure 7A). To account for inflation, duration data were pooled into two groups (0 =2 second interactions, 1 = > 2 second interactions) and the GLMM with a binary error structure showed that difference in size did account for a low but significant amount of variation in contest duration ($\beta$ = -0.05, $\chi^2$ =24.09, $p$ <0.0001, Figure 7B). The larger male size had a weak negative relationship with contest duration ($\beta$ = -0.03, $\chi^2$ = 12.39, $p$ =0.0004, Figure 8B), but there was no significant relationship between smaller male size and duration ($\beta$ = 0.01, $\chi^2$ = 0.63, $p$ = 0.43, Figure 8A).

There was no relationship between grappling duration (the most escalated contest phase) and the mean body size of each pair of opponents ($\beta$ = 0.001, $F_{1,25}$ = 0.000013, $p$ = 0.99, Figure 9).
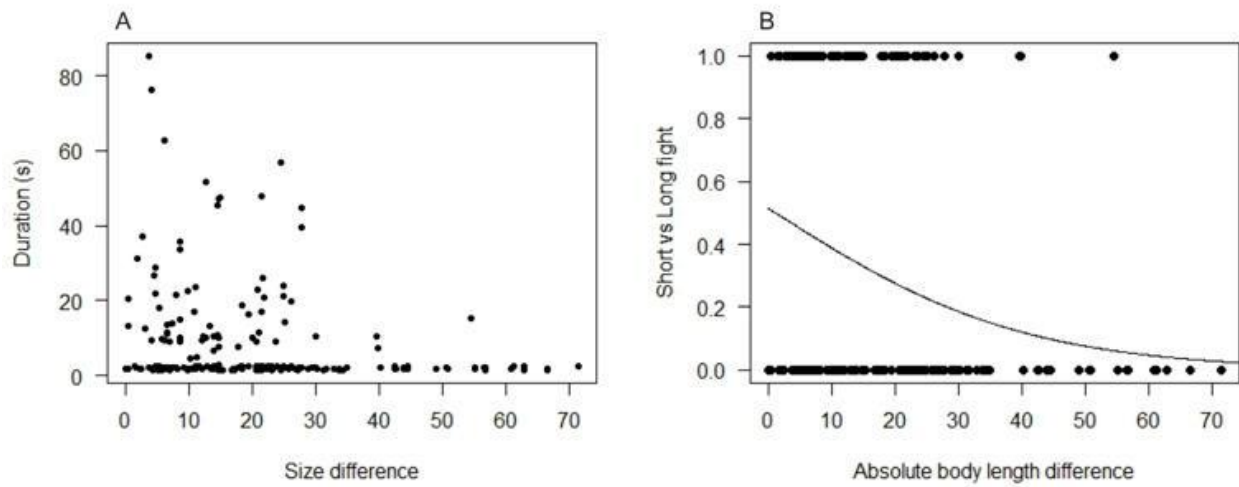
Figure 7. Contest duration in relation to body length difference between contestants using (A) all untransformed data, (B) Contest duration as a binary variable split between short (2 seconds = 0) and longer (>2 seconds = 1) fights.
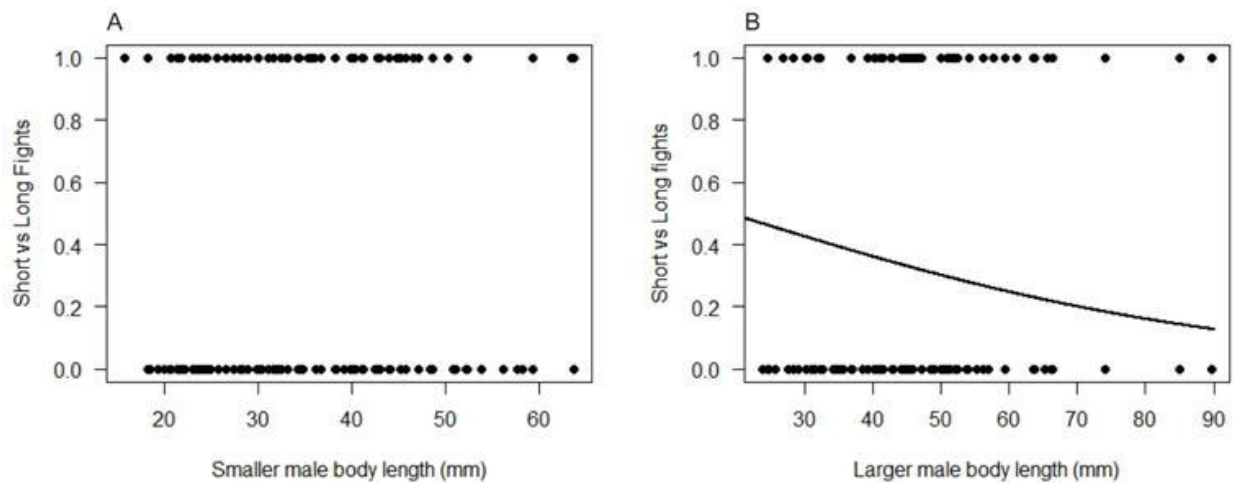


Figure 8. Contest duration in relation to (A) smaller rival size, and (B) larger rival size. Duration was made into a binomial variable of short (2 second bouts) vs. long (>2 second bouts) due to severe inflation in the data.
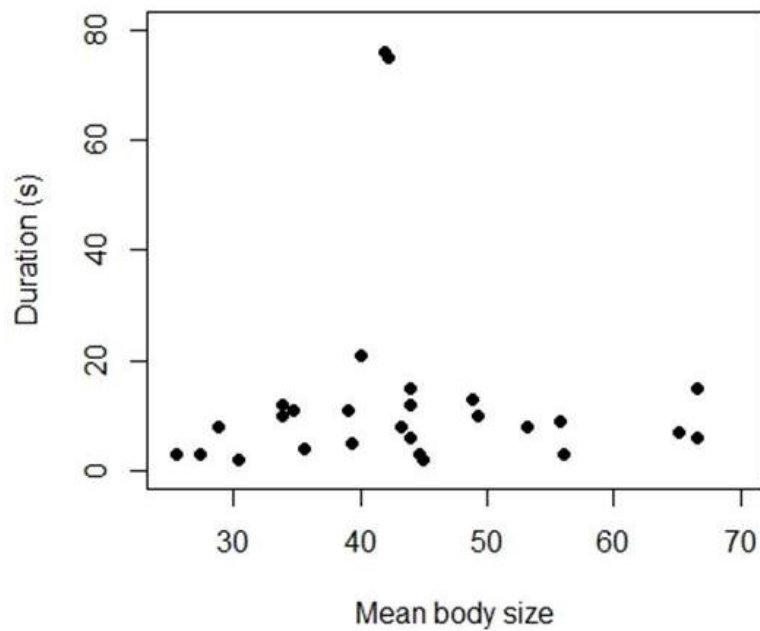
Figure 9. Duration of the grappling phase of contests in relation to the mean body size of opponents.

Mean body size was calculated by taking the mean body size of each pair of grappling weevils.

## 5.5 DISCUSSION

Fighting behaviour between *L. barbicornis* males is prolific, highlighting the intense competition for access to females. Contest outcome in the giraffe weevil was strongly determined by an asymmetry in RHP, where individuals with greater body and weapon size were more likely to win against smaller rivals. Specifically, as the asymmetry in size between the two males increased, the probability of the larger male winning also increased. Weapon (rostrum) and body size (pronotum width and body length) differences were used as measures of RHP, and although all of these body traits were important, weapon size was found to be the best predictor of fight outcome. These results are evidence for directional sexual selection on weapon size in male

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giraffe weevils, such that if two males with closely matched body size were to fight, the male with a relatively longer rostrum would be more like to win.

I used several forms of analysis and documented multiple characteristics of fighting behaviour to determine the assessment strategy used by male *L. barbicornis*, and found most support for the sequential assessment model (SAM) of mutual assessment. The assessment strategies in *L. barbicornis* were analysed by first determining the probability that fights will escalate from stage one and two aggression to stage three grappling in relation to focal male body size, but no relationship between these variables was found. This result shows that males of all sizes will escalate fights to the highest level, irrespective of their body size, where previously it was reported that only large males of this species fight (Meads 1976). This is evidence against both the energetic war of attrition (eWOA) and the cumulative assessment (CAM) models of self-assessment, because if individuals only persist according to their own size, there should be a positive relationship between body size and the probability of escalating the contest (Taylor et al. 2001; Pratt et al. 2003; Yasuda et al. 2012). Taylor (2001) used a similar analysis to determine the assessment strategy employed in *Plexippus paykulli* jumping spider contests, and found that absolute body size positively correlated with the level of escalation, suggesting a self-assessment strategy. Conversely, Pratt et al. (2003) found evidence against eWOA and CAM because the intensity of fiddler crab (*Uca pugilator*) fights were not correlated with the size of contestants, as was the case for giraffe weevils. Furthermore, if fights persist only in relation to an individual's own RHP, then there should be a positive relationship between the mean size of size-matched individuals and contest duration. This is because strong, evenly matched individuals should be able to fight for longer than weak, evenly matched individuals. Although I did not stage contests between rivals, I determined the relationship between the duration of the grappling phase of

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contests (which usually occurs between closely matched individuals, Figure 6B) and the mean size of opponents. In accordance with the SAM, there was no relationship between contest duration and the mean size of opponents, providing more evidence towards a model of mutual assessment.

I found a significant negative correlation between the probability that a fight escalates and body size difference between opponents, showing that escalated fights are more likely to occur between closely matched individuals. If escalated fights occur only between closely matched individuals, regardless of their absolute body size, this indicates mutual assessment of RHP. If only limited information about a rival's RHP is received during initial contact, then more escalated forms of interaction are required for closely matched individuals to determine RHP difference (Enquist et al. 1990). Giraffe weevil male grappling behaviour in relation to morphology also supports and indicates the likelihood of a mutual assessment strategy. During fights that escalate to grapples, males meet side by side with their entire body length including their rostrum alongside that of their opponent. This behaviour may be the mechanism that allows an individual to assess their rival's relative size. Parallels can be made between giraffe weevils and stalk-eyed flies, which are thought to match the length of their eye stalks to an opponent, thus making an accurate measure of their opponents RHP (Panhuis & Wilkinson 1999; Egge et al. 2011).

 While grappling occasionally occurred immediately after approach by an intruder, conflict did not de-escalate. Under the model of sequential assessment (SAM), rivals should perform escalating contest phases that become more costly, but allow individuals to gain more information about their rival's strength and ability (Enquist & Leimar 1987; Enquist et al. 1990). The SAM does not predict de-escalation to occur within or between phases, whereas it can occur

under several models of self-assessment (eWOA: Mesterton-Gibbons et al. 1996; CAM: Payne 1998). My observations of fighting behaviour escalating in phases and not de-escalating once grappling occurs gives further support to the SAM model's relevance to *L. barbicornis*. Furthermore, not every phase of *L. barbicornis* contest showed matching of behaviours between individuals, which is an assumption of the energetic war of attrition (eWOA) (Mesterton-Gibbons et al. 1996). For example, one male would often start the pushing or raking phase, and this would often terminate the contest without the other male pushing back, or would progress into grappling.

Assessment strategy is further determined by analysing contest duration in relation to each opponent's size. An apparent correlation between RHP (size) difference and contest duration may only be due to the weaker rival's ability and persistence to fight, and the tendency for contests between distantly matched individuals to be resolved quickly can simply be because it is more likely for one of the individuals to be one of the weakest in the population (Taylor and Elwood 2003). It is therefore important to address the relationship between the smaller and larger male size and contest duration (Taylor & Elwood 2003). Firstly, only a weak negative relationship between contest duration and rival size difference was found, mostly due to a high proportion of brief, two-second fights. Although brief fights occurred between close and distantly matched individuals, longer duration fights were limited to closer-matched individuals, a pattern clearly seen in the scatterplot of fight duration (Figure 5). An important assumption of the energetic war of assessment (eWOA) model is that contest duration is determined by the male of lower quality, and therefore contest duration should strongly positively correlate with the size of the smaller contestant (Payne & Pagel 1996). There was no significant relationship between smaller male size and duration, which is evidence against the eWOA model in this

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species. Also, a weak negative relationship between larger male size and duration was found, which, despite the large amount of variance, is further evidence against the eWOA model and supports either the sequential assessment (SAM) or cumulative assessment (CAM) models.

The large number of brief interactions that made up more than 70% of all male-male interactions suggests that males are not investing all their time in defending females. Instead, it is likely that scramble competition plays a significant role in driving this behaviour, where although males will guard and fight for access to females, they also spend a large proportion of time searching for unguarded females and attempting to mate as frequently as possible. This is particularly likely when the density of weevils (and therefore intensity of male-male competition) is high or when the frequency of small sneaker males in the population makes it more beneficial to mate as often as possible rather than focus on defence. Also, attempts at sneaking behaviour as an alternative mating tactic by small males could explain a large proportion of the brief agonistic interactions where small males repeatedly attempt access to females guarded by larger males (Chapter 4).

The difficulties in determining assessment strategy encountered in this study are not unique, as many others have found when trying to fit theory around the complex nature of fighting behaviour in their study species (reviewed in Arnott & Elwood 2009; Briffa & Elwood 2009). For example fiddler crabs (*Uca pugilator*) have also been shown to have complicated fighting behaviour, with a series of escalating phases (Pratt et al. 2003). Not all the requirements were met for any of the models tested for *U. pugilator*, as although most evidence was found for the sequential assessment model, they also discussed that the cumulative assessment model might be more appropriate for some aspects of fighting behaviour. Further complications arise when males change from one assessment strategy to another during different phases of a contest. For

example, the hermit crab *Pagurus middendorffii* is thought to use self-assessment during initial contact with an opponent, but switch to mutual assessment during escalated contests (Yasuda et al. 2012). For these reasons it is important to use a combination of methods to determine assessment strategy, including both predictions about the relationship between contest duration and rival size, and specific charactersistics about the fighting behaviour such as whether behaviour matching occurs, and whether contests progress through a series of escalating phases without de-escalation in the intensity of each behavioural phase.

In addition to size asymmetry as a significant predictor of fight outcome, I also predicted that residency would be an important factor, such that in some cases a small defending male may be more inclined to fight more persistently against a larger opponent to retain access to a female. Ownership has been shown to be an important variable in determining fight outcome in other species. For example, in the jumping spider *Phidippus clarus*, ownership overrides the advantage of large body size in determining fight outcome (Kasumovic et al. 2011). Residency was not found to be an important predictor of fight outcome for giraffe weevils. However, it was found that defending males were on average larger and won significantly more contests than intruders. This pattern is likely the result of large males winning previous contests, and therefore having a monopoly over females to make up the largest proportion of guarding males in the population. A similar pattern was also identified in fiddler crab fights, and interpreted as an accumulation of larger males as residents due to earlier fighting success (Pratt et al. 2003).

This study is the first detailed documentation and analysis of fighting behaviour and assessment strategy in the giraffe weevil. Fighting behaviour is highly dynamic, and fight frequency is correlated significantly with total abundance indicating it is also density-dependent and increases as a result of the intensity of intrasexual competition. Despite the difficulties in distinguishing

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between models of assessment strategy that best explain this system, the results from this study indicate that *L. barbicornis* males most likely assess their own RHP in relation to their opponent under a model of mutual assessment. This assertion is supported by analyses that show that males of all sizes escalate contests to the highest degree, the most accelerated contests only occur between closely matched individuals, and there is no relationship between grappling duration and the mean size of rivals. Furthermore, fighting escalates but does not de-escalate, behavioural matching does not occur at all stages, and there was a negative relationship between larger male size and contest duration. This study is an example of fighting behaviour in a dynamic, wild population, and by considering multiple aspects of contest structure, outcome, and duration it is possible to gain a more comprehensive understanding of the assessment strategy used by males in this species. It is recommended that studies attempting to distinguish between assessment strategy models use a combination of analyses as was employed here, to avoid issues with making conclusions from duration data alone.

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# CHAPTER 6

# Geographic variation in body size and weapon allometry along a latitudinal cline

**6.1 ABSTRACT**

Sexual selection has driven the evolution of a diverse range of exaggerated traits including those used as weapons during male-male contests. Exaggerated traits are expected to evolve rapidly in response to environmental variation, but studies documenting this pattern are still relatively rare. In comparison, studies on latitudinal variation in body size according to Bergmann's rule are more common, and here I explore the potential for the combined analysis of these two phenomena. In this chapter, I present data showing latitudinal variation in body size and weapon allometry for the giraffe weevil (*Lasiorhynchus barbicornis*), a large-bodied brentid weevil endemic to New Zealand. Males display an extremely elongated rostrum which is used as a weapon during antagonistic contests with other males for access to females. Among the populations studied, mean body and rostrum size increased with latitude which is evidence for Bergmann's rule, however weapon allometry showed a correlated but opposite trend. Body size and weapon allometry variation is likely due to developmentally plastic responses to environmental change. I speculate that the decreasing allometric slope could be due to a resource allocation trade-off between overall body size and rostrum length such that males investing increasingly more into body size at higher latitudes have fewer resources to allocate to rostrum size. Also, as sexually selected traits are expected to be condition-dependent under environmental stress, cooler climates at high latitudes may result in less investment in rostrum size relative to overall body size. To my knowledge, this is the first study to document a latitudinal cline in weapon allometry, and is therefore a novel contribution to the collection of work on Bergmann's rule and secondary sexual trait variation.

## 6.2 INTRODUCTION

### 6.2.1 Variation in Exaggerated Sexual Traits and Allometry

Sexual selection is responsible for driving the evolution of an incredibly diverse range of exaggerated traits expressed by males across the animal kingdom (Darwin 1871; Andersson 1994). Exaggerated traits are used as either ornaments to attract females, or as weapons in male-male combat, and frequently influence individual reproductive success. While it is recognised that sexually selected traits can evolve rapidly in response to changes in selection pressures (Svensson & Gosden 2007), inter-population variation in exaggerated trait expression is rarely identified. In particular there are few studies that demonstrate inter-population variation in the scaling relationship of exaggerated traits in relation to body size.

Scaling relationships are used to quantify the relative investment into exaggerated traits in comparison to overall body size. These scaling relationships are shown by plotting secondary sexual trait size against body size, and calculating the allometric slope between the two variables. Positive allometries indicate that as body size increases, males invest disproportionately more into secondary sexual trait size than smaller males, which have often been used as an indication that the trait is under directional sexual selection (Kodric-Brown et al. 2006). Recently, Bondurianksy (2007b) raised the importance of supporting allometric data with direct behavioural observations of sexual selection on traits because positive allometries are far from being a universal pattern in all sexually selected traits, and can be observed for traits that should not be under sexual selection.

Once the functional significance of an exaggerated trait is determined, one promising but frequently overlooked avenue towards a better understanding of exaggerated trait evolution is to

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investigate variation between populations in trait expression and allometry. Recently Miller and Emlen (2010) found significant variation in relative weapon (hind femora) size between populations of *Leptoscelis tricolor* bugs. A relationship was identified between the host plant that the bugs were collected from and the relative size of the hind femora, suggesting that population variation in trait size is at least partially driven by the availability of host plant species. Similarly, Moczek and Nijhout (2003) also found that the scaling relationship of male horn sizes varied significantly between native and introduced populations of *Onthophagus taurus*. In this species the scaling relationship is defined by two distinct male phenotypes (horned vs. hornless) separated by a body size threshold. Populations of *O. taurus* show rapid evolution of the body size threshold in less than 40 years after introduction to two new exotic ranges. The extent of threshold divergence between these populations was as great as that normally observed between species. It is not currently known what has driven these rapid shifts in threshold at each population, but the authors suggest population density could be an important factor. Climate has also been shown to affect exaggerated trait size, such as in male red deer (*Cervus elaphus*), where the relative investment in antler tines fluctuated over time such that in years of harsh climatic conditions antler investment was less than in more favourable years (Mysterud et al. 2005). Overall these studies show that scaling relationships can and do evolve rapidly in response to environmental variation.

A major source of environmental variation is latitude, which correlates with factors such as temperature, rainfall, and season length, and has been shown to influence many aspects of species biology, especially body size (see section 6.2.2). We might therefore expect that latitude also correlates with the relative investment in exaggerated trait size (allometry), however, to date this has not been explored.

