BEHAVIOURAL, MICROHABITAT, AND PHYLOGENETIC DIMENSIONS OF INTRASEXUAL CONTEST COMPETITION IN COMBATANT MONKEY BEETLES (SCARABAEIDAE: HOPLIINI)

by

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DECLARATION

I, Ariella Nikia Rink, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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I conceptualised the paper with input from my co-supervisor, Jonathan Colville, regarding the natural history of the study system, with input from my main supervisor, Res Altwegg, concerning statistical approaches to analysing the data, and with input from Shelley Edwards concerning the basic Principals of geometric morphometrics methods. I conducted all analyses and wrote the paper. My co-authors proofread drafts of the paper.

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ABSTRACT

The importance of sexual selection as a driver of evolution, from microevolution to speciation, has overwhelmingly been studied in the context of female choice, but there is evidence that male-male competition can also drive evolution. Recent reviews of the intrasexual competition literature have developed several hypotheses of weapon divergence in both allopatry and sympatry and have suggested means by which weapon divergence may cause reproductive isolation and speciation, both alone and together with mate choice and ecological selection. Here, I assess the role of sexual selection, in the context of environmental variation at the level of the contest substrate and the developmental environment, in contributing to microevolution within the monkey beetles (Coleoptera: Scarabaeidae: Hopliini), a taxonomically and phenotypically diverse group of pollinating insects in the Greater Cape Floristic Region (GCFR) that shows a high degree of sexual dimorphism and mating behaviour driven by male-male competition. I build on previous observations of hind leg use in intrasexual male-male contest for reproductive access to females by showing that, in *Heterochelus chiragricus*, contests occur in the context of a significantly male-skewed sex-ratio and consist of vigorous wrestling and pushing between two males on the flower heads occupied by embedded, feeding females, who apparently exert no mate choice. Contest outcomes are influenced by hind femur size and residency effects, and I apply hypotheses informed by evolutionary game theory to assess how males make decisions regarding persistence versus retreat. I proceed to assess the evidence for the 'divergent fighting contexts' hypothesis which predicts weapon divergence driven by intrasexual contest competition in the context of variation in the contest substrate. I find that hind leg size in another combatant monkey beetle, the species complex *Scelophysa trimeni*, varies across gradients of flower size among several spatially distributed populations, suggesting that variation in flower size (the contest substrate) mediates selection for weapon morphologies that maximise performance under different fighting styles necessitated by differences in the contest substrate. I also find that male elytral colour varies both across gradients in the developmental environment and with variation in flower colour, suggesting that this trait may function as an honest signal of male fitness, but also that it may be under selection to maximise signal transmission against variable backgrounds of contest substrates. Finally, I quantify the extent to which integration, modularity, multivariate allometry, and phylogenetic effects influence the evolutionary lability of male monkey beetle's hind legs, and so mediate the pace of their evolutionary diversification in response to these varying contest substrates. My findings support a two-module pattern of modularity at both static and evolutionary levels, and I find that allometric scaling relationships are conserved within *S. trimeni*. These findings indicate that monkey beetle weapons are relatively unconstrained in their evolutionary diversification across divergent fighting substrates. I conclude by discussing these findings within the broader field of sexual selection and monkey beetle ecology and suggest directions for further work. The findings presented here support a role for sexual selection, interacting with variation in the flower contest substrate, as being an important driver of the diversification of monkey beetles in the GCFR.

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1. INTRODUCTION

1.1 NATURAL AND SEXUAL SELECTION AS DRIVERS OF BIODIVERSITY

Darwin revolutionised our understanding of biodiversity with his theory of evolution by natural selection (1859). Although speciation through natural selection has undoubtedly been a major driver of biodiversity (Maynard Smith 1962; Etienne et al. 2007; Cooke et al. 2012), it has long been apparent that evolution by natural selection does not account for the full extent of biodiversity, specifically, the diverse sexually dimorphic traits that appear to be maladapted to survival (Lyon and Montgomerie 2012), including enlarged horns and tusks in mammals (Lundrigan 1996; Preston et al. 2003), and enlarged and exaggerated mandibles and legs in insects (Eberhard 1998; Kawano 2006; Kelly 2006; Katsuki et al. 2014). The persistence of these traits, despite the fitness costs they may incur, including increased predation risk and higher metabolic costs (Allen and Levinton 2007; Kojima et al. 2014; Somjee et al. 2018), suggests that they do provide a net evolutionary benefit despite being apparently maladaptive (Johnstone 1995). Darwin realised that natural selection could not explain the persistence of these traits and proposed instead that these structures influenced reproductive success through competition for mates (Darwin 1871; Lyon and Montgomerie 2012). His theory of sexual selection proposed that variation in these traits led to differential reproductive success among individuals; those with superior traits would be more successful in competing for mates and thus would contribute more to the proceeding generation, leading to directional selection for ever more successful competitive traits (West-Eberhard 1983; Jones and Ratterman 2009).

Sexual selection may either occur due to intersexual competition, also termed ‘mate choice’ or ‘female choice’ (as females are more often the choosy sex than males), or intrasexual competition, also known as male-male competition since males are commonly the combatant sex (Darwin 1871; Fisher 1930; Murphy 1998; Rosvall 2011; Miller 2013). Sexual selection through mate choice favours elaborately ornamented traits, while intrasexual competition selects for weaponry in the competing sex, either novel (e.g., horns; Emlen et al. 2007; Kijimoto et al. 2012; Zattara et al. 2016) or co-opted from other uses (exaptation, e.g., mandibles and legs; Eberhard 1998; Knell et al. 2004; Okada et al. 2006; Katsuki et al. 2014). Sexual selection is shown to exert strong, directional selection on sexually selected traits in diverse insect taxa such as water striders *Aquarius remigis* (Fairbairn and Preziosi 1994), damselflies *Calopteryx splendens* (Svensson et al. 2006), and horned dung beetles *Onthophagus taurus* (Knell and Simmons 2010). This may facilitate rapid divergence in these traits among populations, leading to reproductive isolation, and ultimately speciation (Bonduriansky 2011; Safran et al. 2013; Grether 2019).

1.2 HOW SEXUAL SELECTION DRIVES EVOLUTIONARY DIVERSIFICATION

Female choice

The importance of sexual selection as a driver of evolution, from microevolution to speciation, has overwhelmingly been studied in the context of female choice (Qvarnström et al. 2012; Lackey et al. 2018; Tinghitella et al. 2018). Linkage disequilibriums and pleiotropic effects binding preference (in the choosy sex) with trait (in the displaying sex) may enable correlated inheritance of genes for both preference and ornament (Andersson 1986; Pomiankowski 1987; Brooks and Couldridge 1999; Brooks 2000; Rick et al. 2011). Correlated divergence of preferences and traits may thereby lead to assortative mating and, ultimately, speciation (Wu et al. 1995; Uy and Borgia 2000; Tobler et al. 2008; Shaw et al. 2011). This genetic coupling between preference and signal was long thought to be essential for evolutionary divergence by sexual selection. In the absence of female choice, the mechanism by which sexual selection would bring about reproductive isolation, which is essential to the biological species concept (Mayr 1942), has been unclear (Burdfield-Steel and Shuker 2018; McCullough and Emlen 2018). Recently, several hypotheses have been proposed concerning the mechanisms through which intrasexual contest competition may lead to speciation (Qvarnström et al. 2012; McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018). These hypotheses consider the roles of interactions between sexual selection and natural selection, the influence of random events, and the importance of the social environment in mediating the strength and direction of sexual selection (Qvarnström et al. 2012; McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018), and provide a framework in which further work can examine the role of intrasexual competition in trait divergence and speciation.

Shift of focus to intrasexual contest competition

Since the turn of the century there has been increasing research interest in male-male contest competition, with several reviews and perspectives on the field published since the early 2000s (e.g., Emlen and Nijhout 2000; Rutte et al. 2006; Arnott and Elwood 2008; Emlen 2008; Hunt et al. 2009; Qvarnström et al. 2012; Lackey and Boughman 2013; Miller 2013; Mesterton-Gibbons and Heap 2014; McCullough et al. 2016; Boughman 2018; Dijkstra and Border 2018; Tinghitella et al. 2018; Rico-Guevara and Hurme 2019). It was long unclear that these exaggerated traits were weapons at all; few researchers had conducted behavioural studies on live specimens in the field before the 1970s (Eberhard 1980). Since then, contest behaviour, sexual dimorphism, and the correlates of contest success have been studied in numerous animal systems, including insects and other invertebrate taxa (Miyatake 1997; Pomfret and Knell 2006; Tedore and Johnsen 2012). Early research noted that, despite the lethal weaponry borne by many species, contests were often ritualised and showed limited escalation. Game theory was recognised by Maynard Smith and Price (1973) as providing a suitable framework in which to understand why it may be in the best interests of individuals to engage in ritualised, minimal-contact contests, rather than inflicting the maximum possible damage on their opponent. Their application of game theory to animal contests, termed evolutionary game theory, has provided key insights into how asymmetries in fighting ability or resource-holding power (RHP; Parker 1974) and ownership influence the strategies, patterns of contest escalation and de-escalation, and the expected outcomes and durations of dyadic animal contests (Briffa 2014). Game theory dictates that an individual's strategy should depend on RHP and ownership asymmetries between it and its opponent (Parker and Stuart 1976; Hammerstein 1981; Arnott and Elwood 2008). Two general classes of contests can occur: wars of attrition, in which contestants do not gather any information to measure these asymmetries, but instead engage in non-physical moves until one is exhausted, and mutual assessment, in which contestants make moves to gather information on the asymmetry between them and after each move retreat or persist accordingly (Parker 1974; Mesterton-Gibbons et al. 1996; Taylor and Elwood 2003; Mesterton-Gibbons and Heap 2014).

Evolutionary game theory has also been applied to the problem of speciation through intrasexual contest competition (McCullough et al. 2016). Assuming that the greater a male's RHP is, the higher his chance of winning a contest, there should be directional selection for increased RHP (Maynard Smith 1982; Maynard Smith and Brown 1986; Härdling 1999). However, more powerful weaponry incurs greater developmental and maintenance costs (Dennenmoser and Christy 2013; Goyens et al. 2015; O'Brien and Boisseau 2018; but see McCullough and Tobalske 2013). At some point the direct fitness cost to the individual of bearing and maintaining this weaponry outweighs the reproductive benefit conferred by winning contests, and males with smaller weapons, being less burdened by costly armaments and thus more fit, should become more successful in gaining mating access to females (Härdling 1999). In allopatric populations, each undergoing evolutionary cycling between high and low weapon investment, chance events at the initiation of each cycle may lead to weapon divergence and thereby divergent evolution among populations (McCullough et al. 2016). Recent reviews of the intrasexual competition literature (Qvarnström et al. 2012; McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018) have developed several other hypotheses of weapon divergence in both allopatry and sympatry and have suggested means by which weapon divergence may cause reproductive isolation and speciation, both alone and together with mate choice and ecological selection. I provide a brief description of each hypothesis below.

1.3 HYPOTHESES OF WEAPON DIVERSIFICATION

Negative frequency-dependent selection

Males bias aggression towards individuals resembling themselves as they are more likely to be competing for the same reproductive resources (e.g., females, oviposition sites; Qvarnström et al. 2012; McCullough et al. 2016; Dijkstra and Border 2018). Males with novel and/or rare competitive traits should therefore experience less agonistic contact with other males and be in better physical condition due to escaping the fitness and time costs of combat (Qvarnström et al. 2012; Dijkstra and Border 2018). If female choice is present, they may be preferred on account of their better physical condition (Lackey et al. 2018; Tinghitella et al. 2018). Novel phenotypes may also be associated with alternative reproductive tactics, e.g., in dung beetles, where there is a threshold body size at which males develop horns, smaller, hornless, males adopt alternate “sneak” mating tactics, while larger, horned, males engage in intrasexual contests (Rasmussen 1994; Emlen 1997). If female choice is present, and female preference co-diverges with male morphotypes, then reproductive isolation may result by the time the increase in frequency of the novel or rare morph erodes its novel advantage (Tinghitella et al. 2018). Negative frequency-dependent selection on male morphs has been demonstrated in cichlid fishes, where males bias aggression towards similarly coloured males in closely related species but not in distantly related species, suggesting a function for negative frequency-dependent selection in the early stages of speciation in this group (Seehausen and Schluter 2004; Dijkstra et al. 2007).

Divergent fighting contexts

If the habitats occupied by populations differ in contest substrate then weapons should be under selection to optimise their contest performance to those conditions, resulting in weapon forms diverging among populations (McCullough et al. 2016). Concomitant changes of fighting style with weapon form are expected, as evidence in rhinoceros beetles indicates that different weapon forms are mechanically suited to different fighting styles (McCullough et al. 2014). If males with the weapons and fighting styles best suited to each population’s environment secure the majority of the mating opportunities, then reproductive isolation may arise on secondary contact with males from other populations as their weaponry and fighting styles will be sub-optimally adapted, and they are therefore unlikely to secure mating opportunities (McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018). This hypothesis has yet to be empirically tested.

Random events

Male competitive traits may diverge in allopatry due to random events, such as genetic drift via founder effects and mutation-order processes (Mendelson et al. 2014; McCullough et al. 2016; Lackey et al. 2018; Rundle and Rowe 2018). To my knowledge, the role of neutral evolutionary processes in driving divergence in male weaponry has not yet been studied empirically.

Divergent costs

If populations inhabit environments in which the fitness costs of developing and maintaining weaponry differ, perhaps due to parasites or predation, then weapon form should be under selection to minimise fitness costs specific to the ecological context of each population, leading to divergence in weapon form among populations that are under different ecological pressures (McCullough et al. 2016; Lackey et al. 2018). In the dung beetle genus *Onthophagus*, horn diversity may reflect adaptations to minimise costs in different ecological habitats (Emlen 2001; Emlen et al. 2005; Moczek 2010; Moczek et al. 2014).

Conspicuous signalling

Weapons with dual use as fitness signals to competitors and choosy females may undergo diversification driven by selection for conspicuousness within divergent signalling environments (Boughman 2002; McCullough et al. 2016). In male *Anolis cristatellus* lizards, whose dewlaps are used to signal to other males as well as to females, divergence in dewlap colour maintains its efficacy as a signal under the distinct light environments occupied by xeric and mesic populations (Leal and Fleishman 2004). Correlated divergence in male signals and female choice may facilitate assortative mating and thereby speciation through sensory drive (Marchetti 1993; Boughman 2002; Seehausen et al. 2008; Mendelson et al. 2014; McCullough et al. 2016; Lackey et al. 2018). It is unclear whether speciation through sensory drive could occur in intrasexual male competition systems lacking female choice (McCullough et al. 2016).

Fighting advantage of novel forms

Males with novel weapon forms may gain an advantage in contests if other males lack the strategies to successfully compete against them (McCullough et al. 2016; Djikstra and Border 2018). Beetle horns may have their developmental origins in final-instar larval head projections that helped to shed the larval head capsule during the transformation to pupa; these are usually resorbed but a novel mutation that resulted in this projection being retained in adulthood may have provided a competitive advantage in beetles that were already exhibiting aggressive intrasexual interactions (Moczek 2008; Hu et al. 2019).

Resource availability

As the population operational sex-ratio becomes more male-skewed, male competition for females should increase, driving stronger selection for male aggression and fitness signals (Kvarnemo and Ahnesjö 1996; Weir et al. 2011; Janicke and Morrow 2018). Male aggression may also be influenced by food availability (Kolluru and Grether 2005). Gradients of resource availability are thus expected to be correlated with the intensity of male aggression and signal conspicuousness (Tinghitella et al. 2018). Jirotkul (1999) found that the relative importance of female choice and male-male competition varies in guppies as a function of the operational sex-ratio (OSR). The OSR may in turn be modulated by the availability of breeding resources, as demonstrated for *Onthophagus taurus* dung beetles, where the number of females able to breed is dictated by dung availability and male competition for females intensifies as dung becomes more limited (Moczek 2003).