*6.2.2 Latitudinal Variation in Body Size and Bergmann's Rule*

Body size variation in relation to latitude and altitude have been well-known since Bergmann's early work in the 19th century, and are generally attributed to variation in temperature because individuals of many species grow larger when they develop at lower temperatures (Atkinson 1994). In general, organisms are found to be larger at higher latitudes and altitudes, both of which are associated with cooler climates. Bergmann (1847) originally described this pattern for endothermic animals, and proposed that large bodied individuals would have smaller surface to volume ratios, allowing them to conserve heat more efficiently in cooler climates than smaller individuals. However, latitudinal size clines are also widely known for ectotherms for which heat conservation is less important because small ectotherms such as insects lack the ability to thermoregulate (Stevenson 1985; Blanckenhorn et al. 2006; Stillwell 2010). Latitudinal clines have been shown for both comparative studies using multiple species within a broader taxonomic unit (Lindsey 1966; Cushman et al. 1993; Barlow 1994; Hawkins & Lawton 1995; Blackburn & Hawkins 2004; Olalla-Tárraga & Rodríguez 2007) and for single species studies across their geographical range (Ashton 2002b; Ashton 2002a; Ashton & Feldman 2003; Blanckenhorn & Demont 2004; Tsuchiya et al. 2012).

Temperature is not always the most important variable in driving Bergmann's clines. For example, Stillwell et al. (2007) found that moisture, seasonality and host seed size were better predictors of variation in seed-beetle (*Stator limbatus*) body size than temperature. Moisture has the potential to drive body size through desiccation resistance, where large-bodied animals will have higher water content and could therefore survive in drier areas (Le Lagadec et al. 1998). Highly seasonal locations could favour larger body size to decrease mortality in relation to long

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periods of starvation or desiccation (Stillwell et al. 2007). In addition many species also show the

opposite pattern, termed the converse Bergmann's rule, where body size decreases towards the

poles. Rather than temperature per se, this pattern is thought to be driven by season length. As

seasons become shorter towards the poles there is less time for foraging, growth and

development, resulting in smaller body sizes (Mousseau 1997; Blanckenhorn & Demont 2004).

A recent review of Bergmann's rule in insects found almost equal number of studies that show

evidence of Bergmann's cline, converse Bergmann's cline, or no cline at all, demonstrating that

these "rules" should not be blindly applied, nor do they necessarily describe patterns observed in

the majority of species (Shelomi 2012).  In addition, we are still far from understanding the

mechanisms behind these patterns, and subsequently the temperature-size rule has been declared

a life-puzzle (Berrigan & Charnov 1994).

*6.2.3 Variation in Sexual Size Dimorphism and Rensch's Rule*

Body size not only varies between species and populations, but can also vary dramatically

between the sexes. Sexual size dimorphism (SSD) is driven by selection pressures acting

differentially on male and female body size.  Large body size in females is most likely the result

of fecundity selection for higher offspring production, while large male size is thought to be

driven primarily by sexual selection through male-male competition for increased mating success

(Andersson 1994; Kraushaar & Blanckenhorn 2002). In invertebrates and ectothermic vertebrate

species, females are generally the larger sex, whereas the size of endothermic vertebrates are

more likely to be male-biased (Fairbairn 1997; Teder & Tammaru 2005). A recent review by

Stilwell et al. (2010) showed that 88% of 1846 insect species surveyed were female-biased, and

only 7% were male-biased. There is, however, variation between insect orders such that the

Odonata (particularly damselflies) have higher levels of male-bias (46% male-biased, 27% female-biased) than Coleoptera, for example, where 72% of species are female-biased and only 9% male-biased (Stillwell et al. 2010).

Although the selective mechanisms driving SSD within species has been reasonably well explained, many species also show variation in the magnitude of SSD between populations (Teder & Tammaru 2005; Blanckenhorn et al. 2006), but we know little about the causes of these differences. In addition, while many studies have looked at body size in relation to latitudinal clines, few have looked at how SSD varies along these clines. Rensch's rule, another common model applied to body size variation, describes the pattern where SSD often correlates with body size (Abouheif & Fairbairn 1997; Fairbairn 1997). In species where males are the larger sex the level of SSD increases with body size, while in species with female-biased SSD the level of SSD decreases with increasing body size (Rensch 1950). This correlation is usually attributed to faster evolutionary divergence of male body size due to sexual selection, because sexual selection can drive the rapid evolution of body and secondary sexual trait size (Fairbairn & Preziosi 1994; Dale et al. 2007). However, evidence from water striders (*Aquarius remigis*) also supports an alternative hypothesis that Rensch's rule is due to differences in phenotypic plasticity between sexes in response to environmental conditions (Fairbairn 2005). Like Bergmann's rule, Rensch's rule was also originally coined to explain variation between species of related animal taxa, however it has recently been used to describe patterns among populations within species (e.g. Fairbairn 2005; Blanckenhorn et al. 2006), and for the first time shown to be evident in dioecious plants (Kavanagh et al. 2011).

While there is an apparent interaction between Bergmann and Rensch's rule for some taxa (reviewed in Blanckenhorn et al. 2006), there is no clear pattern or mechanism for why the

magnitude of SSD should vary along a latitudinal cline. While it is likely that selection pressures could vary between populations, this does not necessarily explain why the level of SSD should follow a latitudinal or temperature gradient. However, it is possible that if one sex is more affected by change in environment than the other, then you would observe differences in SSD that would increase along the cline, either driven by direct selection or developmental plasticity. If sexual and viability selection pressures acting on a species vary over space and time we can expect that exaggerated traits can also vary in expression in response to these changes in selection (Svensson & Gosden 2007). In particular, if sexual selection is stronger in driving male size than fecundity selection is in driving female size, and if those selection pressures vary systematically between populations in response to environmental conditions, then male size will increase faster than female size resulting in divergence in SSD (Blanckenhorn et al. 2006). A complicating factor is that some of the variation observed between populations is likely to be due to developmental plasticity because sexual traits can be expressed differentially in accordance with variation in the quality and quantity of resources and the environment (Stillwell et al. 2010).

*6.2.4 New Zealand giraffe weevils*

The New Zealand giraffe weevil *Lasiorhynchus barbicornis* is an interesting model for addressing questions about geographic size variation because males have an enormously elongated rostrum that is used as a weapon during male-male competition (Meads 1976)(Figure 1). Rostrum length in male *L. barbicornis* scales positively when compared to body size (pronotum width), whereas female rostrum length scaled against body size is weakly negatively allometric (Chapter 3). Although one must take care in interpreting positive allometries

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(Bonduriansky 2007b), rostrum length in males is likely to be under directional sexual selection because of its vital role as a weapon during combat (Meads 1976, Chapter 5).



Figure 1. A large male *L. barbicornis* guards a female below, showing the extreme sexual dimorphism and elongation of the male rostrum used as a weapon during combat with other males.

Using individuals measured at 11 sites spanning the North Island of New Zealand, I assessed if there was significant geographic variation in body size and weapon allometry, and sexual size dimorphism in *L. barbicornis*. The aims of this study were to (1) characterise mean body and weapon size variation between eleven populations, (2) determine if variation follows a latitudinal cline according to Bergmann's rule, (3) determine the magnitude and variation of sexual size dimorphism between populations in relation to latitude, (4) determine if sexual size dimorphism

varies between populations in accordance with Rensch's rule, and (5) determine whether there is variation in the scaling relationship between weapon and body size across populations. To my knowledge this is the first study to determine geographic variation in relation to Bergmann's and Rensch's rule in a species with an exaggerated trait used as a weapon, and the first to document patterns in the relative size of a weapon in relation to a latitudinal cline. Given the strong correlation of body size and rostrum length (Chapter 2), weapon allometry could be expected to show no relationship with latitude, such that the relative investment in rostrum size remains stable across populations. Alternatively, if environmental variation affects rostrum size differently to body size, and this variation correlates with latitude, there could be a shift in allometric slope in relation to latitude. This study therefore provides the first attempts to determine a relationship between latitude and weapon allometry.

## 6.3 METHODS

### 6.3.1 Study species

The giraffe weevil, *Lasiorhynchus barbicornis* (Coleoptera: Brentidae), is endemic to New Zealand and is found across the North Island and in the northern and western regions of the South Island (Kuschel 2003). Larvae of the species are wood-borers and found on a wide-range of host trees such as karaka (*Corynocarpus laevigatus*), mahoe (*Melicytus ramiflorus*), and pigeonwood (*Hedycarya arborea*) (Appendix 1). During the warmer months (October to April), adults are found aggregating on host trees where females use their rostrum to drill holes prior to oviposition. While females drill, males compete for access to females for copulation, and will stand guard over females after copulation to prevent other males from mating. Males are striking

in appearance due to their extremely elongated rostrum that they use as a weapon in conjunction with their mandibles to push, pull and grapple with other males and relative weapon size influences both the outcome and duration of contests between males (Chapter 5). Males do not perform any courtship behaviour and there appears to be no pre-copulatory female choice as females continue to drill during copulation and mate rejection was rarely observed (C. Painting unpublished observations).

Both males and females are extremely variable in body size, ranging from 15-90 mm in total length in males, and 12-50 mm in females (Chapter 2). Males are the larger sex in terms of body length, although due to the large size variation in both sexes it is common to find males that are smaller than females on a tree. Like for many species, characterising sexual size dimorphism (SSD) is also largely influenced by which trait is used as a measure of overall body size. This is of relevance to the giraffe weevil because pronotum width is often a common trait used to describe overall body size in beetles, but in this species females are often of similar size or have larger pronotum widths than males despite being much shorter in length overall (Figure 2). If only using pronotum width to describe size in this species one would be led to believe that females are the larger sex. Because of this problem I have used both body length excluding the rostrum and pronotum width to describe overall size throughout this study.
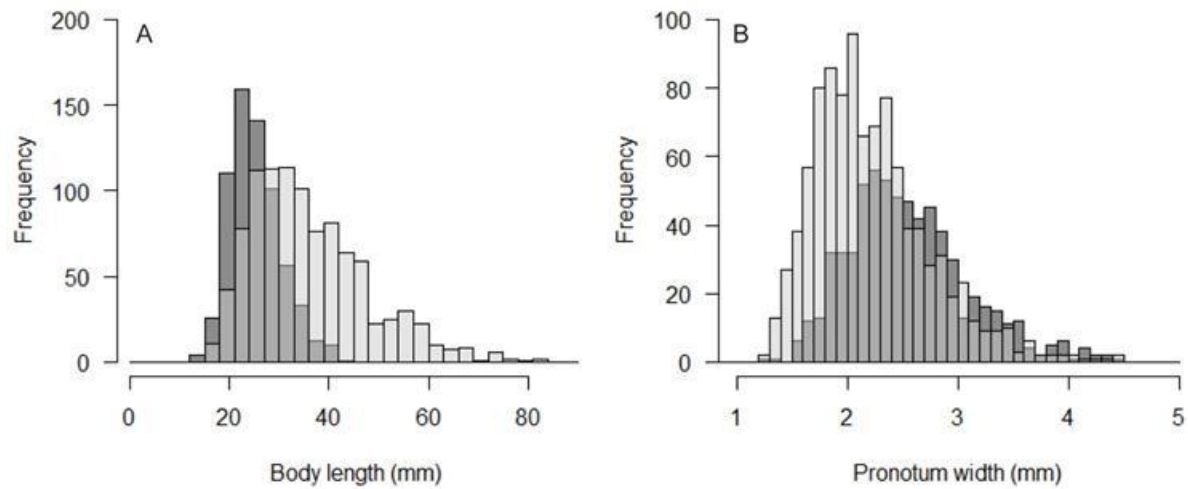
Figure 2. Frequency distribution of (A) body length and (B) pronotum width of females (dark grey; N = 653) and males (pale grey; N = 987) of *Lasiorhynchus barbicornis*. Mid-grey regions of the histogram are where male and female distributions overlap. Data and figures from Chapter 2.

*6.3.2 Collection & measurement*

Between January 2010 and January 2013, I conducted trips during summer months (November to March) to 11 sites around the North Island of New Zealand to locate and measure adult male and female giraffe weevils. These sites cover a latitudinal range of 5.27 degrees, which spans most of the length of the North Island. Although *L. barbicornis* are also recorded from the South Island of New Zealand, they are much rarer and I was unable to locate populations large enough for use in this study. At each location the latitude and longitude was recorded using a Garmin 60CSx GPS.

At each site I searched for giraffe weevils aggregated on dead fallen or dying standing host trees, and then systematically scanned the trunks and along branches of the trees until I was confident that I had located all weevils present at that time. All weevils present were removed and measured using digital calipers to the nearest 0.01 mm. Body size measurements included total body length excluding the rostrum (BL, thorax through to end of elytra), rostrum length (RL, total length from anterior end of mandibles to the base of the head behind the eyes), and pronotum width (PW). Rostrum length was used as a measure of weapon size in male *L. barbicornis*. All body size measurements were previously found to have high repeatability (0.99, Chapter 2).

A total of 308 males and 164 females were measured across 10 populations around the North Island of New Zealand (Table 1, Figure 3). Additionally, a dataset of 987 males and 653 females collected between November 2011 and March 2012 from Matuku Reserve, collected for a previous study (Chapter 3), was incorporated into this study.

Females are found less frequently in the field than males, and therefore fewer females were measured for each population than males. As I was only able to locate four females at Mangamuka Forest, this site was excluded from the analyses of mean female body size and analyses of sexual size dimorphism.

Table 1. Collection locality, latitude, and number of males and females measured at each site.

| Site | Latitude (°S) | N (males) | N (females) |
|------|---------------|-----------|-------------|
| Mangamuka Forest (MAN) | 35.1146 | 18 | 4 |
| Puketi/Omahuta Forest (PUK) | 35.1405 | 44 | 21 |
| Pukenui Forest (WHA) | 35.4208 | 23 | 8 |
| Bream Head (BRE) | 35.5122 | 56 | 25 |
| Matuku Reserve (MAT) | 36.5155 | 987 | 653 |
| Maungatautari Forest (MAU) | 38.0321 | 26 | 14 |
| Pureora Forest (PUR) | 38.3203 | 54 | 43 |
| Ratapihipihi Reserve (RAT) | 39.0616 | 20 | 17 |
| West Ruahine Ranges (RUA) | 40.1108 | 12 | 8 |
| Palmerston North (PAL) | 40.2856 | 30 | 15 |
| Lake Papaitonga (PAP) | 40.3849 | 25 | 9 |
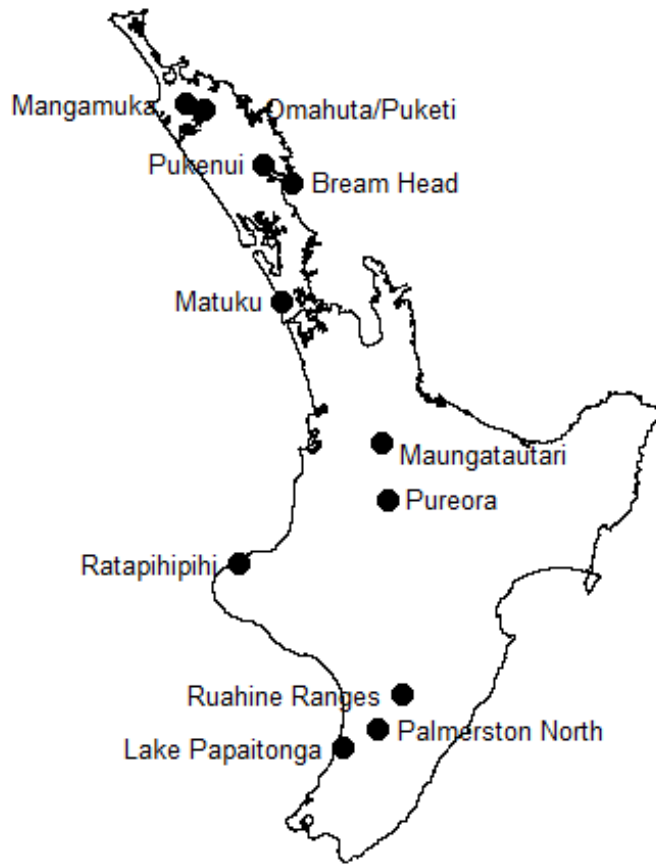
Figure 3. Sites where *L. barbicornis* were sampled across the North Island of New Zealand.

*6.3.3 Statistical analysis*

*Population variation in body and weapon size*

Firstly, I tested for variation between populations in body and weapon size by conducting separate one-way ANOVA's using untransformed data.

*Latitudinal variation in body and weapon size*

A combination of ordinary least squares regression (OLS) and standardised major axis regression (SMA) was used in this study to determine the variation in body and weapon size, and SSD. To determine the relationship between body size and latitude, I used OLS with logged body size. Data sets such as this, where one sex is much larger than the other, are problematic if using raw data because larger means also tend to have higher variances (Tomkins et al. 2005). If using untransformed data, the slopes for males will be much steeper than females due to the difference in body size scale, not necessarily because males are becoming increasingly larger than females. I therefore log transformed male and female body size to remove these statistical artefacts (see Appendix 3; Tomkins et al. 2005; Blanckenhorn et al. 2006).

*Latitudinal variation in sexual size dimorphism*

To investigate the relationship between sexual size dimorphism and latitude, I used the index method recommended by Lovich and Gibbons (1992), where SSD = (mean size of larger sex/mean size of smaller sex) – 1. Values were arbitrarily set to positive when males were larger and negative when females were larger. This index of SSD was used instead of ANCOVA's with latitude-by-sex interactions to avoid problems with scale effects as described above. OLS regression was used to test whether levels of SSD increased with latitude.

*Population variation according to Rensch's rule*

Independent of latitude, I tested whether giraffe weevil populations follow Rensch's rule by determining if male size is more variable than female size. Slopes were calculated using the programme *smatr* Version 3.2.3 (Warton et al. 2012), which uses SMA techniques, and regressed log male size (body length ex. rostrum length, rostrum length and pronotum width) against log female size (Fairbairn & Preziosi 1994). OLS regression was not appropriate for this analysis

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because it assumes that there is no measurement error in the explanatory (independent) variable, and also tends to underestimate the slope, which is important when trying to determine the scaling relationship, rather than just predicting y from x (Fairbairn 1997, 2005). Analyses of sexual size dimorphism will always have measurement error on both axes because male and female size is being compared. Rensch's rule is assessed statistically by regressing male body size against female body size. An observed slope >1 (positive or hyperallometry) supports Rensch's rule and indicates that male size is getting disproportionately larger than female size as overall size increases. *Smatr* is a useful programme for calculating SMA slopes because it also provides 95% confidence intervals around the predicted slopes and intercepts, and can test for deviation from isometry.

*Latitudinal variation in weapon investment*

I previously found that the male scaling relationship was best described by a non-linear Weibull growth curve, which showed a relative decrease in rostrum size for the very largest males (Chapter 3). However, the non-linearity in the model was caused by only the very largest males. Therefore, males longer than 70 mm are associated with the upper asymptote of the predicted scaling relationship, and so, to simplify the analysis here I removed all males larger than 70 mm in body length, to make it possible to treat the male scaling relationship as linear and use SMA regression. The scaling relationships between body and weapon size for each population were calculated using SMA in *smatr* and body and weapon size were log transformed for each individual. Log-transformation also ensured that male and female size could be compared on the same scale (see Appendix 3). The SMA slopes for each population were then regressed against latitude using OLS. There was no further need to account for problems with scale in this analysis

as the raw data was already logged prior to determining the slope for each population. All analyses were conducted in R 2.15 (R Core Team 2013).

## 6.4 RESULTS

### 6.4.1 Population variation in body size and weapon size

Mean body length (ex. rostrum), pronotum width, and rostrum length for both males and females varied significantly between populations (Table 2, Figure 4). The Palmerston North site (PAL) had the highest mean body length, pronotum width, and rostrum length for both males and females, whereas the smallest body sizes were found at Mangamuka (MAN) and Puketi/Omahuta (PUK) (Figure 4). Mean body length was 33.3% smaller at Mangamuka (MAN) than Palmerston North (PAL) for males, whereas Puketi/Omahuta (PUK) females were 19% smaller than Palmerston North (PAL) females.