1.4 HOW DOES DIVERGENCE IN MALE COMPETITIVE TRAITS FACILITATE REPRODUCTIVE ISOLATION?

If recently diverged populations come into secondary contact, and hybridization occurs, hybrids will be selected against if they have lower fitness than the parent lineages (Tinghitella et al. 2018), as occurs in several bird taxa (Hudson and Price 2014). This is speciation by reinforcing selection (Butlin 1987; Servedio and Noor 2003), which can also occur in the case of co-divergence of male competitive phenotype and female preference (see "conspicuous signalling" above). Interlocus sexual conflict, which occurs when competitive advantages in males are harmful to female fitness (Bonduriansky 2011), may drive population divergence as it is expected to be able to drive rapid evolutionary trait change in an arms race between the sexes, generating reproductive isolation as a side-effect (Chapman et al. 2003; Wong and Candolin 2005; Gavrilets 2014; Tinghitella et al. 2018). In experimentally manipulated populations of flies, large, dense populations with high sexual conflict exhibited greater divergence than small, sparse populations with low sexual conflict (Martin and Hosken 2003; Martin and Hosken 2004; but see Bacigalupe et al. 2007). There is thus a potential for divergence in male competitive traits to drive reproductive isolation, and possibly speciation, in the absence of mate choice.

The hypotheses outlined above suggest several scenarios in which male weapons might diverge and drive evolutionary diversification and speciation. However, trait responses to sexual selection are mediated by several types of evolutionary constraints that influence their adaptability (Taute et al. 2014; Hansen 2015; Galis et al. 2018), from, for example, internal factors (e.g., developmental, functional, and phylogenetic effects; Cheverud 1984; Walker 2007), and the external environment (e.g., stabilizing selection on male rhinoceros beetle horns due to predation pressure; Kojima et al. 2014). The potential influence of these constraints on sexually selected trait evolution makes it important to consider these in studies of evolution driven by sexual selection.

1.5 EVOLUTIONARY CONSTRAINTS ON MALE WEAPONRY

The ability of traits to respond to selective pressures, their evolvability or evolutionary lability, is in part determined by how much they are constrained in the rate and direction of their evolution ("resistance to selection"; Futuyma 2010). The terminology of evolutionary constraints lacks consistency, making comparisons of the different hypotheses of evolutionary constraints somewhat difficult (Antonovics and van Tienderen 1991; Burt 2001; Hansen 2015). Furthermore, what is considered an evolutionary constraint depends on the context of the research. For example, in sexual selection research, natural selection may be considered to constrain sexually selected trait evolution if it exerts opposing selective pressure, resulting in stabilizing selection where sexual selection acting in one direction is balanced out by natural selection acting in the other (Stuart-Fox and Ord 2004; Ingleby et al. 2014). In general, evolutionary constraints can be understood as factors that limit or restrain the regions of morphospace (the theoretical space that describes all possible phenotypes; Mitteroecker and Huttegger 2009) that organisms can explore in response to selection, and the rate at which they are able to do so (Futuyma 2010; Hansen 2015; Galis et al. 2018). Below I give an overview of several evolutionary constraints.

Genetic constraints

These are constraints on trait evolution arising from characteristics of the genotype, encompassing limitations due to lack of standing genetic variation, low mutation rates, and pleiotropic effects (Mitchell-Olds 1996; Chenoweth and McGuigan 2010; Futuyma 2010; Roulin 2016; Hu et al. 2017). Limited genetic variance may reflect the past loss of characters and their underlying genetic architecture (Futuyma 2010) and low mutation rates mean that selection has a reduced set of genetic variation on which to act (Baer et al. 2007; Futuyma 2010). Pleiotropic effects may constrain the evolution of a trait under selection when it is genetically correlated with other traits that are under stabilizing or opposing selection compared to the focal trait (Hansen 2003; Futuyma 2010; Sztepanacz and Rundle 2012), but can also facilitate correlated evolution among the components of complex traits (Shaw et al. 2011). Conversely, traits that are under similar selection due to shared functionality but are not underlain by the same genetic architecture will be constrained in exhibiting a coordinated response to selection (Cheverud 1984; Cheverud 1996). The development of genetic modularity can mitigate these effects (Hansen 2003). The evolution of sexual dimorphism requires the overcoming of genetic constraints in the form of the shared genotype of males and females (Lande 1980; Chenoweth et al. 2008), and therefore genetic constraints can hamper the resolution of sexual conflict via the evolution of sexual dimorphism (Bonduriansky and Chenoweth 2009). There is evidence for the genetic constraint of sexually dimorphic plumage in birds where male colour is under sexual selection (Dale et al. 2015). Genetic constraints may also influence the direction of diversification of sexually selected traits Chenoweth et al. (2010). These findings emphasize the importance of considering genetic constraints when studying sexually selected trait evolution.

Modularity and integration

Modules consist of tightly integrated traits that are less well integrated with other such modules (Olson and Miller 1958; Klingenberg 2009). Modularity and integration can either facilitate or constrain evolution, depending on the patterns observed (Adams and Collyer 2019a). While modularity may encourage trait diversification by facilitating the shared evolution of linked traits while reducing pleiotropy between unrelated traits, strong integration between modules constrains their independent evolution (Mitteroecker and Bookstein 2007; Klingenberg 2014). Evolutionary studies examining patterns of modularity and integration have focused on vertebrate skulls (e.g., Drake and Klingenberg 2010; Singh et al. 2012; Evans et al. 2017; Hedrick et al. 2019; Marshall et al. 2019; Watanabe et al. 2019). Relatively little work has been done on sexually selected traits, most of which has investigated ornaments (Badyaev 2004; Badyaev and Young 2004). Studies of integration in the context of male weaponry focus on integration between weapons and supporting traits or traits involved in morphological trade-offs with weapons (Tomkins et al. 2005; Okada and Miyatake 2009; Okada et al. 2012; Rohner et al. 2020). Patterns of modularity and integration, and their roles in constraining evolution, have not been investigated for complex weapons, such as modified jaws and legs, which are comprised of multiple interacting structures, and therefore it is unclear if these complex weapons are under different evolutionary constraints to simple weapons, such as horns.

Functional and developmental constraints

These are constraints on the evolution of traits due to functional trade-offs (Walker 2007; Monteiro 2013; Vermeij 2015) and developmental canalisation (Maynard Smith et al. 1985; Zelditch et al. 1993; van Buskirk and Steiner 2009). Sexual selection for superior weaponry may act in opposing directions to natural selection for other aspects of trait and organismal fitness, resulting in fitness trade-offs. For example, in male crabs *Uca pugnax*, major claw form is constrained by trade-offs between closing speed and closing force (Levinton and Allen 2005), while in *Uca terpsichores and Uca beebei*, male claws experience opposing selection due to their dual functions as weapons and signals (Dennenmoser and Christy 2013), and in the sexually dimorphic stag beetles *Cyclommatus metallifer*, males fight with enlarged mandibles that impose costs on their locomotion (Goyens et al. 2015). Functional constraints impose stabilizing selection on trait evolution, limiting the extent of their exaggeration in response to sexual selection, for example limiting mandible size in stage beetles (Knell et al. 2004), and developmental constraints may bias the direction in which traits evolve, as has occurred in the horned beetle genus *Onthophagus* (Hu et al. 2020). Both sources of constraints are therefore important to study to understand the degree and direction of weapon diversification.