Table 2. Results of ANOVA showing significant variation between populations and sex in mean body length, pronotum width, and rostrum length.

| Variables | df | Sum of squares | $F$ | $p$ |
|---|---|---|---|---|
| *Body length minus rostrum length* | | | | |
| Location | 10 | 4150 | 1931 | <0.0001 |
| Sex | 1 | 5122 | 238.37 | <0.0001 |
| Location:Sex | 9 | 540 | 2.79 | 0.003 |
| Residual | 2103 | 45189 | | |
| *Pronotum width* | | | | |
| Location | 10 | 67.6 | 23.02 | <0.0001 |
| Sex | 1 | 41.34 | 140.73 | <0.0001 |
| Location:Sex | 9 | 5.4 | 2.04 | 0.03 |
| Residual | 2101 | 617.1 | | |
| *Rostrum length* | | | | |
| Location | 10 | 4173 | 14.45 | <0.0001 |
| Sex | 1 | 36466 | 1262.84 | <0.0001 |
| Location:Sex | 9 | 1091 | 4.198 | <0.0001 |
| Residual | 2103 | 60727 | | |
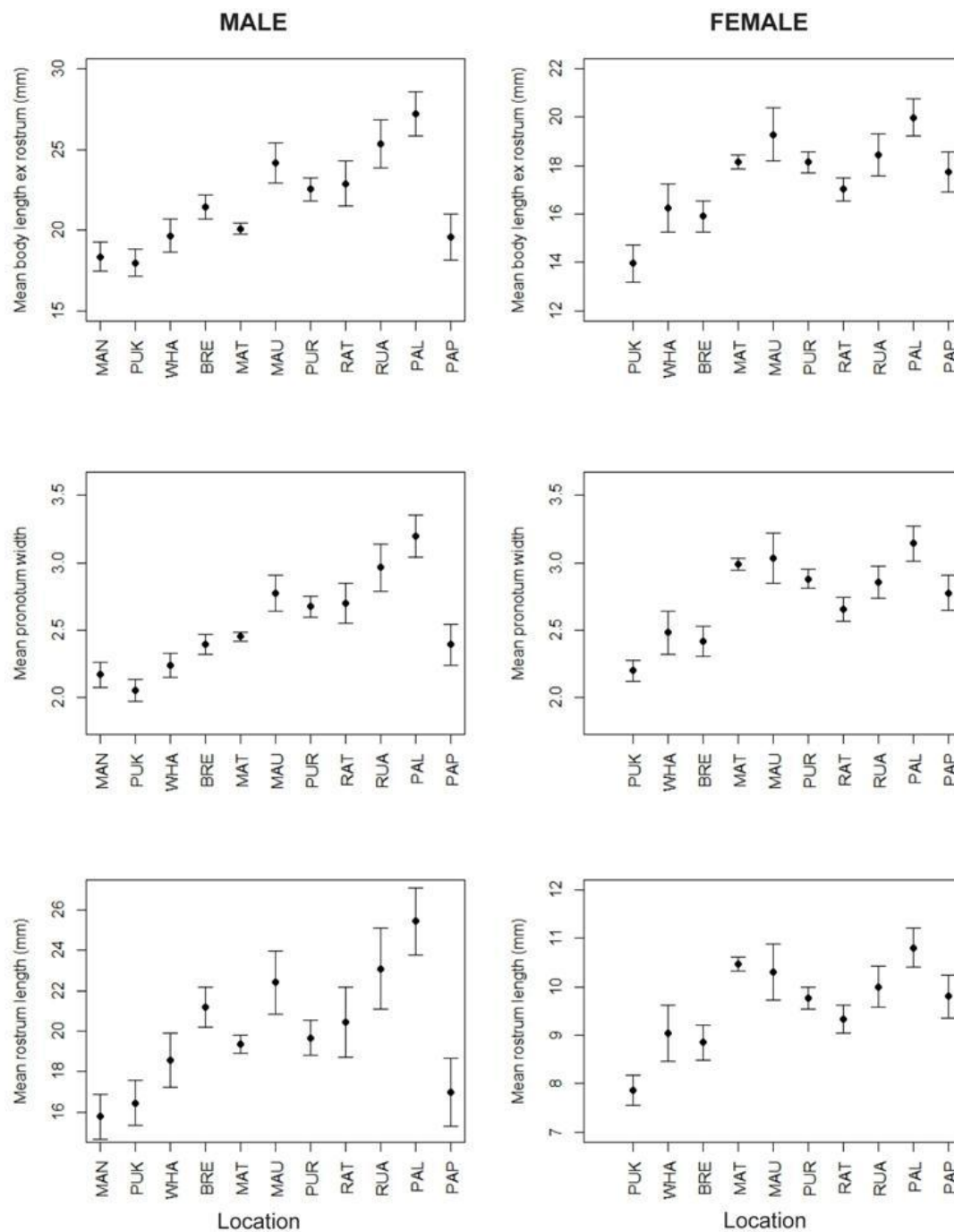
Figure 4. Variation in mean (±SE) body length ex. rostrum length, pronotum width and weapon size (rostrum length) across populations in the North Island of New Zealand (arranged from highest (north) to lowest (south) latitudes. Note that the mean sizes were not calcluated for Mangamuka (MAN) females because of a small sample size. See Table 1 for explanation of location abbreviations.

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*6.4.2 Latitudinal variation in body and weapon size*

The variation in body and weapon size between populations followed a latitudinal cline in accordance with Bergmann's rule, such that mean body and weapon sizes for both males and females were larger at higher latitudes (i.e. body size and weapon size increased the further south specimens were collected; Table 3, Figure 5). The Lake Papaitonga (PAP) site was the only major outlier to this trend, and exclusion of this site increased the significance and strength of the correlation between latitude and mean size for males, in particular changing the relationship between latitude and rostrum length for males from non-significant to highly significant (Body length: $R^2 = 0.86$, $F_{1,8} = 47.78$, $p = 0.0001$; Rostrum length: $R^2 = 0.66$, $F_{1,8} = 15.6$, $p = 0.004$; Pronotum width: $R^2 = 0.91$, $F_{1,8} = 85.56$, $p < 0.0001$).

Table 3. OLS regression results for the correlation of log transformed mean size for three traits in relation to latitude for male and female *L. barbicornis*. Significant correlations are highlighted in bold.

|  | df | Intercept | Slope | $R^2$ | $F$ | $p$ |
|---|---|---|---|---|---|---|
| ***Male*** | | | | | | |
| Body length | 9 | 1.41 | 0.04 | 0.51 | 9.28 | **0.01** |
| Rostrum length | 9 | 1.55 | 0.04 | 0.30 | 3.90 | 0.08 |
| Pronotum width | 9 | -0.98 | 0.05 | 0.64 | 16.29 | **0.0003** |
| ***Female*** | | | | | | |
| Body length | 8 | 1.48 | 0.04 | 0.53 | 9.17 | **0.02** |
| Rostrum length | 8 | 1.14 | 0.03 | 0.44 | 6.32 | **0.04** |
| Pronotum width | 8 | -0.41 | 0.04 | 0.48 | 7.29 | **0.03** |

Figure 5. Variation in (A) mean log body length (ex. rostrum), (B) mean log rostrum length, and (C) mean log pronotum width between populations of male (▲) and female (●) *L. barbicornis* across a latitudinal cline. The outlier site (PAP) is shown circled in red, but was included to calculate the above regression lines.

Specific climate data for each collection location was not available, so it was not possible to test directly for the effect of climate variables on body size and scaling relationship. Instead, broader scale regional weather data was obtained from the National Institute of Water and Atmospheric Research (NIWA 2012). All data are annual means from a 29 year period (1981-2010). All

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temperature variables were strongly negatively correlated with latitude, but there was no

significant correlation for rainfall or relative humidity (Table 4).

Table 4. Correlation between latitude and climate variables.

| Climate Variable | Pearson's correlation (r) | p |
|---|---|---|
| Mean temperature (°C) | -0.87 | <0.0001 |
| Minimum temperature (°C) | -0.80 | <0.0001 |
| Maximum temperature (°C) | -0.82 | <0.0001 |
| Mean annual rainfall (mm) | 0.13 | 0.50 |
| Mean relative humidity (%) | -0.15 | 0.43 |

*6.4.3 Latitudinal variation in sexual size dimorphism*

I previously found a high degree of sexual size dimorphism from a single population of *L. barbicornis* at Matuku Reserve (Chapter 2). Here I calculated the mean body length (ex. rostrum length), rostrum length, and pronotum width for all populations pooled and found that males are, on average, larger in body and rostrum lengths, but are on average smaller in pronotum width when compared to females (Table 5). These findings show how important it is to carefully choose the trait used as a proxy for overall body size. For this species, it is clear that most of the male-bias in body size comes from the extreme elongation of the rostrum and overall elongation of the body. However, if one were to only use pronotum width which is commonly used as a

body size measure for beetles, it would appear that females are marginally but significantly larger in size.

Table 5. Mean trait size (± standard error) for male and female *L. barbicornis*, all populations pooled. F statistics reported are for ANOVA to test difference in mean trait size for each sex.

| Trait | Mean male size | Mean female size | $F_{1,2029}$ |
|---|---|---|---|
| Body length ex. rostrum | 19.67 (0.12) | 16.46 (0.16) | 207.1*** |
| Rostrum length | 17.65 (0.20) | 9.07 (0.06) | 1157*** |
| Pronotum width | 2.29 (0.02) | 2.58 (0.16) | 131.4*** |

*** = $p < 0.0001$

I did find evidence of variation in SSD between populations in general (Figure 6), but did not find a significant relationship between the magnitude of SSD and latitude for any of the body measurements (Table 6). This result was further supported by an ANCOVA comparing body length against latitude and sex, where although the intercept of the slopes differ between the sexes, there was no significant interaction between latitude and sex indicating that the slope is the same for both sexes ($F = 0.17$, $p = 0.69$; See Figure 5). It was important to log the body size values for this analysis to avoid issues around scale effects, as failing to do this can give a false impression that males are getting significantly larger than females at higher latitudes (i.e ANOVA excluding the outlier Lake Papaitonga (PAP) site of raw body length (ex. rostrum) values showed a significant latitude by sex interaction, $F = 7.14$, $p = 0.02$).
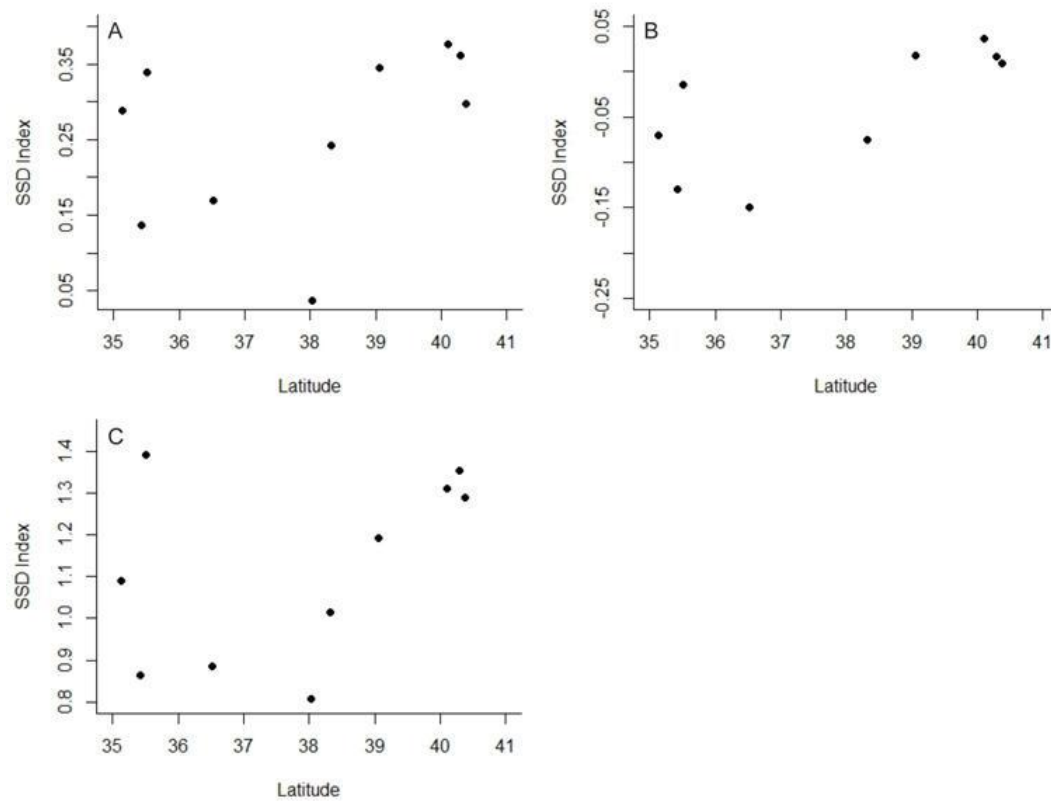
Figure 6. Relationship between SSD and latitude for (A) body length minus rostrum, (B) pronotum width, and (C) rostrum length. Positive values indicate that size was male biased, negative values denote female-bias.

Table 6. Results of linear regression analysis showing relationship between the degree of SSD and latitude for body length, rostrum length and pronotum width.

| Trait | Intercept | Slope | $t$ | $p$ | $R^2$ |
|---|---|---|---|---|---|
| Body length ex. rostrum | -0.52 | 0.02 | 1.18 | 0.27 | 0.15 |
| Rostrum length | -0.59 | 0.05 | 1.36 | 0.21 | 0.19 |
| Pronotum width | -0.86 | 0.02 | 1.14 | 0.29 | 0.14 |

*6.4.4 Population variation according to Rensch's rule*

Population variation in accordance with Rensch's rule was tested by plotting population means of log male size against log female size. It was found that although the means between the sexes were correlated, the slope was not significantly greater than 1 for any of the traits (Table 7, Figure 7). Although each body trait had a predicted SMA slope of greater than 1, the very wide confidence intervals meant that there was not a significant deviation from isometry. Furthermore, if sexual selection was driving any magnitude difference in SSD between populations it would be expected that secondary sexual traits, such as rostrum length, should show a consistent SMA slope >1, and higher than other somatic traits. There was no evidence for this here, as although rostrum length had a slightly higher slope value than other traits, the slope was not significantly greather than 1 (Table 7). These results suggest that there is no covariation between the magnitiude of SSD and body size in this species, such that males are not getting disproportionately larger than females as body size overall increases.

Table 7. Estimated SMA slopes for male against female body size and test for Rensch's rule in ten populations of *L. barbicornis*.

|  | Slope (95% CI) | df | R² | Test for isometry (r) | *p* |
| --- | --- | --- | --- | --- | --- |
| Body length ex. rostrum | 1.24 (0.76 – 2.02) | 8 | 0.61 | 0.33 | 0.35 |
| Rostrum length | 1.45 (0.80 – 2.61) | 8 | 0.41 | 0.44 | 0.20 |
| Pronotum width | 1.17 (0.74 – 1.85) | 8 | 0.66 | 0.27 | 0.45 |
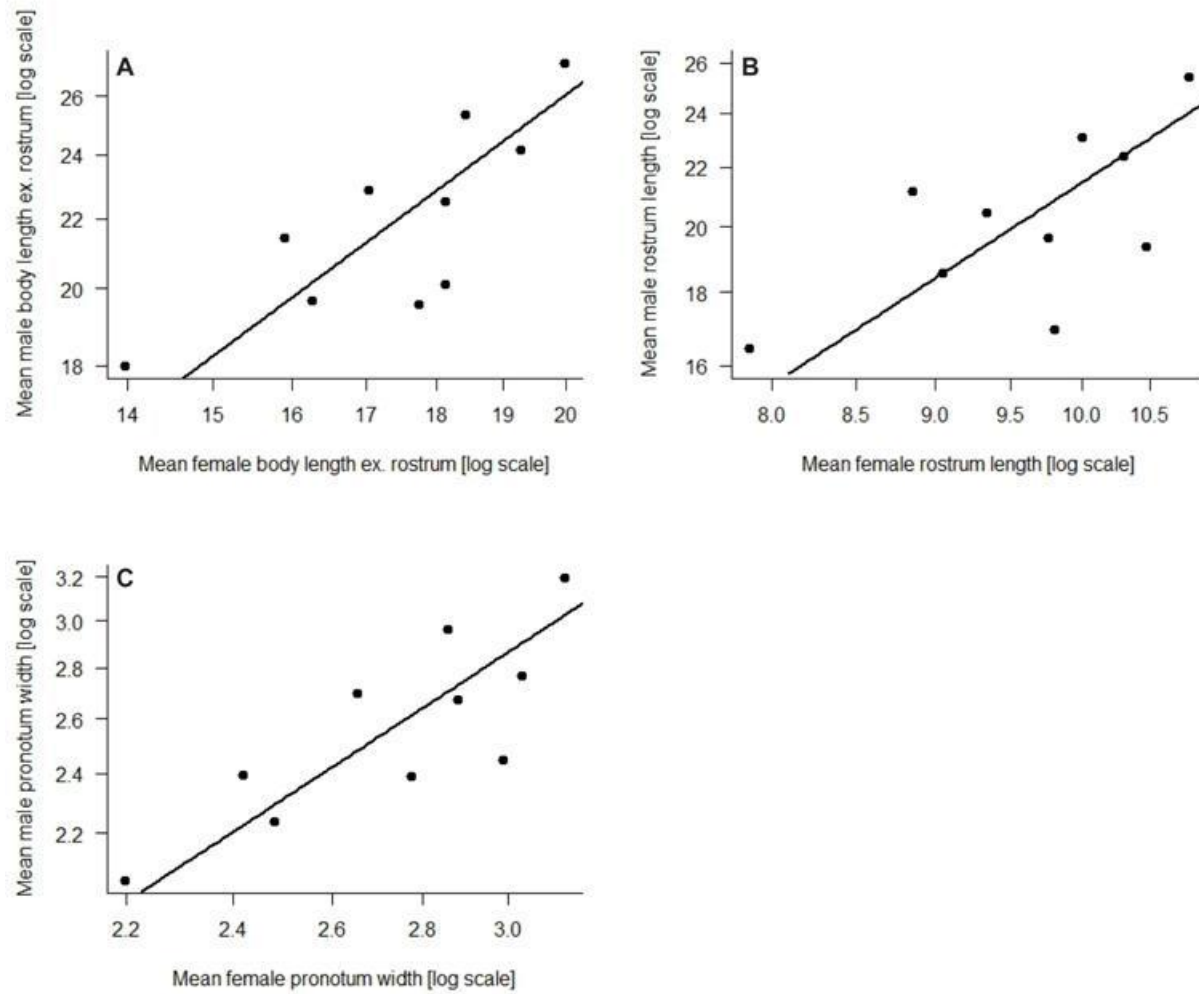
Figure 7. Sexual size dimorphism allometry from ten *L. barbicornis* populations for three body size measurements. (A) Body length ex. rostrum, (B) Rostrum length, (C) Pronotum width.

*6.4.5 Latitudinal variation in weapon investment*

The scaling relationship between weapon size (rostrum length) and body size (pronotum width) for males decreased across the latitudinal cline ($\beta = -0.038$, $F=17.47$, $p = 0.002$, $R^2 = 0.66$; Table 8, Figure 8). There was no change in slope across latitudes for females ($\beta = 0.002$, $F=0.19$, $p$

=0.89, R²=0.002). Least-squares regression was used for these analyses because the data used to calculate the scaling relationships were previously logged, therefore removing any statistical artefacts associated with scale between the sexes. Post-hoc multiple comparisons with a conservative Sidak correction (Warton et al 2012) showed that males from Puketi/Omahuta (north) had a significantly steeper allometric slope than those found in Palmerston North (south, $p = 0.0002$). Males from Matuku Reserve also had steeper allometric slopes than males from Pureora ($p = 0.02$) and Palmerston North ($p = 0.001$). Removing the Sidak correction for multiple comparisons revealed significant differences in allometric slopes between nine other populations.
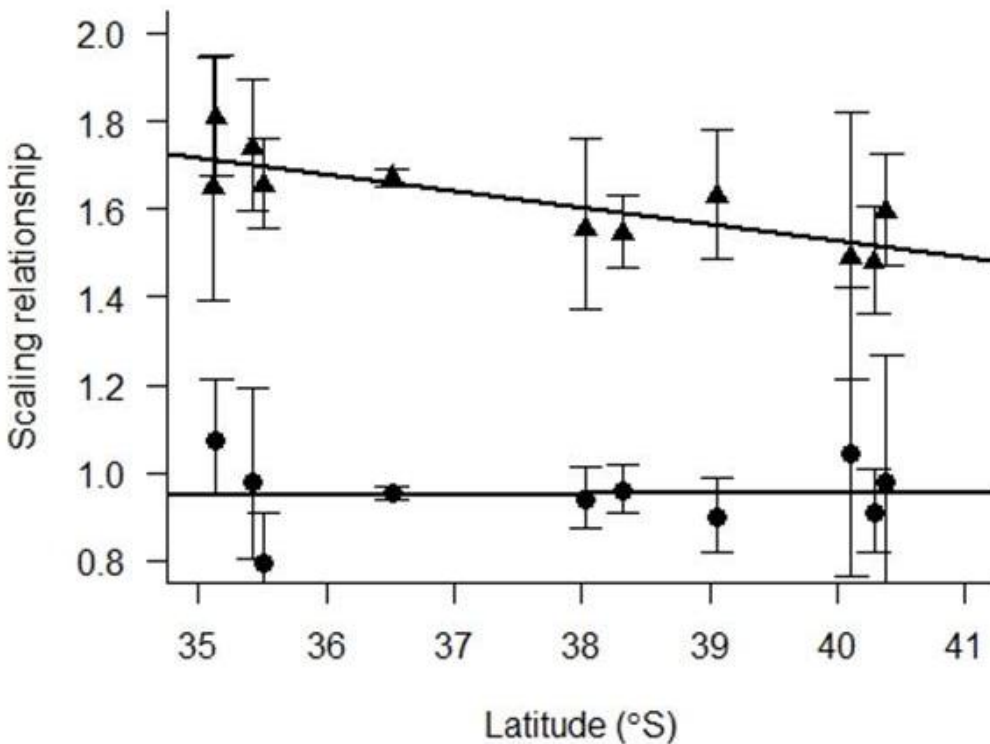


Figure 8. Scaling relationship (± 95% confidence intervals) between weapon and body size varies across latitudes for male (▲) and female (●) *L. barbicornis*.