Allometric constraints

Allometry – the scaling relationships between trait and body characteristics, generally size – has been studied extensively in sexually selected traits, both ornaments and weapons (e.g., Tomkins et al. 2005; Kodric-Brown et al. 2006; Bonduriansky 2007; Simmons and Altwegg 2010; Painting and Holwell 2013; McCullough et al. 2015; Eberhard et al. 2018). Whereas most morphological traits are expected to scale isometrically with body size, sexual selection theory predicts that the slope of the allometric scaling relationship of weapons with body size should exceed one (Emlen and Nijhout 2000; Bonduriansky and Day 2003; Kodric-Brown et al. 2006; Fromhage and Kokko 2014). Allometry may impose a constraint on trait evolution if the allometric slope itself is constrained, meaning that, for example, weaponry will not be able to respond to sexual selection independently of natural selection on body size (Brakefield 2006). Stillwell et al. (2016) found evidence in *Drosophila* wings that the allometric slope can be respond to selection independently of wing or body size. Several studies have examined allometric constraints on the evolution of non-sexually selected traits (Tobler and Nijhout 2010; Voje et al. 2014; Bolstad et al. 2015; Mirth et al. 2016), but in the context of intrasexual contest competition, research on weapon allometry has focussed on quantifying sexual dimorphism, male polymorphisms, and contest outcomes (e.g., Johns et al. 2014; McCullough et al. 2015; Walker and Holwell 2018). Allometry is less often studied from the perspective of being a constraint on weapon evolution (but see Voje and Hansen 2013), and further research on the impact of allometric constraints on microevolutionary processes is a promising topic for understanding how the exaggerated allometries typical of sexually selected traits impact their evolution.

Phylogenetic constraints

The current understanding of phylogenetic constraints is that they arise when past trait adaptations limit the directions in which traits can evolve (Blomberg and Garland 2002; Shanahan 2011) and constrain the extent to which related taxa can diverge from one another due to their shared evolutionary history (Hansen and Martins 1996). However, historically this class of constraints has been poorly defined, and today many authors measure phylogenetic signal, which quantifies the tendency of related taxa to resemble each other more closely than expected by chance, as a proxy of phylogenetic constraint (Blomberg et al. 2003; Shanahan 2011; Monteiro 2013). Several indices of phylogenetic signal have been developed, some requiring the assumption of an underlying model of trait evolution (Münkemüller et al. 2012). The most popular evolutionary model is Brownian motion (BM), a random process which describes the null expectation of trait variation over time within a lineage under pure genetic drift (Pagel 1999; Cooper et al. 2016). BM models are not able to describe trait evolution under directional selection, and although still widely used, are being superseded by more sophisticated models such as the Ornstein-Uhlenbeck (OU) model, which allows for trait means to adaptively evolve over time (Hansen et al. 2008). These models were formulated for univariate trait data inputs, and the rise of multivariate trait data, such as that produced by geometric morphometrics, has necessitated the development of multivariate extensions (Klingenberg and Gidaszewski 2010; Clavel et al. 2015). However, the only multivariate index of phylogenetic signal that produces acceptable rates of Type I errors and statistical power is the multivariate derivation of Blomberg's K assuming BM, and, therefore, BM remains the recommended model of trait evolution for highly multivariate datasets (Adams 2014; Adams and Collyer 2018; Adams and Collyer 2019b).

1.6 INSECTS AS MODEL ORGANISMS TO TEST SEXUAL SELECTION

Insects have proven popular study organisms in sexual selection research, having been used in many studies investigating the functional and mechanistic characteristics of weaponry (e.g., Goyens et al. 2016; Mills et al. 2016; O'Brien and Boisseau 2018; Zhang et al. 2019), hypotheses concerning the allometric relationships of weapon and body size and sexual dimorphism (e.g., Pomfret and Knell 2006; Miller and Emlen 2010; O'Brien et al. 2017), and detailed observational studies that have analysed decision-making and assessments during contests (e.g., Panhuis and Wilkinson 1999; Kelly 2006; Painting and Holwell 2014; Okada et al. 2017). As of 2014, less than half of published papers studying the role of sexual selection in speciation focused on insects (Emlen 2008; Snell-Rood and Moczek 2013; Miller and Svensson 2014; Scordato et al. 2014; Lavine et al. 2015), despite insects accounting for the bulk of terrestrial species diversity (Conrad et al. 2006; Condamine et al. 2016). In the field of evolutionary biology, insects' obvious attraction is their biodiversity; approximately a million species are currently described with many more likely in existence (Mayhew 2007; Stork 2018). The association between polygamy, sexual dimorphism, and species richness in several animal taxa, including insects (Arnqvist et al. 2000), indicates that sexual selection may be a key driver of this biodiversity (Stuart-Fox and Owens 2003; Mayhew 2007; Janicke et al. 2018; Tsuji and Fukami 2019).

Types of insect studies conducted

A considerable portion of sexual selection research on insects has been conducted in laboratory settings using staged interactions (e.g., Emlen 1997; Moczek and Emlen 2000; Bonduriansky and Rowe 2003; Brown et al. 2006; Kasumovic et al. 2010; Worthington et al. 2012). Insects are well-suited to laboratory-based research due to their ease of handling and care, fast generation-time, minimal spatial requirements, and ease of experimental manipulation (Miller and Svensson 2014; Adamski et al. 2019). Staged contests have provided key insights into assessment strategies and the correlates of winning intrasexual contests (Panhuis and Wilkinson 1999; Briffa 2008; Goyens et al. 2015), morphometric measurements have addressed questions concerning weapon-body and weapon shape-size allometry (Eberhard and Gutierrez 1991; McCullough et al. 2015; Pizzo et al. 2015), biomechanical analyses have quantified weapon mechanical performance (McCullough et al. 2015; O'Brien and Boisseau 2018; Buchalski et al. 2019), and molecular analyses have investigated the developmental genetics of exaggerated animal traits and their evolutionary diversification (Emlen et al. 2005; Zinna et al. 2018; Hu et al. 2020). Field-based studies, such as those conducted by Painting and Holwell (2014) and Fea and Holwell (2018), contribute additional insights to the field of sexual selection research as they minimise the disturbance of natural behaviour patterns and maintain the ecological context in which they occur (Lackey and Boughman 2013; Scordato et al. 2014; Tinghitella et al. 2018).

Scarabaeid beetles have proven a popular focal group in sexual selection research, but most work has focussed on horned beetles in artificial settings (e.g., Moczek and Emlen 1999; Emlen et al. 2007; Snell-Rood and Moczek 2013; McCullough et al. 2015). Other scarabs, such as the monkey beetles (Scarabaeidae: Hopliini), contain sexually dimorphic clades characterised by enlarged and exaggerated male hind legs (Ahrens et al. 2011; Colville et al. 2018). These scarabs arguably rival the horned rhinoceros and dung beetles (Colville et al. 2018), but have received little research attention, and their natural behaviour in wild, undisturbed populations is largely unknown.

1.7 MONKEY BEETLES

Development and Phenology

Monkey beetles are predominantly anthophilic scarab beetles whose fossorial larval feed and pupate within the soil before emerging aboveground as adults that feed on nectar, ovules, and pollen, as well as leaves (Steiner 1998a; Steiner 1998b; Colville et al. 2002; Colville et al. 2018). They are univoltine, the eggs hatching at the first rains, and the adults' emergence coinciding with the blooming of spring flowers (Colville 2009). Peringuey (1902) suggested an association of the larvae with termite frass heaps (see also Picker and Midgley 1994), but further work has indicated that their diet consists of detritus and other organic matter, including plant roots (Colville et al. 2002; Mayer et al. 2006). Larvae of the South African fauna were only recently collected and only a few species have been reared in captivity (J. Colville pers. comm.). The physiological requirements of both larvae and adults are largely unknown (Colville 2009). It is possible that variation in the developmental environment, including nutrient availability, soil moisture, and temperature, may influence the speed of larval development and adult morphology including male competitive traits, as found for other holometabolous insects (Kemp and Rutowski 2007; Sentinella et al. 2013; Rohner and Moczek 2020). Emergence cues, dormancy periods in the larval/pupal stage, and the correlates of larval development speed await formal investigation. As of yet, no protocols exist for rearing or breeding monkey beetles in laboratory conditions.