Table 8. Allometric slopes for male and female *L. barbicornis* across 11 populations.

| Males | | | | |
|---|---|---|---|---|
| Population | N * | Slope (95% CI) | Slope elevation (95% CI) | R² |
| Mangamuka Forest | 18 (0) | 1.65 (1.39-1.95) | 0.64 (0.54-0.73) | 0.90 |
| Omahuta/Puketi Forest | 42 (2) | 1.81 (1.68-1.95) | 0.64 (0.60-0.68) | 0.94 |
| Pukenui Forest | 23 (0) | 1.74 (1.60-1.90) | 0.65 (0.60-0.70) | 0.96 |
| Bream Head | 52 (3) | 1.65 (1.56-1.76) | 0.69 (0.66-0.73) | 0.95 |
| Matuku Reserve | 983 (4) | 1.67 (1.65-1.69) | 0.64 (0.63-0.65) | 0.97 |
| Maungatautari Forest | 24 (2) | 1.55 (1.37-1.76) | 0.66 (0.58-0.74) | 0.92 |
| Pureora Forest | 52 (2) | 1.55 (1.46-1.63) | 0.63 (0.59-0.66) | 0.96 |
| Ratapihipihi Reserve | 19 (1) | 1.63 (1.49-1.78) | 0.60 (0.53-0.66) | 0.97 |
| West Ruahine Ranges | 11 (1) | 1.49 (1.21-1.82) | 0.66 (0.52-0.80) | 0.93 |
| Palmerston North | 24 (6) | 1.48 (1.36-1.61) | 0.66 (0.60-0.72) | 0.97 |
| Lake Papaitonga | 24 (1) | 1.59 (1.47-1.73) | 0.61 (0.56-0.66) | 0.97 |
| Females | | | | |
| Population | N | Slope (95% CI) | Slope elevation (95% CI) | R² |
| Mangamuka Forest | 4 | NA[1] | NA[1] | NA |
| Omahuta/Puketi Forest | 21 | 1.07 (0.95-1.21) | 0.53 (0.48-0.57) | 0.93 |
| Pukenui Forest | 8 | 0.98 (0.81-1.20) | 0.57 (0.49-0.65) | 0.96 |
| Bream Head | 25 | 0.80 (0.70-0.91) | 0.64 (0.60-0.68) | 0.90 |
| Matuku Reserve | 653 | 0.96 (0.94-0.97) | 0.56 (0.56-0.57) | 0.96 |
| Maungatautari Forest | 14 | 0.94 (0.87-1.02) | 0.56 (0.53-0.59) | 0.99 |
| Pureora Forest | 43 | 0.96 (0.91-1.02) | 0.55 (0.52-0.57) | 0.97 |
| Ratapihipihi Reserve | 17 | 0.90 (0.82-0.99) | 0.59 (0.55-0.62) | 0.97 |
| West Ruahine Ranges | 8 | 1.04 (0.77-1.42) | 0.52 (0.37-0.67) | 0.90 |
| Palmerston North | 15 | 0.91 (0.82-1.01) | 0.58 (0.54-0.63) | 0.97 |
| Lake Papaitonga | 9 | 0.98 (0.75-1.27) | 0.56 (0.44-0.67) | 0.91 |

* numbers in brackets are the number of males larger than 70 mm total body length not included in calculations of SMA slope
[1] the allometric slope was not calculated for females from Mangamuka due to small sample size

## 6.5 DISCUSSION

This study has shown significant geographic variation in body and weapon size, and sexual size

dimorphism among populations of giraffe weevils. Most interestingly, to my knowledge this

study is the first to determine variation in weapon allometry along a latitudinal cline. This is also one of only a small number of studies that combine aspects of within-species size variation in relation to both Bergmann's and Rensch's rules.

*6.5.1 Latitudinal cline in weapon allometry*

To my knowledge this is the first study to look at how males shift investment in weaponry across populations in relation to a latitudinal body size cline. Although overall mean body size and mean weapon size increased at higher latitudes, relative investment into weaponry by larger males decreased, as shown by the decline in allometric slope. Females however, showed no change in the relative rostrum length across latitude. A handful of previous studies have shown that investment in secondary sexual traits can vary between populations (e.g. Moczek & Nijhout 2003; Svensson et al. 2004; Tomkins & Brown 2004; Miller & Emlen 2010), but these studies do not specifically address temperature as being a possible selection pressure driving variation in exaggerated traits in these species. Instead Miller and Emlen (2010) showed that variation in leg size of *Leptoscelis tricolor* bugs related to the availability of host plants, and Tomkins and Brown (2004) found that population density correlated with a shift in the switchpoint of male morphs in *Forficula auricularia* earwigs. Moczek and Nijhout (2003) also suggest density as a possible driver of weapon size evolution in *Onthophagus taurus* dung beetles, although they did not test explicitly for this. Svensson et al. (2004) measured wing spot variation in relation to the strength of natural and sexual selection between populations of *Calopteryx splendens* damselflies, and suggested that variation in this trait is likely to be due to genetic divergence in response to selection.

There are several explanations for the observed changes in *L. barbicornis* scaling relationship over latitude. Firstly, it is possible that selection pressures vary across the cline which drives increasing body size on the one hand, but decreasing investment into weapon size on the other hand. Selection would lead to genetic differences between the populations, resulting in different phenotypic expression of body and weapon size. For example, Molina-Borja et al. (2010) found that body size of *Gallotia caesaris* lizards varied between populations in relation to the local vegetation structure, suggesting that different ecological pressures between these habitat types has driven adaptation to vegetation type for each population. If an environmental factor important for selection on rostrum length in male *L. barbicornis* such as habitat type, population density, or sex ratio, varied with latitude, this could drive the observed decrease in relative rostrum length.

Alternatively, relative weapon size may be traded-off with overall body size, such that as males invest more into body size at higher latitudes, they invest less into relative weapon size. It isn't clear exactly what would drive this trade-off, although the limitation of resources available for growth in holometabolous species such as this could provide an explanation. Beetles are holometabolous and do not generally grow in size after metamorphosis into the adult stage. Most of the growth in larvae occurs in the stage immediately before pupation, and much of the resources allocated to structures that eventually become adult body parts actually occur after the larva has ceased feeding (Nijhout & Wheeler 1996). The life history of Coleoptera means that the size of each adult body trait derives from a limited pool of nutrients allocated to each trait, and that each body trait grows at the expense of overall size (Nijhout & Wheeler 1996; Bonduriansky 2007b). Some species have been shown to exhibit trade-offs in trait sizes, such that the growth of large weaponry results in the reduction of other traits such as testes, wings or

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eyes (Emlen 2001; Moczek & Nijhout 2004; Simmons & Emlen 2006; Simmons et al. 2007; Yamane et al. 2010; Pizzo et al. 2012). In an experimental study, Simmons and Emlen (2006) found that when horns were prevented from growing in male *Onthophagus nigriventris*, overall body size was larger indicating a resource allocation trade-off between weapon size and overall size. It could therefore be possible that giraffe weevil males investing relatively more into overall body size at higher latitudes have fewer resources to allocate directly to metabolically expensive weapon size (rostrum length). As explained above, while breeding experiments are difficult for *L.barbicornis*, it is possible to look at relative changes in body size and weapon scaling relationships within seasons and over several years, and determine whether these traits fluctuate over shorter periods of time within a single population. For example, if between years a decrease in body size matches an increased slope in the scaling relationship, such as that which was described over a latitudinal cline, this would suggest that individuals are largely responding to environmental conditions through plasticity, rather than through selection. These ideas are explored in a subsequent study for a single population of *L. barbicornis* (Chapter 7).

Another explanation for the decreasing weapon allometry, that is not mutually exclusive from the trade-off hypothesis, concerns the level of condition-dependence of rostrum size. Sexually selected traits are more sensitive to environmental variation and resource stress, and therefore show a high level of condition-dependence (Cotton et al. 2004a; Bonduriansky 2007a; Cothran & Jeyasingh 2010). In regions or in times of high environmental stress, secondary sexual trait size should be more variable in size within the population, because as resources become limited the genetic variation between males in the ability to acquire and allocate resources will become more pronounced as shown by phenotypic variation in trait size (Cotton et al. 2004b). At higher latitudes, where cooler climates presumably inflict a higher level of stress on developing *L.*

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*barbicornis* than in warmer climates, a higher proportion of males should be affected by resource-limitation, and there should be fewer overall resources to acquire and allocate to body parts. As rostrum size is likely to be under direct sexual selection, and therefore highly condition-dependent, any change in size due to stress would be more pronounced than changes in non-sexual traits and overall body size. This would subsequently result in a decreasing scaling relationship along the latitudinal cline if rostrum size is more susceptible to environmental stress, relative to pronotum width as a proxy for overall body size.

### 6.5.2 Latitudinal cline in body size and Bergmann's rule

I found that giraffe weevil body size varies in accordance with Bergmann's rule, such that both males and females have larger mean body sizes at higher latitudes, and this relationship was strong, particularly for males. Despite many taxa following Bergmann's cline, it is not well-understood what mechanism/s drive these patterns. Although I was unable to measure environmental variables at each site, temperature decreases with latitude along the length of the North Island of New Zealand, suggesting its importance in driving body size in the giraffe weevil. Rainfall did not correlate with latitude, so is therefore unlikely to be driving the variation in body size. Although temperature correlates with latitude, it is still unknown exactly how it could drive the size clines observed as Bergmann's rule. The current hypotheses to explain the effects of temperature are divided into adaptive and non-adaptive processes. Some species follow Bergmann's cline in common garden experiments as well as in nature, or have evolved rapidly in new locations, suggesting an adaptive, genetic basis for size variation (e.g. Huey et al. 2000). An overarching explanation for how local adaptation can drive Bergmann's clines is however difficult to explain because while selection (fecundity, sexual, and viability) can drive large size

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it isn't clear why this should follow a temperature gradient and favour large size at cooler

climates and vice versa (Blanckenhorn & Demont 2004). Tsuchiya et al. (2012) found that most

of the differences in body size between populations of *Carabus tosanus* beetles were due to

genetic differences rather than developmental plasticity. They suggest that selection operating

across the cline could be related to adaptive reduction in body size at higher altitudes because of

lower food abundance or limitation during the larval developmental period.

Alternatively, body size according to Bergmann's rule may be the result of differences in

physiological processes at the cellular level which can be affected by temperature. Again these

are not overly well-supported hypotheses, but several authors argue that physiological constraints

in relation to temperature rather than adaptive processes can drive Bergmann clines. Perrin

(1995) in particular shows mathematically that if metabolism is faster at higher temperatures, but

the rate of food acquisition does not change, then individuals at warmer temperatures should

mature faster with overall smaller size than individuals in cooler climates. Similarly, van der

Have and de Jong (1996) showed that the rate of development (cell differentiation and cell

division) is faster at higher temperatures while growth rate is less temperature-dependent, which

could result in individuals in warmer climates maturing faster at a smaller size than their

counterparts at cooler climates. Overall these non-adaptive hypotheses concern constraints on

growth and development in relation to temperature or any other environmental variable that co-

varies with latitude. Atkinson and Silby (1997) also discuss the possibility that the observed

smaller body sizes could be driven by a limitation in another resource that is less available at

higher temperatures. For example, water availability could constrain growth despite overall

growth rate being initially faster at higher temperatures. This could cause a direct constraint on

growth, or could result in selection for the ability of individuals to mature at a smaller size before

resource limitation applies. While this hypothesis could certainly apply to *L. barbicornis*, water availability is unlikely to be causing the observed cline as rainfall did not correlate with latitude across the range of this species.

Although there was strong evidence for increasing overall body size at higher latitudes in accordance with Bergmann's rule, it was not possible to differentiate between the relative contributions of genetic adaptation to local selection pressures, and developmental plasticity and physiological constraint. Studies addressing this complexity have rarely been conducted because of the difficulties in differentiating between these factors, and authors must avoid making conclusions about the adaptive basis to clines using purely phenotypic data (Stillwell 2010). To further understand the role and interactions of genetic versus environmental factors in shaping body and weapon size variation, breeding experiments are necessary, conducted under different temperature regimes using representatives from populations at different latitudes. This has been conducted several times for other species (e.g. Fairbairn 2005; Hallsson & Bjorklund 2012; Tsuchiya et al. 2012). Unfortunately, giraffe weevils have a long larval developmental time of at least two years (Appendix 1), which makes these breeding experiments difficult.

Chown and Gaston (2010) suggested that large-bodied species with long generation times were more likely to show converse-Bergmann clines, because these species are more likely to be affected by season length than short-lived species that have multiple generations per year. Evidence for this hypothesis has been found in several studies (Blanckenhorn & Fairbairn 1995; Blanckenhorn & Demont 2004; Shelomi 2012). The life-history of *L. barbicornis* suggests that, according to this hypothesis, they should follow the converse-Bergmann rule, but this was not found to be the case, despite larvae being long-lived with a generation time of at least two years (Appendix 1). However, the definition of "long development time" is important, as all of the

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species studied in Blanckenhorn and Demont (2004) had development times of less than one year. *L. barbicornis* may not follow the converse-Bergmann rule because development over several years with fluctuating temperatures may actually remove the effect of season length. However, overall lower mean temperatures at southern sites could still drive larger body sizes than northern sites if there was temperature-driven selection or developmental plasticity in response to temperature.

*6.5.3 Latitudinal cline in sexual size dimorphism*

Although sexual selection is likely to be driving the male size bias in *L. barbicornis* (especially in relation to rostrum and overall length), there is not a clear link between latitude and SSD. When controlling for differences in scale between the sexes (i.e. by logging male and female body size and using an index of SSD), there was no evidence that males are diverging in size to a greater extent than females, or that there is directional selection on large size operating primarily on one sex. However, despite the lack of correlation in SSD and latitude, there was high variability in SSD between populations. There could, therefore, be differences in selection pressures such as density or sex ratio between populations that are driving the variability in SSD.

*6.5.4 Variation in accordance with Rensch's rule*

Rensch's rule predicts that in species where males are the larger sex, the level of SSD should increase with body size (Fairbairn & Preziosi 1994). In particular, conformation to Rensch's rule is tested by regressing male and female body size against each other, with an allometric slope >1 providing evidence that male size increases faster relative to female size due to sexual selection (Fairbairn & Preziosi 1994; Fairbairn 2005). In this study the allometric slope comparing male to

female size was not found to be significantly different from 1 (isometry). The very wide

confidence intervals found could be due to issues with power because of the relatively small

sample sizes found for each population combined with the level of extreme size variation in the

species. However, assuming the data are representative of population differences and Rensch's

rule does not apply to the giraffe weevil, the isometric relationship in body size between the two

sexes suggests body size in both sexes are evolving at the same rate over evolutionary time,

possibly due to equally strong fecundity selection for large female size in relation to sexual

selection for large male size. Alternatively, body size may be highly genetically correlated

between the sexes (Kraushaar & Blanckenhorn 2002). The positive correlation between *L.*

*barbicornis* male and female mean body size does, however, show that environmental conditions

and selection operate in the same direction for both sexes across populations (Teder & Tammaru

2005).

Rensch's rule was first used to describe variation in SSD at the interspecific level, and has only

rarely been used in intraspecific studies to determine patterns in among-population variation in

SSD. These studies have found mixed results (Fairbairn & Preziosi 1994; Kraushaar &

Blanckenhorn 2002; Fairbairn 2005; Young 2005; Blanckenhorn et al. 2006; Pyron et al. 2013).

Blanckenhorn et al. (2006) found that only around half of the species studied in their meta-

analysis conformed to Rensch's rule, and therefore concluded that intraspecific variation in SSD

does not generally conform to Rensch's rule. For Rensch's rule to be apparent at the intraspecific

level there must be genetic differences between populations that are consistent enough over time

to drive stable evolutionary divergence in SSD. However, phenotypic plasticity, gene flow

between populations, and temporal variability in selection acting differently on each sex can all

reduce the likelihood of this occurring (Blanckenhorn et al. 2006). Therefore, it is more likely to

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find evidence of Rensch's rule at the interspecfiic level, because there is a much longer time for evolutionary divergence to occur (Fairbairn 2005).

*6.5.5 Conclusions*

This study provides a novel contribution to the literature relating to Bergman's rule and secondary sexual trait variation, as this is the first example demonstrating that weapon allometry can vary across a latitudinal cline. While I can only speculate at the likely mechanisms driving these patterns, it is likely that variation in body size and the correlated but opposite trend in weapon allometry variation are due to developmentally plastic responses to environmental change. Combining the study of variation in exaggerated trait allometry, and Bergmann's and Rensch's rules, provides a unique avenue to the investigation of each of these. It also highlights the possibility that the patterns identified here for *L. barbicornis* may also be important for other species with exaggerated traits. This is an exciting area of research and could provide a new focus for future studies on the variation and plasticity of exaggerated traits.

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# CHAPTER 7

## Temporal variation in body size and weapon allometry in the giraffe weevil

## 7.1 ABSTRACT

Sexually selected traits are expected to evolve rapidly in space and time as selection pressures can vary both temporally and spatially. Along with temporal variation among generations, the expression of these traits can also fluctuate significantly within a breeding season in response to decreasing energy and resources available for allocation to their development and maintenance. This study documents patterns of temporal variation in body and weapon size, and in weapon allometry for the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*). *L. barbicornis* are highly sexually dimorphic, mostly due to the extreme elongation of the male rostrum used as a weapon during contests for access to females. Temporal variation was studied across three years for a single wild population of *L. barbicornis*, and at a finer-scale within each breeding season. Specifically it was predicted that body size and rostrum allometry would decrease during a breeding season, but little evidence was found for either of these hypotheses. Instead it was found that body size and rostrum allometry varied significantly both between years, and between months during the breeding season, but did not follow a consistent pattern. There was weak evidence to suggest that body size is driven by climate factors such as temperature and rainfall. Weapon allometry in males was more variable between years and over the breeding season than females, suggesting that rostrum size in males may be more susceptible to environmental change than in females. I speculate that temporal variation in this species is a plastic response to changes in environmental conditions, with some evidence of a trade-off between body and weapon size during times of resource stress.

## 7.2 INTRODUCTION

Males of many animal taxa display exaggerated secondary sexual traits that are used as either ornaments to attract mates, or weapons to fight for them (Andersson 1994). Darwin (1871) identified early on that these elaborate structures had evolved via sexual selection, through intense male-male competition and female choice. However, it is also recognized that exaggerated secondary sexual traits represent a balance between natural (viability) and sexual selection (Endler 1980; Andersson 1994). This is because while sexual selection often drives traits to become more conspicuous for the attraction of females and for signaling to rivals, natural selection also favours traits that maximize survival and viability, for example by reducing conspicuousness to predators (Endler 1980, 1982).