Distribution and behaviour

Global Hopliini diversity is concentrated in South Africa, where there are approximately 1040 species in 51 genera, of which 98% of species and 80% of genera are endemic (Colville et al. 2014).

Within South Africa, monkey beetle diversity is centred in the Greater Cape Floristic Region (GCFR; Vernon 1999; Colville et al. 2014; Colville et al. 2018). This winter-rainfall fauna is characterised by a high rate of sexual dimorphism with regards to elytral colouration and hind leg form (Colville et al. 2014; Colville et al. 2018), with male hind legs functioning as weapons in intrasexual contests for mating access to females that occur on flower heads (Rink et al. 2019). The GCFR monkey beetle fauna is divided into two flower-feeding guilds (Picker and Midgley 1996). The non-embedders are associated with non-disc-shaped flowers (Picker and Midgley 1996; Colville et al. 2002; Colville et al. 2018) and are highly mobile, frequently flying between the flowers on which they feed. This guild shows lower rates of hind leg dimorphism and less aggressive male competition than the embedding guild (Picker and Midgley 1996; Colville et al. 2018). Embedders are associated with the disc-shaped flowers of the Asteraceae and Aizoaceae, in which the sedentary females embed themselves while feeding (Picker and Midgley 1996; Colville et al. 2018). Embedders show a high frequency of sexually dimorphic species with regards to hind leg shape and size (Picker and Midgley 1996; Colville et al. 2018), and males compete with one another using their enlarged hind legs as weapons in one-on-one contests for mating access to the sedentary females, who lack any apparent mate choice (Rink et al. 2019). These contests occur on the disc-shaped Asteraceae and Aizoaceae flower heads, which form a relatively flat and stable contest arena on which males can fight (Colville et al. 2018). In contrast, non-embedders feed predominantly on non-disc-shaped flowers and therefore lack a stable contest arena (Colville et al. 2018). Both guilds are intimately associated with their host plants and monkey beetle diversity in the GCFR is linked to plant species turnover (Colville et al. 2002), suggesting that they have undergone some co-diversification with their host plants (Colville et al. 2018). At a broader scale, they have diversified along gradients of rainfall seasonality and habitat vegetation (Colville et al. 2002). This diverse fauna has in turn driven adaptive radiations of several of the region’s geophytes and other floristic components that have become specialised for monkey beetle pollination (Goldblatt et al. 1998; Steiner 1998a; Steiner 1998b; Goldblatt et al. 2005; Bernhardt and Goldblatt 2006; Manning and

Goldblatt 2006; van Kleunen et al. 2007). Monkey beetles therefore constitute an important component of the biodiversity of the GCFR.

Plant-insect interactions

The GCFR flora exhibits high rates of endemism, restricted ranges, and high alpha and beta diversity, partly due to specialisation along abiotic gradients (Linder 1991; Goldblatt 1997; Goldblatt and Manning 2002; Born et al. 2007; Desmet 2007; Colville et al. 2018). Specialised coevolutionary plant-pollinator relationships, such as those between *Lapeirousia* (Iridaceae) and their long-tongued fly (Diptera: Nemestrinidae) pollinators (Goldblatt et al. 1995; Manning and Goldblatt 1996; Forest et al. 2014), are thought to be important drivers of the region's floristic diversity (Goldblatt and Manning 2002; de Jager and Ellis 2017; Forest et al. 2018). Hopliines are important pollinators of several GCFR Iridaceae, such as the peacock moraeas, *Babiana*, and *Gladiolus* (Steiner 1998b), as well as several orchids (Steiner 1998a; Johnson et al. 2007), and Asteraceae and Aizoaceae (Mayer et al. 2006). Several Hopliinid-pollinated Asteraceae and geophytes exhibit convergent evolution, having developed 'beetle markings', portions of the inner petals that may have evolved to mimic monkey beetles and so attract them to flowers (Midgley 1993; Goldblatt et al. 1998; Bernhardt 2000; Johnson and Midgley 2001; van Kleunen et al. 2007). While the importance of plant-pollinator mutualisms is well accepted as a driver of diversification in the GCFR flora, the richness of the pollinator fauna, and whether it has diversified in concert with the flora, is debated (Giliomee 2003; Procheş and Cowling 2006; Procheş et al. 2009; Kemp and Ellis 2017). However, several pollinating insect taxa have undergone adaptive radiations in the GCFR and exhibit high levels of taxonomic and phenotypic diversity in the region (Colville et al. 2018). Monkey beetle diversity is not fully explained by co-diversification with their host plants or specialisation across ecological gradients (Colville et al. 2002; Colville 2009; Colville et al. 2018), and the high prevalence of sexual dimorphism and evidence for male-male contest competition (Midgley 1992; Rink et al. 2019), especially in the embedding guild, suggest that sexual selection may have contributed to the taxonomic and phenotypic radiations of monkey beetles in the region

(Colville 2009; Colville et al. 2018). Little work, however, has been done to describe the characteristics of sexual selection in monkey beetles or to understand its contribution to the considerable taxonomic and phenotypic diversification of these beetles.

Drivers of monkey beetle diversity

Monkey beetle taxonomic diversity is incompletely explained by natural selection i.e., coevolution with their host plants, but is associated with high levels of sexual dimorphism, especially with respect to hind leg form, in the embedding guild. This guild has spatially clumped, easily defensible, sedentary females (Midgley 1992; Colville et al. 2018; Rink et al. 2019), and the disc-shaped Asteraceae and Aizoaceae on which embedders feed and mate form a stable substrate on which one-on-one intrasexual male contest competition can take place (Colville et al. 2018; Rink et al. 2019). This close association with their host flowers means that the high floristic diversity and turnover within the GCFR may be driving monkey beetle diversification across divergent contest substrates, in line with the ‘divergent fighting contexts’ hypothesis proposed by McCullough et al. (2016). Since the larvae are soil-dwelling, selection pressures are likely modulated by both vegetation and edaphic heterogeneity (Colville et al. 2002). This group thus presents an ideal opportunity to address the role of intrasexual contest competition, in combination with natural selection acting through habitat heterogeneity, in driving taxonomic diversification.

1.8 THESIS AIMS, STRUCTURE, and METHODS

In this thesis, I aim to address questions relating to the role of sexual selection, in association with ecological selection gradients and variation in the contest substrate, in driving microevolutionary patterns of weapon divergence in monkey beetles. To achieve these aims, I combine field-based observational data collection with molecular and geometric morphometric methods to assess the role of sexual selection, in the context of habitat heterogeneity, in driving phenotypic and taxonomic diversity in two combatant, sexually dimorphic monkey beetle taxa, *Heterochelus chiragricus* Thunberg, 1818 and the cryptic species complex, *Scelophysa trimeni* Péringuey, 1885.

My aims are as follows:

Chapter 1: In this introductory chapter I aim to provide a broad overview to the topic of my thesis, to introduce the study system, and to summarise the aims, structure, and methods of the thesis.

Chapter 2: Using the sexually dimorphic monkey beetle *Heterochelus chiragricus*, I aim to assess the empirical evidence for the occurrence of intrasexual contest competition over mates in leg dimorphic monkey beetles. Within the framework of evolutionary game theory, I quantify the importance of motivational disparities between competitors in contributing to winning contests. I also quantify shape and size variation in male hind-legs using geometric morphometric methods and assess the importance of these hind leg traits in determining contest success using generalized linear models.

Chapter 3: In recent years, several hypotheses have been developed concerning the mechanisms by which diversification and speciation may occur due to intrasexual contest competition. In this chapter, I aim to assess support for the 'divergent fighting contexts' hypothesis (*sensu* McCullough et al. 2016) of taxonomic diversification by weapon form divergence in response to variation in the contest substrate. I quantify male monkey beetle weaponry and colouration within several populations of the cryptic species complex *Scelophysa trimeni.* I then utilize linear mixed effects models to quantify variation in these traits in relation to variation in the size and colour of the flowers on which they compete and variation in the abiotic environment, whilst accounting for spatial and phylogenetic autocorrelation among populations. I estimate the phylogenetic relationships among populations with a maximum-likelihood tree built with sequences extracted from individuals from each population using next-generation techniques.