Natural and sexual selection pressures can vary over space and time, and will therefore vary in how much they contribute to the evolution of trait expression (Svensson et al. 2004). In addition, if there is a shift in the balance between these two selective forces then it might be expected that the evolution of the trait could follow a new trajectory (Svensson & Gosden 2007). For example the removal of an important predator from a population could relax natural selection against a secondary sexual trait, allowing sexual selection to drive its elaboration as it is no longer constrained by natural selection (Svensson & Gosden 2007). Changes in exaggerated trait size can occur rapidly over only several generations if changes in selection pressures are strong. For example, Hegyi et al. (2006) showed a linear decrease in forehead patch size in collared flycatchers (*Ficedula albicollis*) over 15 years and showed that this was likely a response to directional selection as a result of decreased survival of males with large patch sizes. While changes in trait size can also be the result of phenotypic plasticity in response to environmental

change, for this species this was unlikely because forehead patch size is not condition dependent and did not vary within individuals over time.

Several studies have shown that secondary sexual traits can vary significantly between populations, which suggest that these traits can evolve rapidly in response to changes in selection pressures (e.g. Moczek & Nijhout 2003; Svensson et al. 2004; Tomkins & Brown 2004; Miller & Emlen 2010). However, studies looking at temporal changes in absolute and relative trait size are much rarer (but see Møller & Szép 2005; Hardersen 2010; Miller & Emlen 2010; Hardersen et al. 2011). Secondary sexual traits are costly to produce and as honest signals of quality and resource holding potential, should also be condition dependent (Cotton et al. 2004a; Bonduriansky 2007). Therefore, exaggerated traits should be highly responsive to changes in environmental conditions, which has been shown in several breeding experiments (Emlen 1996, 1997; Bonduriansky 2007). Variation in the allocation of resources to the development of secondary sexual traits should be an adaptive plastic response to variation in environmental condition, therefore allowing individuals to adjust their investment and maximize fitness (Hardersen 2010).

The production of secondary sexual traits often comes at the expense of the size of other body parts, such as testes and eyes (Emlen 2001). Holometabolous insects are particularly susceptible to these trade-offs because most of the growth of structures that will become adult body parts develop in a closed system after feeding has ceased (Nijhout & Wheeler 1996). Resource allocation is therefore constrained by the quality and quantity of nutrition that was acquired during earlier larval stages. Of particular importance to studies of the variation in body and weapon size over time and space is the trade-off between secondary sexual traits and overall body size. This was demonstrated clearly in a study that experimentally reduced weapon size by

cauterizing the cells that would otherwise have grown into a horn in adult *Onthophagus nigriventris* dung beetles, causing an overall increase in body size (Simmons & Emlen 2006). Therefore, any observed variation in weapon size between populations or within populations could be coupled with a relative shift in body size. Furthermore, exaggerated traits have also been shown to be condition dependent and are highly responsive under resource stress (Cotton et al. 2004a; Cothran & Jeyasingh 2010). Overall investment into weapon size under stressful conditions is often lower than in optimal conditions, and if weapon size is more affected by stressful conditions than overall body size, there could be an observed shift in weapon allometry.

While studies on temporal variation in secondary sexual traits are rare, the body size of insects has generally been shown to decline in size across an emergence season, such that early emerging individuals are on average larger than those that emerge late (Rowe & Ludwig 1991; Hardersen 2010; Hardersen et al. 2011; Steele et al. 2011; but see Rantala et al. 2001; Miller and Emlen 2010). Rowe and Ludwig (1991) proposed that this decrease was an adaptive response to the conflict between size and age, such that plasticity in development time should allow individuals to emerge at the best size according to a trade-off of costs and benefits. Consequently larvae that are large in size at the beginning of the season should emerge early as the benefits of gaining further mass during the season are outweighed by the costs of delaying emergence. In comparison, larvae that are small at the beginning of the season should delay emergence to increase body mass. This is despite the increased risk of larval mortality and the possibility of finding fewer mates late in the season. Wood-boring species face a particular conflict in that they are long-lived and larvae that are still small late in an emergence season must trade-off eclosing at a small size, or delaying maturation to the following season at potential risk of mortality (Walczyńska et al. 2010).

An alternative hypothesis is that body size variation is a physiological response to temperature, because at warmer temperatures development rate is faster relative to growth rate such that individuals mature at a smaller size than if they had developed in cooler temperatures (Perrin 1995; van der Have & de Jong 1996). In relation to seasonal variation, warmer temperatures in the later summer months may accelerate development, such that individuals emerge at smaller sizes (Rowe & Ludwig 1991). Variation in weapon or body size over time can also be a simple non-adaptive response to fluctuations in environmental conditions, such as the availability of nutrients in years of resource stress (Steele et al. 2011). Distinguishing between adaptive responses to selection or environmental pressure in comparison to physiological and developmental constraints in development requires breeding experiments, although these are rarely conducted and many studies remain correlational and speculative about the mechanism driving variation (but see Fairbairn & Preziosi 1994; Fairbairn 2005).

In this study, the extent of temporal variation in body and weapon size was determined for the giraffe weevil *Lasiorhynchus barbicornis* (Coleoptera: Brentidae), a large-bodied, wood-boring beetle from New Zealand. *L. barbicornis* adults are highly size variable, with males varying from 14.7 – 90 mm and females from 12.5 to 50.4 mm in total body length (Chapter 2; Kuschel 2003). Adults also show extreme sexual dimorphism, mostly due to the elongation of the male rostrum and overall body length (Chapter 2). Males use their rostrum as a weapon during agonistic contests with other males, and both overall body size and rostrum length are important predictors of fight success (Chapter 5). Large rostrum size in males is therefore an indicator of resource holding potential and quality, and honest signals such as weapons are expected to be costly to produce (Zahavi 1975). The costliness of rostrum production is supported by the previous finding that the scaling relationship begins to asymptote at the upper end of the slope due to a

decrease in the relative rostrum size for the largest males, likely due to resource limitation (Chapter 3). Variation in environmental conditions within and between seasons could therefore have a large effect on resource acquisition, and subsequent allocation shown as a trade-off between overall body size and the energetically costly rostrum.

Both body and weapon size were previously found to be highly variable between populations of *L. barbicornis*, and that the populations with the largest mean body size also had the smallest relative rostrum sizes in males (Chapter 6). Here I follow on from the previous study by documenting patterns of temporal variation in the size and scaling relationship between body and weapon size for adult *L. barbicornis* both within and between seasons for a single wild population. I first determine annual variation in the size and scaling of weapon and body size over three years, and then focus on fine-scale seasonal variation between months over the three year period. By replicating the measurements of finer-scale, mean monthly body and weapon size over three years, it is possible to determine if variation is consistent and predictable. Specifically I determine whether body size and weapon allometry vary according to the Rowe and Ludwig (1991) hypothesis, which predicts that individuals are on average larger at the beginning of the season. Similarly, the relative investment in weapon size (slope of scaling relationship) is expected to decline during the breeding season as predicted by Hardersen (2010). It is also determined whether in years of increased mean body size there is an observed decrease in the allometric slope for rostrum length and vice versa, which would suggest a trade-off between overall body size and relative weapon size. This analysis of body size and weapon allometry over three years can be used to show that these traits can vary over multiple temporal scales, and can be used to compare to larger-scale geographic patterns of intraspecific variation in *L.barbicornis.*

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## 7.3 METHODS

### 7.3.1 Morphological measurements

This study was conducted at Matuku Reserve, which is 120 hectares of predominately lowland coastal broadleaf forest west of Auckland, New Zealand. Surveys were conducted during three breeding seasons between November and April in the summers of 2010-2011, 2011-2012, and 2012-2013. In October 2010 I located 16 karaka (*Corynocarpus laevigatus*) trees throughout the reserve that had reliable aggregations of adult *L. barbicornis*. Between November 2010 and April 2011 surveys were carried out approximately every seven days (weekly), although this was weather-dependent and field work was avoided during days of, and immediately after heavy rain as *L. barbicornis* become difficult to find. Due to logistical difficulties, surveys were less frequent in March and April, and were conducted up to fortnightly. During these surveys I visited each of the 16 trees, and located and removed all weevils present. All weevils were measured, and released back onto the tree after being painted with a unique combination of colours using Queen Bee Marking paints (Lega, Italy; Appendix 1). Marking the weevils ensured that each individual was only measured once, and therefore I can be confident that the data set for the following analyses represents different individuals. Measurements were made using digital calipers to the nearest 0.01 mm. Rostrum size (RL, weapon size) was measured in males and females as the total length from the anterior end of the mandibles to the base of the head behind the eyes. Body size measurements were total body length minus rostrum length (BL, thorax through to end of elytra) and pronotum width (PW). Pronotum width was used in subsequent analyses as an overall measure of body size, as this is the most common body size character used in other studies of beetles (Eberhard & Gutierrez 1991; Moczek & Emlen 2000;

Tomkins et al. 2005; McCullough et al. 2012). As previously reported, pronotum width was highly correlated with all other body size measurements, and all measurements were made with high repeatability (0.99, Chapter 2 & 3). Weekly surveys between November and April were repeated in the 2011-2012 and 2012-2013 seasons, although the subset of sampling trees varied as some trees no longer hosted aggregations of adult weevils after the 2010-2011 season. In 2011-2012 there were 15 trees, and in 2012-2013 eight trees were used. As I was only interested in temporal patterns in mean body and weapon size it was not important if overall abundance varied between seasons. Only newly encountered weevils (unpainted) were included in every weekly survey as an attempt to only include recently emerged adults in each analysis of mean body size and scaling relationship.

*7.3.2 Statistical analysis*

Analyses were conducted to compare temporal variation in body and weapon size and scaling relationship at two time scales: (1) annual variation over three years and (2) monthly variation over six months between November and April in 2010-2011, 2011-2012, and 2012-2013. All statistical analyses were conducted in R 2.15 (R Core Team 2013).

The variation in mean (untransformed) body length, pronotum width, and rostrum length between years and months was analysed using one-way ANOVA's and Tukey post hoc comparisons.

The difference in scaling relationship of rostrum length and body size (pronotum width) was then characterised for males and females between years and months by calculating the slope using the R package *smatr* Version 3.3 (Warton et al. 2012). This package was developed to analyse allometry data, where simple linear regression is not suitable because the aim is to estimate the

line of best fit between two variables, rather than try to predict the value of one variable from another (Warton et al. 2006). Instead, this package uses standardized major axis regression (SMA), and has inbuilt functions for testing whether slopes deviate from isometry, and for determining if groups share a common slope. Pronotum width and rostrum length were log-transformed, which made the relationship linear and made it possible to use a simple power equation to determine the scaling relationship (rostrum length = $\alpha$*pronotum width$^b$). Log-transformation also allowed the comparison of male and female on the same scale (see Appendix 3 for further discussion). Importantly, all males larger than 70 mm in total body length were removed, because previously it was found that the male scaling relationship was best described by a non-linear Weibull growth curve (Chapter 3). However, the non-linear component of this model was due to a relative decrease in rostrum length only for the very largest males, and removal of these individuals' results in a linear relationship between the variables, and made it possible to use the more straightforward option of comparing slopes using SMA.

Likelihood ratio tests in *smatr* were used to test for significant differences in scaling relationships (SMA slopes) between groups (months and years) that are analogous to analysis of covariance (ANCOVA) models. Instead of following an *F* distribution, the likelihood ratio tests use a chi-square distribution (Warton et al. 2006). When significant differences were found between groups, multiple comparison tests with Sidak correction were used to determine specific differences between those groups.

The larval development period of *L. barbicornis* is at least two years (Appendix 1); however it is not known when specific climate variables are important for growth. As a preliminary investigation into the relationship between climate (temperature and rainfall) and body size I chose the annual mean of each climate variable in the year prior to emergence. These data were

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obtained from the National Institute of Water and Atmosphere Research CliFlo database (NIWA 2014). The nearest weather station with complete data for those years studied was located in Mangere, Auckland (-36.9618, 174.7764), approximately 29 km in a straight line from Matuku Reserve. The climate variables chosen were mean annual air temperature (°C), mean annual daily minimum temperature (°C), mean annual daily maximum temperature (°C), and total annual rainfall (mm). I conducted Pearson's correlation coefficient tests for each of these variables to the mean body length of males and females.

## 7.4 RESULTS

### 7.4.1 Body and weapon size variation between years

There was no significant difference between mean body size (body length and pronotum width) across years for males or females at Matuku Reserve (Table 1), although the $p$ values were only marginally above the 0.05 threshold for males. There was however significant variation in mean male rostrum length between years (Table 1). The Tukey's post hoc test showed that the mean male rostrum length was significantly smaller in 2011-2012 than in 2012-2013 ($p = 0.009$). Despite this significant variation, the effect size is small (Cohens $d = 0.14$).

There was significant variation in the slope of the scaling relationship between years for male *L. barbicornis* (Likelihood Ratio = 18.63, df = 2, $p < 0.0001$), but no difference was found for females (Likelihood Ratio = 0.34, df = 2, $p = 0.85$) (Table 2). Posthoc pairwise multiple comparisons showed that the scaling relationship for males collected in 2010-2011 was significantly shallower than the slope for 2011-2012 (Test statistic = 18.46, $p < 0.0001$), while there was no significant difference between any other comparisons.

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Table 1. Annual variation in body size (pronotum width, body length) and weapon size (rostrum length) of male and female *L. barbicornis* at Matuku Reserve between 2010 and 2013.

| | Year | | | $F^{\dagger}$ | $p^{*}$ |
|---|---|---|---|---|---|
| | 2010/2011 | 2011/2012 | 2012/2013 | | |
| *Males* | | | | | |
| Body length (ex. rostrum) | 19.41 (0.17) | 19.13 (0.15) | 19.72 (0.20) | 2.63 | 0.07 |
| Pronotum width | 2.29 (0.02) | 2.23 (0.02) | 2.27 (0.02) | 2.76 | 0.06 |
| Rostrum length | 17.63 (0.21) | 16.99 (0.19) | 17.95 (0.26) | 4.81 | **0.008** |
| *Females* | | | | | |
| Body length (ex. rostrum) | 16.11 (0.13) | 16.44 (0.12) | 16.13 (0.14) | 2.06 | 0.13 |
| Pronotum width | 2.56 (0.02) | 2.58 (0.02) | 2.51 (0.02) | 2.31 | 0.09 |
| Rostrum length | 8.91 (0.07) | 9.05 (0.06) | 8.91 (0.08) | 1.31 | 0.27 |

$^{\dagger}$ Degrees of freedom for ANOVA: Males = 2, 3144; Females = 2, 2262.

* $p$ values <0.05 highlighted in bold.


Table 2. Variation in the scaling relationship between rostrum length and pronotum width for male and female *L. barbicornis* over three breeding seasons at Matuku Reserve.

| | N | Slope (95% CI) | Elevation (95% CI) | $R^2$ | df | r | $p$ |
|---|---|---|---|---|---|---|---|
| *Males* | | | | | | | |
| 2010/2011 | 1218 | 1.60 (1.58 - 1.62) | 0.66 (0.65 - 0.67) | 0.95 | 1216 | 0.91 | <0.0001 |
| 2011/2012 | 1134 | 1.66 (1.64 - 1.68) | 0.64 (0.63 - 0.65) | 0.96 | 1132 | 0.94 | <0.0001 |
| 2012/2013 | 748 | 1.64 (1.61 - 1.68) | 0.66 (0.65 - 0.67) | 0.90 | 746 | 0.85 | <0.0001 |
| *Females* | | | | | | | |
| 2010/2011 | 898 | 0.95 (0.93 - 0.96) | 0.56 (0.56 - 0.57) | 0.94 | 896 | -0.21 | <0.0001 |
| 2011/2012 | 768 | 0.95 (0.94 - 0.97) | 0.56 (0.56 - 0.57) | 0.96 | 766 | -0.22 | <0.0001 |
| 2012/2013 | 599 | 0.96 (0.94 - 0.97) | 0.57 (0.56 - 0.57) | 0.96 | 597 | -0.22 | <0.0001 |

The allometric slope is the SMA regression slope (± 95% confidence interval), and the elevation is the slope intercept (± 95% confidence interval). Each slope estimate was tested for deviation from isometry (test statistic = r), $p$ values <0.05 indicates that the slopes are not isometric.

I correlated four climate variables (mean annual temperature, mean annual daily maximum temperature, mean annual daily minimum temperature and total annual rainfall) with mean body length over the three years measured. There was no significant correlation with any of the climate variables measured, probably due to weak power with a sample size of only three data points (Table 3). Despite the lack of significant correlation, there does appear to be a negative relationship between temperature, rainfall and mean body size (Figure 1). Mean body size appears to decrease with increasing temperature and rainfall for males, while there is a less obvious pattern for females.

Table 3. The correlation between climate variables and mean body length of male and female *L. barbicornis*

|  | Males | | | Females | | |
|---|---|---|---|---|---|---|
|  | r | *t* | *p* | r | *t* | *p* |
| Mean temperature | 0.96 | -3.59 | 0.27 | 0.94 | 2.87 | 0.21 |
| Min temperature | 0.99 | -5.7 | 0.11 | 0.91 | 2.16 | 0.28 |
| Max temperature | 0.91 | -2.24 | 0.27 | 0.98 | 5.29 | 0.12 |
| Total rainfall (mm) | 0.89 | -1.9 | 0.30 | 0.99 | 7.69 | 0.08 |
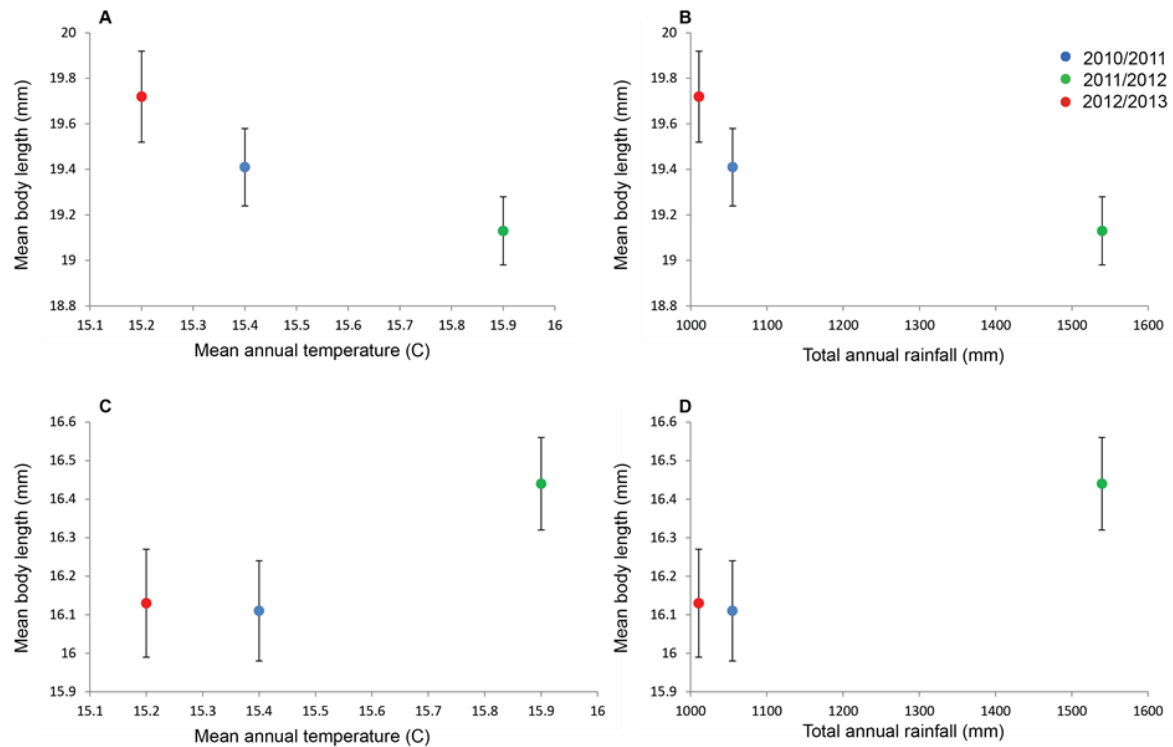
Figure 1. Correlation between body size and climate factors. Mean body length of male *L. barbicornis* compared to (A) mean annual temperature and (B) total annual rainfall. Mean body length of female *L. barbicornis* compared to (C) mean annual temperature and (D) total annual rainfall. Note that the relationship between mean body length and minimum and maximum temperature showed a similar relationship to mean temperature so is not shown here.