Chapter 4: Evolutionary constraints may impact the ability of traits to respond to variation in the selection regime. Highly constrained traits should exhibit evolutionary inertia and should be conserved across taxa, but little is known about the influence of evolutionary constraints on animal weapon diversification. In this chapter, I aim to quantify developmental, functional, and phylogenetic constraints on the diversification of male monkey beetle weaponry to estimate their evolutionary lability. Using geometric morphometric methods, I quantify patterns of morphological modularity, integration, and shape-size allometry for four components of male *S. trimeni* hind legs from the same populations studied in **Chapter 3** and utilize the phylogeny to calculate the phylogenetic signal present in patterns of hind leg shape and size divergence among these populations. Using these data allow me to assess whether the phylogenetic diversity of the group is reflected in the diversity of its male weaponry as an indication of the extent to which male monkey beetle weaponry is constrained in its response to sexual selection.

Chapter 5: In this concluding chapter I aim to synthesize the findings from chapters 2 – 4 and discuss their contributions to the current theoretical and empirical body of work concerning the role of intrasexual contest competition in driving evolutionary diversification in general, and to our understanding of monkey beetle evolutionary ecology specifically.

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2. CONTEST DYNAMICS AND ASSESSMENT STRATEGIES IN COMBATANT MONKEY BEETLES (SCARABAEIDAE: HOPLIINI)

2.1 ABSTRACT

Some of the most striking examples of intrasexual contest competition are to be found in the insects, whose weaponry and contest behaviours have become highly intricate and diverse. Game theory has been used as a basis to develop models of the competitive assessment strategies that may be used by males to either judge their probability of winning by comparing their own fighting ability to that of their opponents, or to persist in contests for a period determined only by their own fighting ability. Conclusions from empirical studies about the means of assessment in their study systems have not, however, always been clear. In view of this, some authors have suggested that utilizing a broad suite of data concerning multiple facets of the study system may assist in gaining clearer insights into animal contests and assessment strategies. The present study integrates data on contest behaviour, weapon morphology, residency effects, cost accumulation, and correlates of contest success, to test game theory-informed models of competitive assessment strategies in the sexually dimorphic monkey beetle *Heterochelus chiragricus*. We found that males of all sizes engaged aggressively in intrasexual contests for mating access to sedentary females, utilizing their hypertrophied hind legs as weapons. Contest outcome was determined by hind femur size and strongly influenced by residency effects. We found mixed support for both pure self-assessment and mutual assessment contest strategies. Such inconclusive findings are not uncommon in animal contest assessment studies, even when contest cost and resource-holding power data are contextualized with behavioural and ecological data.

2.2 INTRODUCTION

Intrasexual contest competition amongst males for mates has driven the evolution of highly exaggerated ‘weaponised’ traits in a diverse range of animal taxa (Kodric-Brown and Brown 1984; Blanckenhorn 2005; Plard et al. 2011; McCullough et al. 2014). The physical contests in which these weapons are wielded may incur significant costs to contestants (Enquist and Leimar 1987; Briffa and Sneddon 2007; Lane and Briffa 2017), and they are generally won by the individual with the greatest fighting ability, often referred to as the greatest ‘resource holding power’ (RHP; *sensu* Parker 1974). RHP may be influenced by several factors, including weapon and body size, and quantifying the relative importance of each is integral to increasing our understanding of decision-making by combatants (Vieira and Peixoto 2013; Palaoro and Briffa 2017). Maynard Smith and Price (1973) published the first applications of game theory to the study of decision-making and assessment strategies in animal contests. This development has been highly influential on the growth and direction of research on animal contest systems, giving rise to more refined evolutionary game theory models (Parker and Rubenstein 1981; Austad 1989; Kokko et al. 2006; Arnott and Elwood 2008). Three classes of competitive assessment models have been developed, each predicting different correlations between the RHP and contest cost (generally contest duration) of a contestant: pure self-assessment, cumulative self-assessment, and mutual assessment strategies (Arnott and Elwood 2009; for predictions and assumptions of the assessment models see Table 1 of Painting and Holwell 2014; and Table 1 of Green and Patek 2018). Models of animal contest assessment have been applied to empirical studies for a range of taxa to assess the applicability of these models e.g., newts (Verrell 1986), fiddler crabs (Pratt et al. 2003), ungulates (see review in Jennings, Dómhnall and Gammell 2013), and many studies in insects and spiders (e.g., Parker and Thompson 1980; Kelly 2006; Small et al. 2009; Elwood and Prenter 2013; Walker and Holwell 2018).

Identifying the assessment strategy that is operational in a particular study system has, however, proven to be challenging across different animal groups (e.g., Turner and Huntingford 1986; Palaoro and Briffa 2017; Green and Patek 2018; Walker and Holwell 2018). Several authors have suggested that collecting data on a broad suite of traits used in contests may provide a potential solution to this challenge (Briffa and Elwood 2009; Kasumovic et al. 2011; Briffa, Hardy, and Mowles 2013; Palaoro and Briffa 2017). Such data may include more accurate quantification of RHP-related traits, or whole-body measures of fitness (Briffa, Hardy, and Mowles 2013). Field-based studies may also contribute valuable insights, as they provide the behavioural and ecological context of the study organism and how these define contest dynamics (Rodríguez-Muñoz et al. 2010; Painting and Holwell 2014). We investigate contest dynamics using game theoretic predictions in a naturally behaving wild population of insects with limited and easily defendable females and intense physical contests between males. This approach allows us to identify the physical determinants of RHP, and to quantify the role of fighting ability in shaping contest dynamics, within the behavioural context of resource ownership asymmetries.

We quantify the relative importance of RHP and residency effects in determining the outcome of intrasexual male contest competitions in the monkey beetle *Heterochelus chiragricus* (Thunberg, 1818), which occurs in high densities, emerging *en masse* in the Southern Hemisphere’s spring (August-October). Monkey beetles are a speciose flower-visiting scarab clade (>1600 species) in which sexual dimorphism is marked and prevalent in both hind leg form and body colour (Colville et al. 2018). Picker and Midgley (1996) noted their high abundance and easily observable behaviour on flower capitula. Males have been observed to use their enlarged hind legs as weapons in intrasexual contests (Louw 1987; Midgley 1992), and there is putative evidence for these fights to be concerned with obtaining access to females (Colville et al. 2018). Females of sexually dimorphic species are generally sedentary, spending extended periods of time feeding on flower heads (Picker and Midgley 1996), and are therefore defendable by resident males against intruder males (i.e., defence of a localised resource *sensu* Emlen and Oring 1977).

In this study, we aim to verify the occurrence of intrasexual contest competition, to establish the determinants of RHP, and to identify the competitive assessment strategy used by male monkey beetles *Heterochelus chiragricus*. Due to the lack of research into contest dynamics and behaviour in monkey beetles, we were unable to make *a priori* predictions concerning the most likely mode of assessment used by competing males. Instead, we set out to quantify the relationships between RHP, contest outcome, and contest duration, and infer from these the best-fitting model of contest assessment. We follow Taylor and Elwood (2003) in utilizing larger RHP/smaller RHP contestant asymmetries in our analyses of the correlations between contest cost, fighting ability and resource ownership asymmetries. If pure self-assessment is operational, we expect a negative correlation between contest duration and smaller rival RHP but not larger rival RHP (Taylor and Elwood 2003). For cumulative self-assessment, we expect a negative correlation between the difference in RHP between rivals and contest duration because the lower the RHP of the smaller rival, the more quickly it reaches its energetic threshold, and the larger its rival is, the more quickly it inflicts costs on the smaller contestant. For mutual assessment strategy, we expect a lack of correlation between mean size in size-matched contests with contest cost (duration) (Green and Patek 2018). Prior studies have shown that males guard females from rival males (Colville et al. 2018), and therefore, we predict residency effects on both assessment strategy and contest outcomes.