## 7.4.2 Seasonal variation in body and weapon size

Evidence of seasonal (monthly) variation in body and weapon size was found across the three sampling years (Table 4, Figure 2). Female body length, pronotum width and rostrum length showed significant variation between months for all years, whereas I only found significant variation in male pronotum width during the 2010-2011 season. Tukey's post hoc tests showed that in 2010/2011 males collected in November had larger mean pronotum widths than in the middle of the season in February. In the 2010/2011 season, females collected in November and

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January were larger than those collected in February for all three body measurements. In 2011/2012 females collected in November and April were larger than those collected in December for all body size traits. Specimens collected in April were also on average longer in body length than those collected in January and February. Finally, in the 2012/2013 season patterns were similar, with females collected in November and April on average larger than those collected in December for all body traits. Females collected in March also had a longer mean body length than those collected in December. The effect sizes for pairwise comparisons between significantly different months were variable, from medium to large Cohens $d$ values (0.25 – 1.1, Appendix 5).

Table 4. ANOVA $F$ statistics showing seasonal variation in body and weapon size during a six month period over three years at Matuku Reserve.

|  | 2010/2011 | 2011/2012 | 2012/2013 |
|---|---|---|---|
| *Males* | $F_{5,1129}$ | $F_{5,1141}$ | $F_{5,753}$ |
| Body length ex. rostrum | 1.57 | 1.57 | 0.75 |
| Pronotum width | 3.39** | 1.1 | 1.24 |
| Rostrum length | 1.86 | 0.63 | 0.44 |
| *Females* | $F_{5,882}$ | $F_{5,762}$ | $F_{5,593}$ |
| Body length ex. rostrum | 4.52*** | 5.94*** | 4.35*** |
| Pronotum width | 4.27*** | 4.70*** | 4.51*** |
| Rostrum length | 4.59*** | 4.06** | 3.27** |

Significant $p$ values are indicated as follows: * <0.05, ** <0.01, *** <0.001

Table 5. Tests for seasonal (monthly) variation in SMA slope across three years at Matuku Reserve.

|  | Males | | Females | |
|---|---|---|---|---|
|  | Likelihood Ratio | $p$ | Likelihood Ratio | $p$ |
| 2010/2011 | 5.55 | 0.35 | 10.3 | 0.07 |
| 2011/2012 | 17.85 | 0.003 | 13.72 | 0.02 |
| 2012/2013 | 20.84 | 0.0009 | 6.81 | 0.24 |
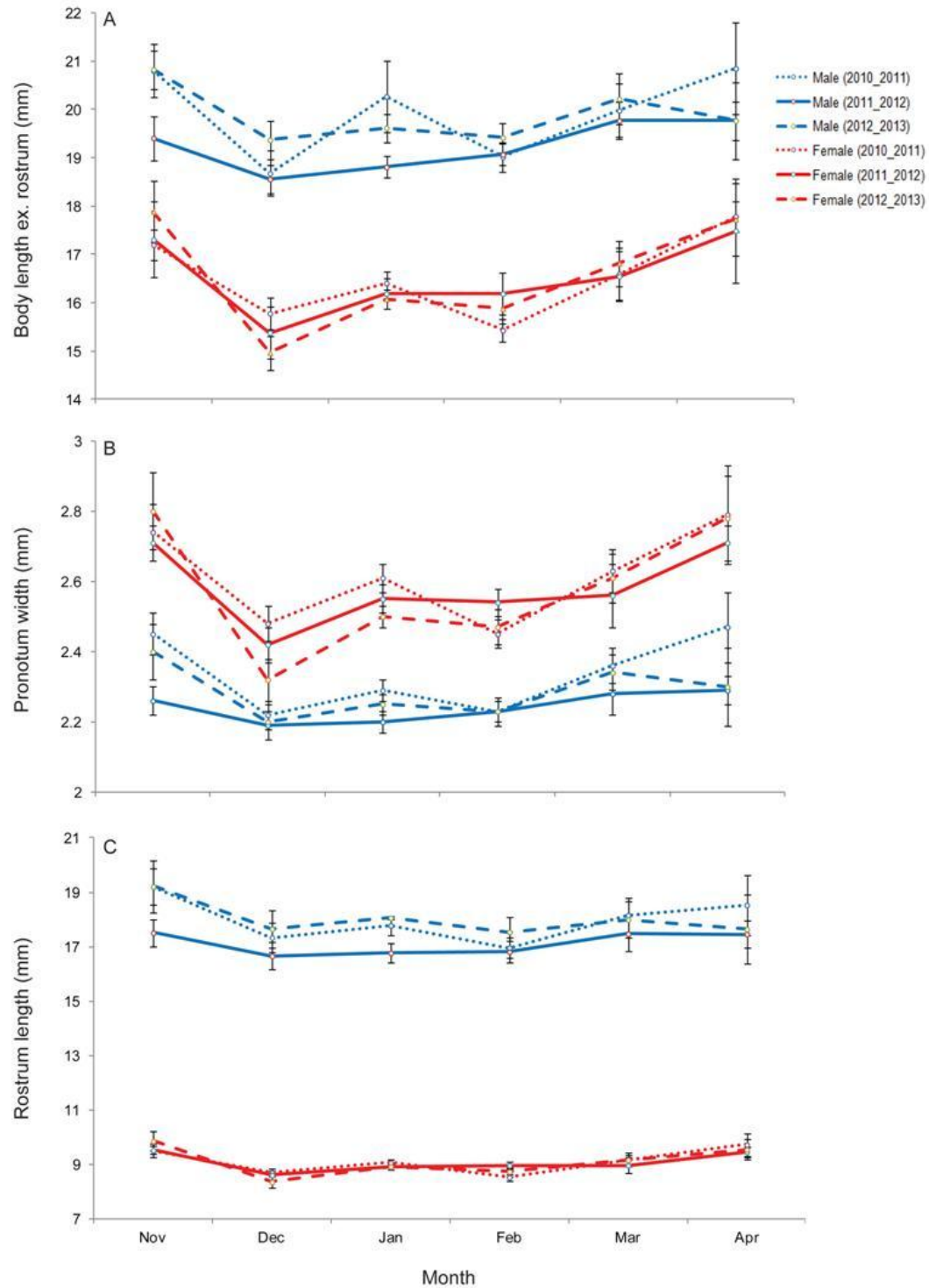
Figure 2. Seasonal variation in (A) body length minus rostrum length, (B) pronotum width, and (C) rostrum length over three years at Matuku Reserve.

Relative investment in rostrum length (i.e. scaling relationship) showed significant monthly variation in some years, as indicated by the likelihood ratio tests for a common slope (Table 5, Figure 3). Overall, the allometric slopes for males were more variable than females, shown both by the significant variation in the scaling relationship between months, and also by the generally wider 95% confidence intervals (Figure 3). Adjusted multiple comparison tests for years with significant monthly variation showed that for males, the scaling relationship was significantly shallower in March 2012 than December and January 2011, and the November 2012 scaling relationship was shallower than December 2012, January, February and March 2013. There was significant monthly variation in the female scaling relationship in 2011-2012, but the adjusted multiple comparison test determined only a marginally significant difference ($p = 0.07$) between December 2011 and February 2012. A detailed table of all SMA slopes with 95% confidence intervals is presented in Appendix 6.
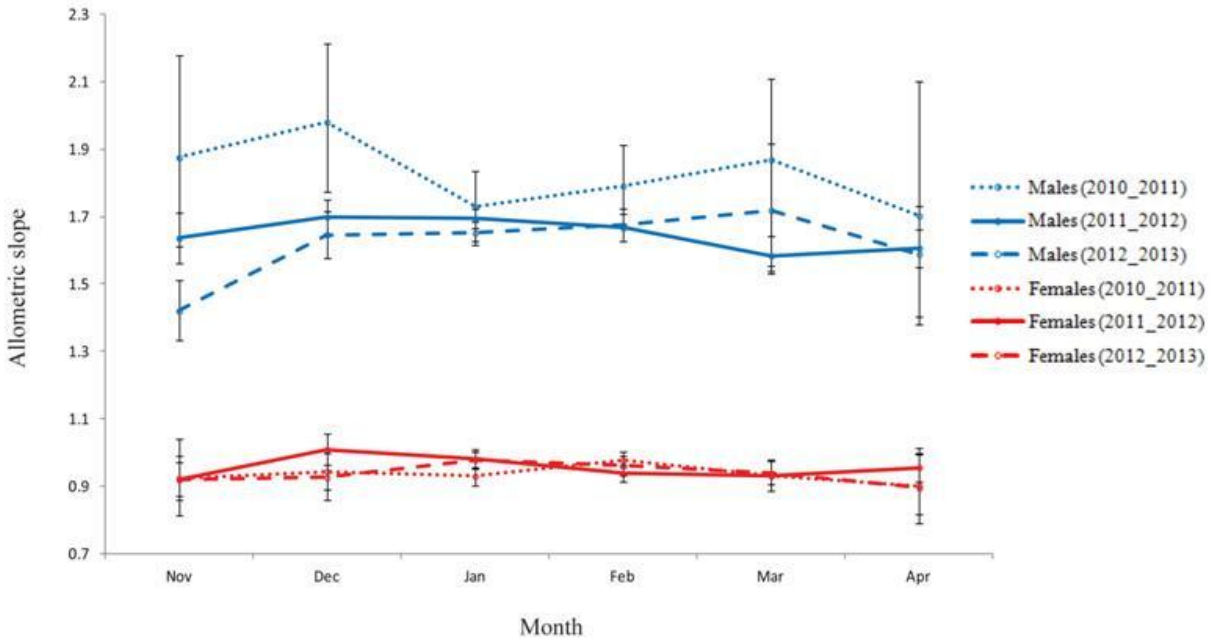
Figure 3. Temporal variation in the allometric slope (± 95% confidence intervals) for rostrum length for male and females *L. barbicornis* between November and April over three years.

## 7.5 DISCUSSION

In this study I found significant temporal variation in mean absolute body and weapon size, and in the scaling relationship of body to weapon size. Variation in body and trait size can simply represent a non-adaptive response to environmental conditions or resource availability, and the subsequent ability to acquire resources (e.g. individuals are able to grow larger in years of abundant food resources). Alternatively, phenotypic plasticity in body and weapon size can be an adaptive response to environmental change, as individuals shift phenotype to maximise fitness (Steele et al. 2011).

Scaling relationships (allometries) show the relative investment in trait size for a group of individuals, and therefore comparisons over time and space can inform us about differences in selection pressures on trait size, and how changes in environmental conditions drives phenotypic plasticity. This study is unique in that it incorporates annual variation, but also determines monthly changes within three breeding seasons to determine fine-scale variation in body and weapon size.

*7.5.1 Seasonal body size variation*

Rowe and Ludwig (1991) predict that the commonly observed declining size of insects during an emergence period is an adaptive response to the conflict between the size and age of an individual. In this study there was little support that early emerging *L. barbicornis* adults were on average larger than individuals emerging later in the season. Females were more variable in size than males, as generally males showed very little variation in mean body and rostrum size over the season. Female body size was on average larger at the beginning of the season than during the peak emergence months, which does lend support towards the Rowe and Ludwig (1991) hypothesis. However, body size increased again towards the end of the season in April. It is possible that some support for the emergence of larger individuals at the beginning of the season will be seen, and that the large body size found in April is a consequence of size-biased mortality of smaller bodied individuals as the weather cools.

The extreme size variability shown by giraffe weevil adults of both sexes may overshadow any effect of seasonality on mean body size. Wood-boring Coleoptera are known to demonstrate exceptional levels of intraspecific size variation (Andersen & Nilssen 1983), which has been shown to be due to the multiseasonality of larval development (Walczyńska et al. 2010). Wood-

boring larvae develop slowly, often over several years, due to the low quality of wood as a food source. Larvae that are small towards the end of a season experience a trade-off between emerging late in the season and suffering relatively lower egg production, or remain in the tree until the following season and risk early mortality as larvae. This trade-off explains the high level of intraspecific size variation observed in many wood-feeders, and could decrease the likelihood of finding a pattern of decreasing body size over the season if individuals can delay emergence until the following season.

*7.5.2 Seasonal weapon allometry variation*

There was little evidence that the relative investment into rostrum length decreased as the season progresses. Hardersen (2010) predicts that the relative investment into secondary sexual traits should decrease over a season as individuals emerging late in the season should have less energy to allocate to costly exaggerated traits. This was found for male *Calopteryx splendens* damselflies, which had shallower allometric slopes for wing spot size later in the season (Hardersen 2010).

In the current study it was found that in one year the scaling relationship was significantly shallower in March in comparison to the slopes from December and January, but in a subsequent year weapon allometry was most shallow at the beginning of the season in November. Overall there was little evidence of a temporal pattern in relative rostrum investment in males, and little evidence that individuals should invest less in weapon size as the season progresses. The absence of any pattern in seasonal fluctuations in weapon allometry for males suggests that any variation is likely to be a response to changing environmental conditions through the ability to acquire resources.

Interestingly, despite the lack of seasonal pattern in rostrum allometry, males did have higher levels of variation than females. Over the three years females showed very little change in rostrum allometry both between and within seasons, which shows that in comparison to males, the female rostrum is likely to be less costly and therefore less affected by shifts in resource acquisition over time. Secondary sexual traits are more sensitive to changes in environment and resource stress than non-sexual traits, and are therefore highly condition dependent (Cotton et al. 2004a). Cothran and Jeyasingh (2010) showed the differences in variability between sexual and non-sexual traits, and between males and females, in *Hyalella* amphipods. They found that male gnathopod size (a secondary sexual trait) showed much higher variability in size under conditions of high food stress in comparison to male pereopod size (non-sexual trait), and female gnathopod size. Males of high quality are expected to be able to produce larger or more elaborate secondary sexual traits than those of poor genetic quality, and therefore under stressful conditions the ability of males to acquire and allocate resources becomes more variable in accordance with male quality (Cotton et al. 2004b). If male *L. barbicornis* are allocating a large proportion of available resources to rostrum production, which is an indication of the costliness of this trait, rostrum length should also be highly variable under conditions of stress. In comparison, female rostrum length is less likely to be affected by environmental change because it is a non-sexual trait and less condition dependent.

*7.5.3 Relative investment in body and weapon size*

It was previously found that mean body and rostrum size increased along a latitudinal cline in accordance with Bergmann's rule (Chapter 6). In that study it was also found that the allometric slope for male rostrum length declined along the same cline, showing for the first time that

weapon allometry can also follow a latitudinal cline. Interestingly the populations with the largest mean body size had the smallest relative rostrum size, suggesting a trade-off between overall body size and secondary sexual trait size. While I can only speculate at this stage, a trade-off between overall body size and secondary sexual trait size could occur due to the way resources are allocated to adult body parts prior to pupation. In holometabolous insects, most of the growth of imaginal discs, which will eventually become adult body parts, occurs after the larvae has stopped feeding (Nijhout & Wheeler 1996). This means that each disc competes for a limited allocation of resources. Trade-offs between secondary sexual traits and other body parts, such as wings and testes, have been shown in multiple studies (Emlen 2001; Moczek & Nijhout 2004; Simmons & Emlen 2006; Simmons et al. 2007; Yamane et al. 2010; Pizzo et al. 2012). In addition, a trade-off between overall body size and secondary sexual trait size was shown by Simmons and Emlen (2006), such that overall body size increased when horns were experimentally prevented from growing. A trade-off between overall body size and rostrum length could, therefore, occur if *L. barbicornis* males invest more into body size at higher latitudes, thus leaving fewer resources available to allocate to weapon size.

I predicted that if this apparent trade-off was due to a plastic response to environmental change, then any observed shift in the mean body size of weevils between years should see a corresponding shift in weapon allometry following this same pattern. In the current study there was some evidence of this, as weevils measured in the 2011/2012 season had the smallest mean body size, and correspondingly steepest allometric slope. This pattern did not however follow through to within-season variation, although males showed less variation in both body size and weapon allometry at this fine temporal scale.

An alternative but not mutually exclusive hypothesis is that investment in rostrum length is highly condition-dependent, and in years of stress due to environmental change, investment in rostrum size decreases at a higher rate than overall body size causing a decrease in the scaling relationship. As already explained, resource stress can drive high variability in secondary sexual trait size because of differences between males in their ability to acquire and allocate resources (Cotton et al. 2004b). If the majority of males are negatively affected by resource stress, such that they have fewer resources to invest in rostrum size at certain times, then this would result in a decreased scaling relationship, especially if overall body size is less responsive to resource stress than weapon size.

*7.5.4 Body size in response to climate factors*

Finally, a comparison of the mean body size of males in each season to mean annual temperature and rainfall variables showed a negative correlation, while there was no consistent trend for females. Although these correlations were not statistically significant, probably due to the small sample size of only three years, the pattern shown does suggest that at least for males, individuals emerge on average smaller in warmer and wetter years. This can be compared to the spatial pattern identified in this species, which found that L. barbicornis are on average larger at higher latitudes in accordance with Bergmann's rule (Chapter 6). There was a strong negative correlation between latitude and temperature, such that individuals from cool areas were on average larger than those from warmer areas. This could be preliminary evidence to suggest that body size is the result of a physiological response to temperature. If individuals develop under warm conditions their development rate may be accelerated, despite food acquisition and therefore growth rate remaining the same (Perrin 1995; van der Have & de Jong 1996). This

would result in individuals maturing at a faster rate, but at smaller sizes. Alternatively there could be selection on body size to be smaller in years of warm temperatures and vice versa. Further evidence is required to support these ideas, both through the collection of a longer-term dataset and through breeding experiments.

*7.5.4 Conclusions*

At this stage the predictions of what drives seasonal variation in body size and rostrum scaling relationship are non-conclusive, as breeding experiments are required to differentiate between shifts in selection pressures, adaptive phenotypic plasticity, and non-adaptive responses to environmental variability. However, as it was not possible to identify any strong or consistent patterns in body size over time, I suggest that variation is due to a non-adaptive response to environmental change, either due to the ability to grow larger in years of more abundant food resources, or due to changes in cellular processes driven by temperature variation. Weapon allometry variation is also likely to be due to developmental plasticity, most likely due to trade-offs with body size in times of resource stress. Although variation in body size itself may not be adaptive, phenotypic plasticity in the relative size of weapons could be adaptive as a way of increasing fitness through survival (through increased overall body size) rather than through increased competitive ability. Overall it is unlikely that changes in natural or sexual selection explain temporal variation in body and weapon size in *L. barbicornis*. While I can only speculate at this stage, it is more likely that temporal variation is due to phenotypic developmental plasticity in response to environmental change.

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# CHAPTER 8

## General discussion

In this thesis, I have brought attention to the New Zealand giraffe weevil, *Lasiorhynchus barbicornis*, as an ideal model system for the investigation of alternative reproductive tactics (ARTs) and the evolution of exaggerated animal weaponry. Prior to this study, little was known about this species and I have contributed significantly to our understanding of its ecology, evolution and behaviour. I found that the giraffe weevil mating system is characterised by intense male-male competition for access to a limited number of females. The remarkable sexual dimorphism in this species (**Chapter 2**), and the exaggeration of the male rostrum (**Chapter 3**), is likely to be due to sexual selection acting on both rostrum length and body size, driven by their importance during contests (**Chapter 5**). Rostrum size in males was found to be steeply, positively allometric in comparison to females, and although non-linear in shape, was not found to be characterised by the dimorphism found in many other beetles with exaggerated traits (**Chapter 3**). I have shown that small males adopt sneaking behaviour in addition to guarding and fighting behaviour, and can readily switch between these behaviours (**Chapter 4**). I have also shown that weapon size can vary significantly in time and space, and have contributed a novel addition to the field by showing for the first time that weapon allometry follows a latitudinal cline (**Chapter 6 & 7**).

## 8.1 Why would ARTs evolve in *L. barbicornis*?

The main aim of this thesis was to determine if alternative reproductive tactics (ARTs) had evolved in *L. barbicornis*. ARTs are expected to evolve in species where there is intense sexual selection, a large disparity in reproductive success between groups, and the ability of some males to monopolise access to potential mates (Gadgil 1972; Shuster & Wade 2003; Brockmann et al.

2008). ARTs are particularly common in fish, which has in part been attributed to the extreme

size variation between male groups, as well as the presence of opportunities for sperm

competition due to external fertilisation and the exploitation of parental care (Taborsky et al.

2008). In **Chapter 2** I documented the sex ratio, seasonal abundance, intrasexual size variation,

and sexual size dimorphism of *L. barbicornis* as a first step towards understanding their biology.