2.3 METHODS

Study site and field observations

We conducted fieldwork within the Hantam National Botanical Gardens on the Bokkeveld Plateau in the Northern Cape Province of South Africa. The Bokkeveld Plateau is situated on the ecotone of two global floristic hotspots - the Succulent Karoo Biome and the Cape Floristic Region (Goldblatt et al. 1989; Snijman 2013).

We carried out data collection at a mixed stand (ca. 100m by 200m) of *Berkheya glabrata* (Thunb.) and *Berkheya pinnatifida* (Thunb.) during the period 26-29 October 2015. By this time of the year, the vast majority of spring-flowering plants had ceased to flower – the main bloom period for this winter-rainfall region being August-September (Cowling et al. 1999). The two *Berkheya* species, which are mid- to late spring flowering species, were the primary species in flower at the time. They provide an important floral resource for later-emerging species of monkey beetle, including our study species *Heterochelus chiragricus* (Thunberg, 1818) (Fig. 2.1).

Observational protocol

Four researchers, acting independently, made observations of intrasexual contests between males of *H. chiragricus*. Beetles were highly concentrated and active (flying between flowers, and feeding, fighting, and mating) on the available *Berkheya* inflorescences. Data collection commenced mid-morning (ca. 09h30) and continued for as long as beetles were highly active, concluding when activity was noticeably reduced, around late-afternoon (ca. 16h00).

We identified contests in one of two ways. Either we monitored a plant, or stand of plants, which bore flowers occupied by beetles, or we moved between plants, examining them for beetles alighting on flowers occupied by *in copula* or mate-guarding pairs. We considered contests to have begun at first contact between individuals. If a contest was already underway, we began timing immediately (although we observed ~85% of fights from first contact). As such, our findings apply only to the physical contact phase of monkey beetle contests, and we cannot extend our conclusions to pre-contact agonistic behaviour, should any exist, in *H. chiragricus*. Timing was terminated when one competitor left the inflorescence or did not re-engage in combat once disposed from the flower. The duration of each contest was timed to the second. Contests were always dyadic.

Sex ratio

The sex ratio of the population within our chosen mixed stand of *Berkheya* species was estimated by conducting a census of all observed individuals either flying or stationary on flowers within the 100m by 200m stand. All counted individuals were marked with a single small white dot of nontoxic acrylic paint to avoid re-counting. Males and females collected during contest observations, or for comparative measures of sexual dimorphism, were included in the calculation of sex ratios.

Size measurements

Individuals of both sexes were measured to quantify sexual dimorphism in hind leg size (length and muscle mass) (Fig. 2.1). The males used for these analyses included those from contest observations. For both males and females, body length (distance from tip of clypeus to tip of pygidium) and right hind leg length were measured using digital callipers accurate to 0.01 mm. Body and right hind leg mass were obtained using a Mettler AE100 analytical balance accurate to 0.1 mg.

Statistical analysis

All analyses were conducted in R v.3.5.1 (R Core Team 2018). The disparity in numbers of males and females in the population was quantified using an exact binomial test with a null probability of p=0.5. The allometric relationships between leg and body size in males and females were described using nonlinear least-squares (NLS) regressions. The allometric relationship, developed by Huxley (1932), is given by:

$$y = kx^{a}$$

where y is leg size, k is a constant, x is body size, and a is a scaling exponent. The parameters k and a are estimated from the data. If $a > 1$, then the relationship between the variable of interest (leg size) and body size is positively allometric, if $a = 1$, the relationship is isometric. If $a < 1$, the relationship is negatively allometric. To assess differences in the allometric relationship due to sex, the above equation was modified following Simmons and Altwegg (2010):

$$y = (k+bs)x^{(a+cs)}$$

Where s is a factor that indicates sex (0 = male, 1 = female) and b and c estimate differences in the constant k and exponent a, respectively, that are due to sex.

Intersexual differences in allometry were assessed by testing whether *b* and *c*, respectively, were significantly different from zero. We fitted this equation to the data using the function 'nls' in R (R Core Team 2018) to construct a nonlinear least squares regression.

Weapon size and shape (i.e., form) have been traditionally quantified using linear morphometrics (e.g., Tiainen 1982; Vollrath and Parker 1992; Herrel et al. 1999). These methods conflate shape and size and are not amenable to complex analyses (Rohlf and Marcus 1993; Zelditch et al. 2004) and are limited for quantifying trait-based measures of RHP. Geometric morphometric methods have superseded these (Adams et al. 2004; Adams et al. 2013). These multivariate, landmark-based techniques have become popular tools for quantifying weapon size, especially in invertebrates, such as beetles (Eldred et al. 2016), megaloptera (Ramírez-Ponce et al. 2017), spiders (Fernández-Montraveta and Marugán-Lobón 2017), and lobsters (Claverie and Smith 2010), and produce information-rich data that can provide additional insights into trait performance (e.g., in insects Bots et al. 2012; Worthington et al. 2012; Pizzo et al. 2015). The first step in geometric morphometric analysis is to digitize the trait of interest. We photographed the right hind leg of each male using an Olympus SZ61 stereomicroscope fitted with an Olympus SC30 digital camera. A stage micrometer was used to calibrate the images for scale. The *tps* series of morphometric software (Rohlf 2015) was used to place landmarks on anatomically homologous points of biological interest following the criteria for geometric morphometric landmark selection as described in Zelditch et al. (2004) e.g., the femoral spine (Fig. 2.1). Fifteen landmarks were placed on each femur and thirteen on each tibia. Each tps file was converted into an nts file and then imported to MorphoJ v1.06d (Klingenberg 2011) for the analysis of landmark coordinate data. Each individual received a code that identified the contest it participated in and whether it won or lost. A full Procrustes fit was performed on the landmarked image sets (Zelditch et al. 2012). This process quantifies pure shape by removing all information concerning rotation, position, and size from the set (Zelditch et al. 2012). A covariance matrix based on the Procrustes-aligned coordinates (shape metric) was constructed and a Principal Components Analysis (PCA) was performed using this matrix.

Wireframes representing mean shape, and the shapes represented by the most extreme negative and positive PCA1 coefficients (eigenvectors), were constructed for each leg segment (Klingenberg and Marugán-Lobón 2013). We quantified the shape-size allometry of each leg component using major axis (MA) regressions of centroid size against the Principal component scores for each of the first three Principal Component axes using package *smatr* in R (Warton et al. 2012). To quantify the allometric scaling relationship of leg shape with body size in males, we implemented major axis (MA) regressions of the scores of each of the first three shape PC axes for each leg segment against body length.

Binomial generalised linear models (GLMs) with a logit link were used to identify the body and hind leg shape and size covariates that predict success of the resident males in contests. Each model explored a different hypothesis related to the probability of success of the resident correlated with: (1) femur shape; (2) tibia shape; (3) femur shape and tibia shape; (4) femur size; (5) tibia size; (6) whole leg size; and (7) body length. Our response variable was binary: 1 if the resident male won the contest, and 0 if he lost.

Leg segment size was quantified by centroid size, and scores of the first three PC axes of each leg segment represented shape. All covariates (except PC axis scores) were log-transformed prior to analysis. The log-transformed trait values of the intruder males were subtracted from the log-transformed trait values of the resident males to obtain relative pairwise differences. Models were run with the relative pair-wise differences in trait values as explanatory variables and the success of the focal individual as the response variable. We compared models using their Akaike Information Criterion (AIC) values (Akaike 1974; Arnold 2010).