Several aspects of giraffe weevil biology were identified that suggest that there would be high

levels of male-male competition, and the opportunity for ARTs to evolve. Most importantly, the

adult sex ratio was found to be consistently male-biased, suggesting that competition between

males for access to females should be strong. Furthermore, I found high size variation in both

sexes, with a six-fold difference identified between the smallest and largest males. Behavioural

observations showed that males spent a large proportion of time guarding females before or after

copulation (**Chapter 4**), and use their rostrum as a weapon to defend access to females (**Chapter

5**). These observations suggest that the high level of sexual size dimorphism observed, mostly

due to the elongated male rostrum (**Chapter 2**), is likely to have been driven by intense sexual

selection to allow males to better defend females during male-male interactions. These aspects of

*L. barbicornis* ecology all give evidence towards this species as a candidate for ART evolution,

to reduce the potentially large disparity in competitive ability between males across the size

spectrum.

## 8.2 Alternative reproductive tactics in *L. barbicornis*

### *8.2.1 Flexible ARTs*

In a recent review, Brockmann et al. (2008) called for accurate descriptions of alternative tactics, as an essential means to increase our understanding of how ARTs have evolved. In **Chapter 4** I describe size-related ARTs in *L. barbicornis*, using focal male observations in the field. Small males (less than 40 mm long) were found to readily switch between sneaking and fighting behaviour, while larger males relied solely on fighting behaviour to secure mates. Few studies to date have shown that ARTs can be so highly flexible, such that individuals choose the tactic most suitable to the immediate situation. I used logistic regression analysis to predict the likelihood of sneaking or fighting in relation to body size, but I also took this a step further to look at decision making in relation to male-opponent size. Sneaker-sized males were shown to be able to assess their opponent's body size in male-male interactions, because they chose to sneak or fight in relation to the relative size of their opponent. This study, therefore, gives preliminary evidence for a threshold mechanism used in decision making by male *L. barbicornis*.

### *8.2.2 Mating success*

One of the main assumptions for the conditional model of ARTs is that the average fitness of the subordinate tactic will be lower than the dominant tactic (Gross 1996). However, Shuster and Wade (2003) have criticised this assumption, in particular suggesting that previous studies have mismeasured the reproductive success of each tactic, and overrepresented the success of the dominant tactic. I measured the relative mating success of males across all sizes and found no difference in success between small and large males. During antagonistic contests, resident males

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were found to be larger than intruder males, suggesting that large males are able to monopolise a larger proportion of females than smaller males (**Chapter 5**). However, small males were still able to gain access to females via snaking and use this in combination with fighting behaviour in some situations, which gives a likely explanation for why there was no disparity in mating success across the size spectrum. By following focal males for one-hour periods, I was able to accurately gauge mating success across all size categories by including males that spent time away from the aggregation, searching or hiding.

*8.2.3 Post-copulatory competition and female choice*

In my study I measured mating success as a proxy for reproductive success and found no difference between males of different sizes. However, this measure of success does not take into account post-copulatory processes such as sperm competition and cryptic female choice (Parker 1970, 1990; Eberhard 1996; Simmons 2001). I found no evidence that sneaker-sized males increase relative testis size to compensate for any increased risk in sperm competition (**Chapter 3**), but this aspect of giraffe weevil biology requires further attention. It is notoriously difficult to determine the importance of sperm competition and cryptic female choice, however I highly recommend that attempts be made for giraffe weevils, to be able to state with more certainty whether there is any size-dependent reproductive success. I recommend that the female reproductive tract is described in reference to where sperm is stored post-copulation and to determine last male precedence. This should be coupled with behavioural observations to determine the frequency at which sneaker and fighter males are the last male to mate (Parker 1970; Zeh & Zeh 1994). Efforts should also be made to determine the relative ejaculate size of males across the size spectrum, in relation to the risk of sperm competition (Simmons et al. 1999;

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Kelly 2008). Lastly, paternity analyses could be applied to determine size-related reproductive success of males using amplified fragment length polymorphism (AFLP) or microsatellite techniques (Brockmann et al. 1994; Simmons et al. 2004; Umbers et al. 2011; Cogliati et al. 2013). Determining paternity would, however, be difficult because of the long larval life-span in this species, and would therefore require a long term experiment.

*8.2.4 ARTs and threshold dimorphism*

ARTs in invertebrates are commonly associated with a threshold dimorphism in male exaggerated trait size (Goldsmith 1987; Danforth 1991; Emlen 1997; Moczek & Emlen 2000; Okada et al. 2007; Buzatto et al. 2011; Zatz et al. 2011). However, for *L. barbicornis,* there was no evidence of a dimorphism present between males, despite the presence of discrete behavioural differences between males of different sizes. Instead, my analysis of the scaling relationship of rostrum length in *L. barbicornis* found it to be continuous and non-linear in shape, best described by a Weibull growth curve because of the asymptote associated with the largest males in the population (**Chapter 3**). Sneaking behaviour ceased to occur at a body size of approximately 40 mm, which was roughly associated with the size of the largest females. Weapon allometry in the giraffe weevil, therefore, does not relate to the use of mating tactics, and ARTs occur without the presence of distinct male morphs.

## 8.3 Weapon evolution and competitive assessment strategy

### 8.3.1 The rostrum as a weapon

Guarding and fighting behaviour evolves in species where females are aggregated in space or time in such a way that allows them to be easily defended by males (Emlen & Oring 1977). In a similar way, males are predicted to evolve weaponry when resources or females are able to be monopolised by a few males (Emlen 2008). Giraffe weevil females aggregate around oviposition sites in summer months, therefore providing an ideal opportunity for males to monopolise access to them. I showed that the male rostrum is important as a weapon used during aggressive contests between males, and determines fight outcome (**Chapter 5**). The extreme sexual size dimorphism observed in this species (**Chapter 2**), is therefore due to the evolution of the rostrum as a weapon used to increase the success of males at guarding females post-copulation.

### 8.3.2 Evidence for mutual assessment

In many species showing evidence of alternative reproductive tactics, fighting has been identified as an important tactic used by at least one group of males. The competitive assessment strategy used, however, is rarely determined when fighting is just one of several possible tactics used to secure mates.

In **Chapter 5** I described fighting behaviour in *L. barbicornis*, and conducted numerous analyses to determine the best model to describe the competitive assessment strategy used by males during contests. By describing aspects of contest structure in addition to analysing the correlation between contest duration and contestant size, I was able to find strong support for the sequential assessment model (SAM). Males, therefore, are able to mutually assess their rivals in relation to

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their own resource holding potential. Furthermore, the larger male won 90% of contests, and rostrum length (weapon size) was found to be more important as a predictor of fight outcome than overall body size. This study, therefore, provides the first evidence that rostrum length is likely to be under sexual selection in *L. barbicornis*.

*8.3.3 Proximate mechanisms of competitive assessment*

The proximate mechanisms used to assess rivals during contests are currently unknown for *L. barbicornis*. My observations suggest that males are able to visually assess their opponents to some extent before contests escalate to grappling, because only closely matched males escalate fights to the grappling phase. Also, small males appear to have the ability to visually assess their rival's size from a distance before physically interacting with them, and subsequently choose whether to sneak or challenge their opponent in relation to size disparity. However, it is likely that further information about an opponent's size and strength are gathered during the grappling phase by matching the length of their bodies alongside each other. The mechanisms used by *L. barbicornis* may therefore be a composite of visual and tactile cues.

Understanding the proximate mechanisms used in competitive assessment are vital, but are very rarely addressed to date (Arnott & Elwood 2009). Manipulative experiments can help us to understand the role of visual and tactile cues used in assessment. For example, experiments manipulating the facial patterns of *Polistes dominulus* wasps showed that males visually assess these patterns to determine the relative quality of the rival male (Tibbetts & Lindsay 2008; Tibbetts et al. 2010). Hermit crabs (*Pagurus bernhardus*) have been shown to use tactile cues during contests over access to shells, as the shell-owner assesses the rapping power of a challenging male to determine whether to defend or leave the shell (Briffa et al. 1998; Briffa &

Elwood 2000a, b, c). Future studies on *L. barbicornis* could address the role of visual and tactile cues to increase our understanding of the proximate mechanisms used in competitive assessment. A first step could be to observe how small males approach rivals and determine if they conduct a ritualised behaviour that allows them to match their size against their opponent. For example, they may always approach from a certain angle or walk past the opponent first to assess size. Manipulative experiments adjusting rostrum length relative to body size could also be used to determine the role of visual cues used by males when choosing whether to sneak or fight.

## 8.4 Weapon variation in *L. barbicornis*

Studies on geographic and temporal variation in exaggerated trait size are rare, despite the widely held view that sexually selected traits should evolve rapidly (West-Eberhard 1983; Svensson & Gosden 2007). In this thesis I found significant geographic (**Chapter 6**) and temporal variation (**Chapter 7**) in mean body and weapon size. The most novel finding from this part of my thesis was the discovery of a latitudinal cline in weapon allometry. I found that although mean body and rostrum size increased with latitude, the relative size of the male rostrum decreased, as shown by the significant decline in weapon allometry. At this stage it is only possible to speculate as to the factors driving this variation, but I suggest that rostrum size is traded-off with overall body size at higher latitudes. As overall body size increases with latitude, it is possible that there are fewer resources available to allocate directly to rostrum length. Alternatively, at higher latitudes giraffe weevils may be under more resource stress, and therefore unable to invest as much into rostrum size than at lower latitudes. This is an exciting new area of research that certainly requires further attention. My recommendation is that

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researchers explore the possibility of latitudinal clines in weapon allometry in other species, particularly those that can reared easily, because breeding experiments are the best way to determine possible sources of this variation.

**8.5 Giraffe weevils as a model species**

Brentid weevils are rarely studied in the field of behavioural ecology (but see Johnson 1982, 1983; Sanborne 1983; Johnson & Hubbell 1984; Garcia-C 1989), despite being a diverse family with approximately 1700 species described, and showing an incredible range of morphology (Sforzi & Bartolozzi 2004) (Figure 1). Until this study, surprisingly little was known about the life history of *L. barbicornis,* despite being large-bodied, highly charismatic, and the longest weevil in the world. This study was the first to document weapon allometry, size-related alternative reproductive tactics, competitive assessment strategy, and geographic and temporal variation in trait size in a brentid weevil. Giraffe weevils were found to be an excellent model species for behavioural ecological study because they were easily observed in the wild in large numbers. Unlike many insect species that are potentially difficult to observe because they are highly mobile (e.g. flying species such as the odonates), or conduct at least some behaviour underground (e.g. dung beetles; Emlen 1997; Moczek & Emlen 2000), adult *L. barbicornis* aggregate on the surface of dying or dead trees making them easily observable. Copulation and fights were all conducted around drilling females, which were generally aggregated on one section of a tree, such that many animals could be observed at one time. Studies conducted on wild populations are important to gain an accurate understanding of how mating systems evolve,

and are particularly pertinent for species such as *L. barbicornis* for which very little is known about their natural history.

*8.5.1 Limitations*

I did identify several limitations to studying the behavioural ecology of *L. barbicornis*. Multiple attempts at running laboratory-based experiments were made, to manipulate variables such as density and sex-ratio, but these experiments ultimately failed due a lack of success at observing copulating and fighting in captivity. Both of these behaviours largely relied on females being stationary during drilling episodes to allow males to defend them. However, despite providing them with pieces of dead karaka (*Corynocarpus laevigatus*) logs, females spent most of their time trying to escape. Subsequently, male fighting behaviour was rarely observed in the lab, despite being highly frequent in the wild (**Chapter 5**). Giraffe weevils are also very long-lived as larvae (at least 2 years; **Appendix 1**), and would therefore be difficult to use in quantitative genetic studies and breeding experiments.

Figure 1. Diversity among the Brentidae (A) *Ectocemus decemmaculatus*; (B) *Prophthalmus planipennis*; (C) *Brentus anchorago*. (D) *Prodector laminatus*; (E) *Eterodiurus singularis*; (F) *Calodromus malgosiae*. Images A-E by S. Bambi, Mueso di Storia Naturale Firenze. Image F by M. Zilioli, Civic Natural History Museum Milan. All images used with permission from Luca Bartolozzi, Mueso di Storia Naturale Firenze.

(Figure removed due to copyright)

*8.5.2 Sexual selection and ARTs in other brentid weevils*

While single species studies are important for increasing our understanding of how sexual selection operates, it would be useful to put the current study into context with other brentid species. To date there are no comparative analyses of behaviour and morphology in this family. However, given the ease of observing *L. barbicornis* and other brentids in the wild (Johnson 1982, 1983; Johnson & Hubbell 1984), the reports of exaggerated traits and sexual selection in other species (Figure 1; Johnson 1982, 1983; Sanborne 1983), and high variation in the extent of sexual dimorphism (Figure 1; Sforzi & Bartolozzi 1998), this family should make a useful system for research on weapon evolution. I recently photographed and measured a large number of specimens from across the Brentidae using the extensive collection of brentids in international museum collections. These collections open the door for comparative analysis of exaggerated trait evolution and sexual dimorphism as a precursor to detailed behavioural research.

**8.6 Concluding remarks**

In summary, this thesis has highlighted the giraffe weevil as a useful model to address questions about weapon evolution and variation, sexual selection, alternative reproductive tactics, and competitive assessment. In this thesis I have shown that the giraffe weevil mating system is complex, competitive, and dynamic. By collecting and measuring large numbers of animals across multiple levels in space and time I have been able to describe novel patterns in body and weapon size variation. Furthermore, my extensive field-based observations of behaviour have allowed me to document for the first time the use of flexible sneaking behaviour by small males as an alternative reproductive tactic, and determine mutual assessment used by males during

agonistic contests. It is my hope that this study will have implications for our understanding of the evolution of weaponry and ARTs, and will be used as a springboard for future studies in this field.

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# APPENDIX 1 Life history notes and observations on the ecology and behaviour of the New Zealand giraffe weevil (*Lasiorhynchus barbicornis*)

**INTRODUCTION**

The New Zealand giraffe weevil, *Lasiorhynchus barbicornis*, (Coleoptera: Brentidae) is endemic to New Zealand, and is found across the North Island and in the north western regions of the South Island (Kuschel 2003). *L. barbicornis* are generally thought to be rare and cryptic and it is likely that it is for this reason, that to date they are virtually unstudied and consequently very little is known about their life history. It is known, however, that there is remarkable sexual dimorphism in adults. This is mostly due to the extreme elongation of the male rostrum, which makes up approximately half of its total body length (Kuschel 2003). Males use their rostrum during fights with other males for access to females, whereas females use their rostrum to drill holes in wood prior to oviposition (Meads 1976). In addition, both sexes are highly size variable, with males ranging in body length from 16 – 90 mm and females from 18 – 47 mm (Kuschel 2003). High size variation in males can lead to disparity in reproductive success, which in many species has led to the evolution of alternative reproductive tactics (Gadgil 1972; Shuster & Wade 2003; Brockmann et al. 2008). This, in combination with the presence of an exaggerated trait (the elongated rostrum) in males suggests that *L. barbicornis* would make an interesting new model species for research on sexual selection and behavioural ecology. It is hoped that these natural history notes will be of use to anybody wishing to observe or collect *L. barbicornis* in the future.

**METHODS**

Behavioural observations were conducted at Matuku Reserve (36° 51.92'S, 174°28.32'E), west of Auckland city over a three year period (2010-2013) between late October and April in each year. Adult *L. barbicornis* aggregate on host trees in the warmer months, during which females drill holes prior to oviposition while males locate and copulate with them. To prepare for a large-scale study on their behavioural ecology, I first located trees around the reserve which hosted aggregations of adult weevils, and then established the best methodology for observing the behaviour of this species. I conducted observations of *L. barbicornis* and report some notes on their behaviour here. Detailed documentation of mating and fighting behaviour is reported elsewhere (Chapters 4 & 5). All images were taken by the author, unless otherwise credited.

**RESULTS AND DISCUSSION**

*Host trees & locating giraffe weevils*

*L. barbicornis* were commonly found by identifying trees that had clear signs of injury such as sap bleeds and piles of sawdust gathering at the bottom of the tree. Matuku Reserve is a predominantly coastal broadleaf forest (Figure 1B), with an abundance of karaka trees (*Corynocarpus laevigatus*) (Figure 1A), which were the most common tree species found to host giraffe weevils. At Matuku Reserve, giraffe weevils were also found on mahoe (*Melicytus ramiflorus*), rangiora (*Brachyglottis repanda*), ti kouka (*Cordyline australis*), and tarata (*Pittosporum eugenioides*) trees. During this same three year period, I also observed *L. barbicornis* aggregating on numerous other tree species during collection trips around New Zealand (Table 1). Only trees that hosted more than one individual (including a drilling female)

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are reported here, to ensure that only those trees likely to be larval hosts are listed here, not those used as resting or adult feeding sites. A total of 16 tree species from 15 families were observed to host giraffe weevils, significantly increasing the record of previously known host trees (Kuschel 2003).

Table 1. *L. barbicornis* host trees from around New Zealand.

| Scientific name | Family | Common names |
|---|---|---|
| *Aristotelia serrata* | Elaeocarpaceae | Makomako, wineberry |
| *Beilschmiedia tarairi* | Lauraceae | Taraire |
| *Beilschmiedia tawa* | Lauraceae | Tawa |
| *Brachyglottis repanda* | Asteraceae | Rangiora |
| *Coprosma grandifolia* | Rubiaceae | Kanono |
| *Cordyline australis* | Asparagaceae | Ti kouka, cabbage tree |
| *Corynocarpus laevigatus* | Corynocarpaceae | Karaka |
| *Dysoxylum spectabile* | Meliaceae | Kohekohe |
| *Entelea arborescens* | Tiliaceae | Whau |
| *Hedycarya arborea* | Monimiaceae | Pigeonwood |
| *Laurelia novae-zelandiae* | Atherospermataceae | Pukatea |
| *Melicope ternata* | Rutaceae | Wharangi |
| *Melicytus ramiflorus* | Violaceae | Mahoe, whiteywood |
| *Pittosporum eugenioides* | Pittosporaceae | Tarata, lemonwood |
| *Schefflera digitata* | Araliaceae | Pate |
| *Sophora microphylla* | Papilionaceae | Kowhai |
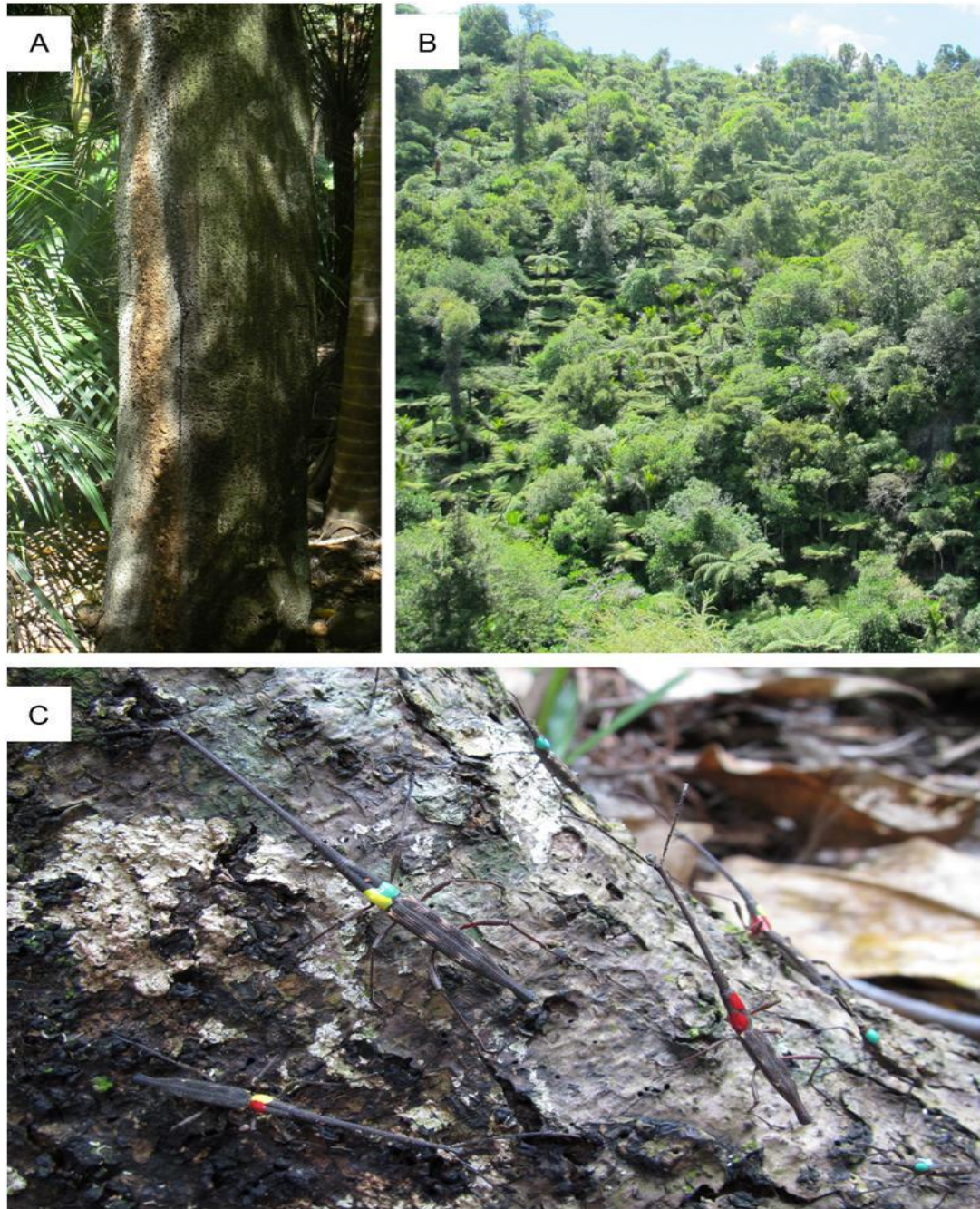
Figure 1. Matuku Reserve, west of Auckland, hosts an unusually high abundance of *L. barbicornis*
present, making it an ideal site for behavioural observation. (A) A karaka tree (*Corynocarpus laevigatus*)
infested with *L. barbicornis*. (B) Matuku Reserve is dominated by coastal broadleaf forest, with kauri
(*Agathis australis*) and tanekaha (*Phyllocladus trichomanoides*) ridges. (C) Giraffe weevils marked with
coloured paints to identify individuals during observations.

*Observation techniques*

To ensure the success of conducting a large-scale study of mating and fighting behaviour of *L. barbicornis,* I first determined the possibility of observing this species in the wild. I found that that observations of the behaviour of adult *L. barbicornis* in the field are relatively easy because large aggregations of both sexes can be found on dead fallen, or dying standing trees throughout the reserve. Once a tree was identified to host *L. barbicornis*, one could make repeat visits to the tree and reliably find a population of weevils to observe. Observations were therein simple to conduct because copulation occurs on the trunks or branches of the trees while females drill holes to prepare for oviposition.

Standing karaka trees were found to be the most useful for behavioural observations, because they would reliably host aggregations of adult *L. barbicornis* throughout at least one breeding season. Standing trees were also best for ease of observation 360° around the tree, although occasionally a focal weevil would disappear into the canopy during observations. While I regularly found dead, fallen trees hosting *L. barbicornis,* these dehydrated rapidly in dry years, and were only useful for observing adults over one breeding season. However, fallen trees are useful for short-term behavioural observations or collection. Observations were best undertaken on dry days, as *L. barbicornis* were very difficult to find during heavy rain, particularly if the tree trunks became wet.

*Marking system*

To enable a large field-based study to be developed, I designed a system to mark individual weevils so that they could be reliably and uniquely identified during observations. This method was adapted from von Frisch (1993), who originally developed a system to identify bees during

his behavioural studies. Queen bee marking paints (Lega, Italy) were used as they are non-toxic, do not rub off, and last the life-time of the individual. Each of the five colours used (blue, yellow, red, green, white) were coded to two numbers from 0-9, in relation to where they were placed on the pronotum (Figure 2). Numbers 1 – 99 were made on the pronotum, and hundreds and thousands were marked on the top and bottom of the elytra, respectively. During field observations this method was found to be an ideal way of distinguishing and identifying each individual, so that multiple individuals could be observed at one time (Figure 1C).

(Figure removed due to copyright)

Figure 2. Marking system used during the study of *L. barbicornis*. Original drawing by D. W. Helmore, © Landcare Research, used with permission. Modifications by V. Ward and C. J. Painting.

*General behavioural observations*

Although observing behaviour in the wild was relatively simple, it was possible to disturb giraffe weevils during observations. If disturbed suddenly, both male and female *L. barbicornis* will fall backwards from the tree and feign death in the leaf litter, sometimes for more than an hour before climbing back up the tree. If an observer came too close to an aggregation, most of the group would ascend the tree and hide amongst the canopy or inside cracks in the tree. It was therefore important to develop a technique that ensured normal behaviour was not affected by observer presence. I ensured that all observations were made standing at least two metres from the tree, using close-range binoculars (Pentax Papilio 8.5 x 21, focus to 0.5 metres) to identify individuals and their behaviours.

*L. barbicornis* were found to be diurnally active, although mating pairs could still be found immediately after dusk. When it became very dark (at approximately 2200 hours), all weevils would climb into the canopy of the tree and hide on the underside of leaves. No pairs were observed copulating at this time; instead all individuals were motionless and solitary under the leaves. *L. barbicornis* pairs copulating around midnight were observed at Paengaroa Scenic Reserve near Taihape (A. Dennis personal communication), but I visited all our previously marked trees regularly after dark and did not observe any active pairs after 2200 hrs at Matuku Reserve.

On occasion, I have observed both males and females gathered around sap deposits on dying karaka (*Corynocarpus laevigatus*) trees. It was assumed that they were feeding on the running sap because their mouthparts were pushed into the liquid (Figure 3). *L. barbicornis* have also

been found feeding on the nectar of male nikau flowers (*Rhopalostylis sapida*) (J. Waite pers. communication).



Figure 3. A male *L. barbicornis* feeds on sap dripping from a karaka (*Corynocarpus laevigatus*) tree.

*Larval life-span experiment*

Brentid larvae are generally known to feed on fungi (Sforzi & Bartolozzi 2004), and a dissection of the gut contents of a *L. barbicornis* specimen was found to contain fungal material (May 1993). Little is known however, about the length of time taken for brentid larvae to develop. To determine the length of time taken for *L. barbicornis* larvae to emerge as adults, I set up an experiment in the field at Matuku Reserve. On the 4th January 2011, a previously healthy karaka

tree was felled and cut into 12 x 400 mm long pieces. Each log was left on the forest floor, spaced approximately two metres apart. After approximately three weeks, adult *L. barbicornis* began arriving on the logs, including females that were observed ovipositing eggs into the wood. At the end of the breeding season, in April 2011, the logs were placed into mesh cages (Bioquip Rearing and Observation Cage, 90 x 60 x 60 cm) with three logs per cage. Air could pass freely through the cages, but the mesh was extremely fine to exclude any giraffe weevils from entering or exiting the cages. The cages were left on the forest floor to ensure ambient humidity and temperature conditions. No adult *L. barbicornis* were found to emerge from the logs between December 2011 and April 2012. However, the following year from 11th December 2012, *L. barbicornis* were found to emerge from the logs, and the cages were checked weekly until 2nd April 2013 (Figure 4). *L. barbicornis* therefore require at least two years as larvae before emerging as adults. Each week all weevils were counted, removed, and released onto a nearby tree.

A total of 430 adult weevils (206 male, 224 female) emerged from the logs over the 15 week period. The overall adult sex ratio was 0.52, which did not deviate significantly from a 1:1 ratio (binomial exact test, $p = 0.4$). Interestingly, surveys conducted over the whole emergence season for three years at Matuku Reserve showed that the adult sex ratio in the field is consistently male-biased (Chapter 2). These caged experiments, therefore, suggest that the sex ratio at emergence is equal, and that the observed male-biased ratio in the field could be due to differences in survival or dispersal rates between the sexes. Further research is required to establish the reason for the apparent male-biased sex ratio observed in the field.
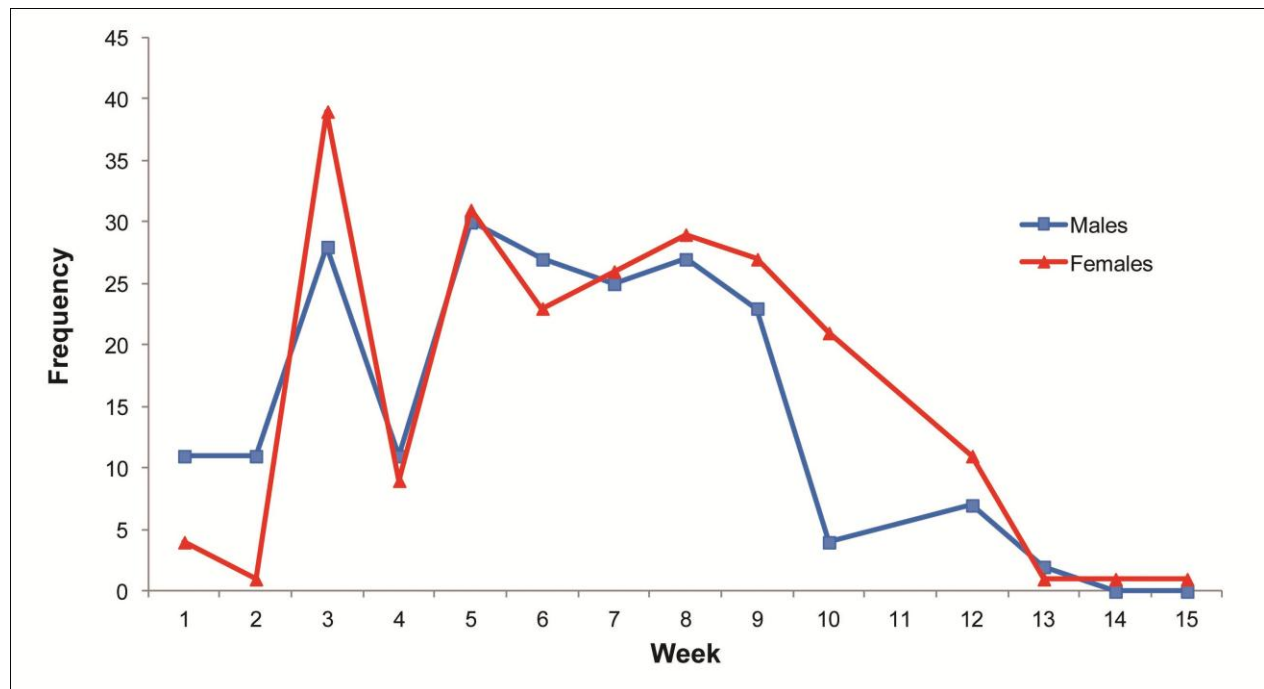
Figure 4. Number of adult *L. barbicornis* emerging from karaka logs between 11 December 2012 and 4 April 2013

*Conclusions*

It was established that observations of *L. barbicornis* behaviour at Matuku Reserve were straightforward, due to the high abundance and reliable number of specimens found on dead or dying karaka trees. I have also provided evidence that giraffe weevil larvae require at least 2 years to mature. It is my hope that these life history notes will be useful to anyone wishing to study these fascinating creatures, and that I can dispel the myth that giraffe weevils are rare and difficult to observe.

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# APPENDIX 2 Model fitting of the scaling relationship for male *L. barbicornis*

Table 1. Models fitted to describe the scaling relationship of natural log-transformed rostrum length and pronotum width of male *Lasiorhynchus barbicornis.* The best models are highlighted in bold, and models are arranged by increasing values of AIC and BIC.

| Model | AIC | ΔAIC | BIC | ΔBIC | Model Parameters |
|---|---|---|---|---|---|
| **Weibull growth function** | **-2597.5** | **0** | **-2572.6** | **0** | **a (asymptote) = 4.11** |
| | | | | | **drop (asymptote minus y intercept) = 2.35** |
| | | | | | **lrc (ln rate constant) = -0.12** |
| | | | | | **power (power x is raised to) = 1.77** |
| **Four parameter logistic** | **-2597.5** | **0** | **-2573.0** | **0.44** | **a (lower asymptote) = 1.15** |
| | | | | | **b (upper asymptote) = 4.11** |
| | | | | | **c (scale) = 0.69** |
| | | | | | **d (x value for inflection point) = 0.42** |
| Breakpoint | -2588.9 | 8.6 | -2564.4 | 8.18 | intercept = 1.47 |
| | | | | | slope left = 1.68 |
| | | | | | breakpoint = 1.1 |
| | | | | | slope right = -0.61 |
| Quadratic | -2556.3 | 41.2 | -2536.7 | 35.87 | intercept = 1.34 |
| | | | | | slope 1 = 2.06 |
| | | | | | slope 2 = -0.27 |
| Linear | -2483.4 | 114.1 | -2468.7 | 103.85 | intercept = 1.51 |
| | | | | | slope = 1.62 |

# APPENDIX 3 Analysis of allometry data: The log-transformation debate

Log-transformation was originally employed to allow the use of an exponential model to describe allometries, at a time when computational power was not sufficient so that these calculations were done by hand (Packard 2012). Regardless of the shape of the scaling relationship and the subsequent analyses used to characterise the scaling relationship, log-transformation of body and trait size has remained the norm (Warton et al. 2006; Knell 2009). However, in recent years a series of publications have raised issues with the use of log-transformation in allometry (Packard 2009, 2011; Packard et al. 2011; Packard 2012). Packard (2009) claims that log-transformation can cause misinterpretation of the model estimates due to bias when parameters are back-transformed to the arithmetic scale, because the resulting means are geometric rather than arithmetic. Furthermore, Packard (2009) points out that log-transformation can change the relationship between the two variables such that outliers are no longer obvious, which he demonstrates using the brain mass allometry of 24 carnivores. Subsequent publications further discuss and support these claims (Packard 2011; Packard et al. 2011; Packard 2012).

While the Packard papers make some interesting points, log transformation is necessary to make traits scale-independent and unitless, allowing comparisons of scaling relationships between traits measured at different scales, or between groups (e.g. species, sex, population, time), particularly if there are magnitudes of variation in trait size between groups (Kerkhoff & Enquist 2009). Allometric slopes are largely influenced by the scale at which a trait is measured, because large traits inherently have more morphological variation than small traits and therefore a steeper slope value (Shingleton & Frankino 2013). Kerkhoff and Enquist (2009) provide a simple

example using the metabolic rates of shrews and elephants to demonstrate why log-transformation is necessary to allow comparison of *proportional* variation not possible using an arithmetic scale. On a logarithmic scale, the variation in metabolic rate for both species is around 0.175, but on arithmetic scales this corresponds to variation of 0.035 W for shrews, and 284 W for elephants. On an arithmetic scale the variation in elephants appears to be much higher than the variation in shrews, despite the fact that variation is proportionally the same. Furthermore, many biological phenomena such as growth and metabolism are also multiplicative processes, so while Packard and co-authors claim that allometric data should be analysed using arithmetic calculations, this goes against the very nature of the data being analysed (Kerkhoff & Enquist 2009). To my knowledge, currently there is only a single publication using untransformed data in weapon allometry (Santos et al. 2013). In this thesis I am exploring weapon allometry variation between males and females, and over space and time, so I therefore have chosen to log-transform data to allow for simple comparison. However, I have followed Packard's recommendations when first describing the shape of weapon allometry, and present data for both log-transformed and untransformed data.

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# APPENDIX 4 Injury rates of adult giraffe weevils

During fights males use their mandibles to bite onto the tarsi, antennae and elytra of their competitors in an attempt to pull the individual away from the female or dislodge them from the tree (Chapter 5). A low percentage of males and females surveyed over two seasons at Matuku Reserve were found to have sustained injuries due to missing body parts (Table 1). These data were collected as part of another study (Chapter 2), but for every weevil collected and measured, the presence and location of any injuries were recorded.

Table 1. Relative injury rates for male and female *L. barbicornis* over two seasons at Matuku Reserve

|        | 2010/2011 | | 2011/2012 | | Total | |
|--------|------|--------------|-----|--------------|------|--------------|
|        | N    | No. injuries | N   | No. injuries | N    | No. injuries |
| Male   | 1201 | 40 (3.33%)   | 994 | 46 (4.63%)   | 2195 | 86 (3.92%)   |
| Female | 879  | 18 (2.04%)   | 664 | 23 (3.46%)   | 1543 | 41 (2.66%)   |

During focal observations of fighting behaviour collected as part of Chapter 5 I did not witness any loss of body parts of males during fights, but I did not closely examine them after fights so as not to disturb their behaviour. However the proportion of males with body parts missing was significantly greater than females ($Z = 2.09$, $p = 0.02$), suggesting that males may be sustaining injuries during conflict. For both sexes the majority of injuries were due to loss of all or part of a leg, but damage to elytra and antennae was also found (Table 2). All of these body parts are frequently bitten by competitors during fights, so the observed damage is likely to be due to these conflicts.

Table 2. Injury types for male and female *L. barbicornis*

|  | Antennae | Elytra | Legs | Multiple injury |
|---|---|---|---|---|
| Male | 14 (16.28%) | 11(12.8%) | 57 (66.3%) | 4 (4.65%) |
| Female | 5 (12.19%) | 2 (4.88%) | 33 (80.5%) | 1 (2.43%) |

# APPENDIX 5 Cohens *d* effect sizes showing pairwise comparisons of significant differences in mean body and weapon size between months over three years.

| Year | Sex | Trait | Comparison | Cohens *d* |
|------|-----|-------|------------|-----------|
| 2010/2011 | Males | Pronotum width | Nov-Feb | 0.36 |
| | Females | Body length ex. rostrum | Nov-Feb | 0.48 |
| | | | Jan - Feb | 0.25 |
| | | Pronotum width | Nov-Feb | 0.46 |
| | | | Jan - Feb | 0.25 |
| | | | Nov-Feb | 0.47 |
| | | Rostrum length | Jan - Feb | 0.26 |
| 2011/2012 | Females | Body length ex. rostrum | Nov-Dec | 0.63 |
| | | | Apr-Dec | 0.64 |
| | | Pronotum width | Nov-Dec | 0.61 |
| | | | Apr-Dec | 1.10 |
| | | Rostrum length | Nov-Dec | 0.57 |
| | | | Apr-Dec | 0.49 |
| 2012/2013 | Females | Body length ex. rostrum | Nov-Dec | 1.06 |
| | | | Apr-Dec | 1.01 |
| | | | Mar-Dec | 0.56 |
| | | Pronotum width | Nov-Dec | 1.04 |
| | | | Apr-Dec | 1.01 |
| | | Rostrum length | Nov-Dec | 0.94 |
| | | | Apr-Dec | 0.80 |

**APPENDIX 6 Monthly variation in the scaling relationship of rostrum length for male and female *L. barbicornis* over three years at Matuku Reserve. Values are the SMA slope, with lower and upper 95% confidence intervals in parentheses**

| | November | December | January | February | March | April |
|---|---|---|---|---|---|---|
| *Males* | | | | | | |
| 2010/2011 | 1.87 (1.61 - 2.18) | 1.98 (1.77 - 2.21) | 1.73 (1.63 - 1.84) | 1.79 (1.68 - 1.91) | 1.87 (1.55 - 2.11) | 1.70 (1.38 - 2.10) |
| 2011/2012 | 1.64 (1.65 - 1.75) | 1.70 (1.65 - 1.75) | 1.69 (1.67 - 1.72) | 1.67 (1.63 - 1.71) | 1.58 (1.53 - 1.64) | 1.61 (1.55 - 1.66) |
| 2012/2013 | 1.42 (1.33 - 1.51) | 1.65 (1.58 - 1.72) | 1.65 (1.62 - 1.69) | 1.67 (1.63 - 1.72) | 1.72 (1.54 - 1.92) | 1.59 (1.40 - 1.73) |
| *Females* | | | | | | |
| 2010/2011 | 0.92 (0.86 - 0.99) | 0.94 (0.89 - 1.00) | 0.93 (0.90 - 0.96) | 0.98 (0.96 - 1.00) | 0.93 (0.89 - 0.98) | 0.90 (0.82 - 0.99) |
| 2011/2012 | 0.92 (0.87 - 0.97) | 1.01 (0.97 - 1.05) | 0.98 (0.95 - 1.01) | 0.94 (0.91 - 0.96) | 0.93 (0.89 - 0.98) | 0.96 (0.91 - 1.00) |
| 2012/2013 | 0.92 (0.81 - 1.04) | 0.93 (0.86 - 1.00) | 0.98 (0.96 - 1.00) | 0.96 (0.93 - 0.99) | 0.94 (0.90 - 0.97) | 0.89 (0.79 - 1.01) |