As the assumptions of assessment models are based on RHP and contest cost, it is essential for the descriptive power of the models that suitable proxies for these variables are identified and accurately quantified (Prenter et al. 2006; Vieira and Peixoto 2013), as generally we cannot measure them directly (Payne 1998; Taylor and Elwood 2003). RHP asymmetries are generally characterised in one of three ways: either as winner versus loser, resident versus intruder, or larger RHP versus smaller RHP opponents (Parker and Thompson 1980). The third approach appears to better facilitate a stepwise unpacking of the system by avoiding conflating assessment strategy with fighting success. To infer the competitive assessment model utilized by males during fights, we examined the relationship between the RHP of each individual within each dyad and contest duration. RHP is generally approximated with a measure of weapon or body size, although factors such as energy reserves and age are also important (Kemp and Alcock 2003; Arnott and Elwood 2008). Contest duration is often used as a metric of contest cost, based on the reasonable assumption that energetic costs and physical damage to contestants accrue with time (Jennions and Backwell 1996; Mesterton-Gibbons et al. 1996; Morrell et al. 2005). Asymmetry in resource value should also be considered. We tested the effect of residency status of the winning beetle on contest duration using a two-sample t-test with unequal variances (data were heteroscedastic: Bartlett's K-squared = 12.826, $df = 1$, $P < 0.001$). To assess whether monkey beetles employed mutual assessment, we implemented a Generalized Linear Mixed Model with contest duration as the dependent variable, centroid sizes of bigger and smaller hind femurs as measures of RHP, residency status of bigger RHP rival as a random effect, and a Gamma distribution with an identity link, after Walker and Holwell (2018). Secondly, we tested for a cumulative assessment strategy using a Generalized Linear Model with a Gamma distribution and the canonical link. To do so, we examined the correlation between contest duration and absolute relative difference in RHP between contestants (absolute value of the difference between femur size of rival with greater RHP and femur size of rival with smaller RHP divided by mean femur size of rivals). Lastly, we implemented a Generalized Linear Model to assess the relationship between contest duration and mean size for size-matched contests (≤5% difference in RHP between contestants, $n = 41$), with a Gamma distribution and the canonical link to test for mutual assessment. These analyses were conducted with a reduced dataset that excluded contests not timed from initiation produced results that were qualitatively similar to those of analyses performed on the full dataset (see results section below).

2.4 RESULTS

Population sex-ratio

Within our study area, we counted 853 individual beetles, 213 of which were females and 640 of which were males. The sex ratio was male-skewed (observed proportion of males = 0.75, 95% confidence interval (*CI*) = 0.71-0.77, $P < 0.001$).

Description of fighting behaviour

Contests between males occurred only on the capitula of the flowers of the two species of *Berkheya* on which a female was present. Patrolling males alighted on flower heads occupied by a conspecific female. If she were guarded by a resident male, the intruding male would engage the resident in one-on-one combat. During combat, the female remained passive, and never rejected the advances of the successful male. Sometimes a mate-guarding or *in copula* male would lift his hind legs and vibrate his feathery tarsi at the appearance of an intruding male (Fig. 2.1). Facing away from each other, opponents lashed out with their hind legs, reflexively clenching their tarsi in a lever-like action, attempting to dislodge their opponent either off a female and/or out of the flower arena. Resident males would cling to females using their fore- and mid-legs while battling with their hind legs. Fight duration ranged from 2s to 120s (mean duration ± SE = 18.68s ± 1.68). Contests were generally won when one contestant used his hind legs to grasp his opponent and lever him off the female and out of the flower. In some instances, intruders simply gave up and left the flower. Males utilized their enlarged tibia and femurs in conjunction as levers when lifting opponents.

Sexual dimorphism and allometry

Leg length exhibited a negative allometric relationship with body length in both males (Fig. 2.2a; $k = 0.69$, 95% $CI = 0.59\text{-}0.80$) and females (Fig. 2.2b; $k = 0.82$, 95% $CI = 0.67\text{-}0.98$). There was no significance difference in slopes between the sexes ($t_{428} = -1.37$, $P = 0.17$), but the difference in intercepts was significant ($t_{428} = 3.94$, $P < 0.001$). Leg mass exhibited an isometric relationship with body mass in males (Fig. 2.2b; $k = 1.07$, 95% $CI = 0.97\text{-}1.18$) and a negative allometric relationship in females (Fig 2.2b; $k = 0.63$, 95% $CI = 0.52\text{-}0.74$), with significant differences in slopes between sexes ($t_{439} = 5.77$, $P < 0.001$).

Geometric morphometrics

Femur centroid size explained 35.05% of the shape variation accounted for in femur Principal Component axis 1 (Fig 2.3; PC1: $R^2 = 0.35$, $P < 0.001$). Principal Component axis 2 (PC2: $R^2 = 0.003$, $P = 0.32$), and Principal Component axis 3 (PC3: $R^2 = 0.073$, $P < 0.001$) accounted for minimal amounts of variation. Tibia centroid size explained nominal amounts of the shape variation accounted for in each of the first three tibia Principal Component axes (PC1: $R^2 = 0.029$, $P < 0.001$; PC2: $R^2 = 0.073$, $P < 0.001$; PC3: $R^2 = 0.06$, $P < 0.001$). Femurs exhibited notable variation in both shape and size, whereas tibias were less variable (Figure 4). Body length explained 24.81% of the change in femur shape along Principal Component axis 1 (Fig. 2.5a; PC1: Major axis regression: $R^2 = 0.25$; $P < 0.001$). Body length did not explain significant portions of the variation in femur shape along Principal Component axis 2 and Principal Component axis 3 (PC2: Major axis regression: $R^2 = 0.0108$; $P = 0.064$; PC3: $R^2 = 0.0404$; $P < 0.001$, respectively), neither did it account for significant portions of shape variation along the first three PC axes of tibia shape variation (Major axis regression: PC1: $R^2 = 0.03$; $P < 0.01$; PC2: $R^2 = 0.033$; $P < 0.01$; PC3: $R^2 = 0.084$; $P < 0.001$). There was a strong correlation between femur and tibia size, whether considering length (Fig. 2.5c; $R^2 = 0.84$, $P < 0.001$) or centroid size (Fig. 2.5d; $R^2 = 0.78$, $P < 0.001$).

Correlates of contest outcome

The candidate model for contest outcome ($n = 172$) incorporating femur size had the lowest AIC score (Table 2.1) and showed a significant effect of femur size on the probability of a resident winning (Fig. 2.6; $Z = 5.21$, $P < 0.001$). Furthermore, the model also showed that for battles where the two contestants were of similar size (difference in femur size = 0), the resident was 2.81 times more likely to win than the intruder. In contrast, if the relative difference in femur size was about

10%; that is, when the intruder had relatively larger hind femurs than the resident, each contestant was equally likely to win. Mean femur size difference within dyads ranged from 0.002% to 38.89%. Residents had significantly larger hind femurs than did intruders (t_{148} = -1.86, $P < 0.05$), but were not significantly larger-bodied (t_{157} = 1.54, P = 0.062).

Relationship between contest duration, RHP and residency effects

Resident-won contests were significantly shorter in duration than those won by intruders ($t_{63.61}$ = -2.61, $P < 0.01$; mean duration ± SE of resident-won contests = 15.41s ± 2.401, mean duration ± SE of intruder-won contests = 26.77s ± 3.92), and residents won more than twice as many contests as did intruders (114 contests were won by residents versus 52 by intruders). Contests in which the rival with greater RHP was resident were significantly shorter than contests where residents had lower RHP than intruders ($t_{104.2}$ = -1.96, $P < 0.05$). In our Generalized Linear Mixed Model, contest duration exhibited a significant positive correlation with smaller rival RHP (full dataset: β = 6.47, SE = 2.11, $P < 0.005$; reduced dataset: β = 4.69, SE = 2.11, $P < 0.05$) and a non-significant negative correlation with larger rival RHP (full dataset: β = -1.85, SE = 2.103, P = 0.38; reduced dataset: β = -1.484, SE = 2.103, P = 0.48). There was no significant relationship between contest duration and absolute relative difference in RHP between contestants (full dataset: β = 0.12, SE = 0.063, P = 0.055; reduced dataset: β = 0.11, SE = 0.074, P = 0.13). For the subset of contests in which rivals were RHP-matched, there was no significant correlation between mean contestant RHP and contest duration (full dataset: β = 0.0106, SE = 0.019, P = 0.57; reduced dataset: β = -0.0034, SE = 0.014, P = 0.802).

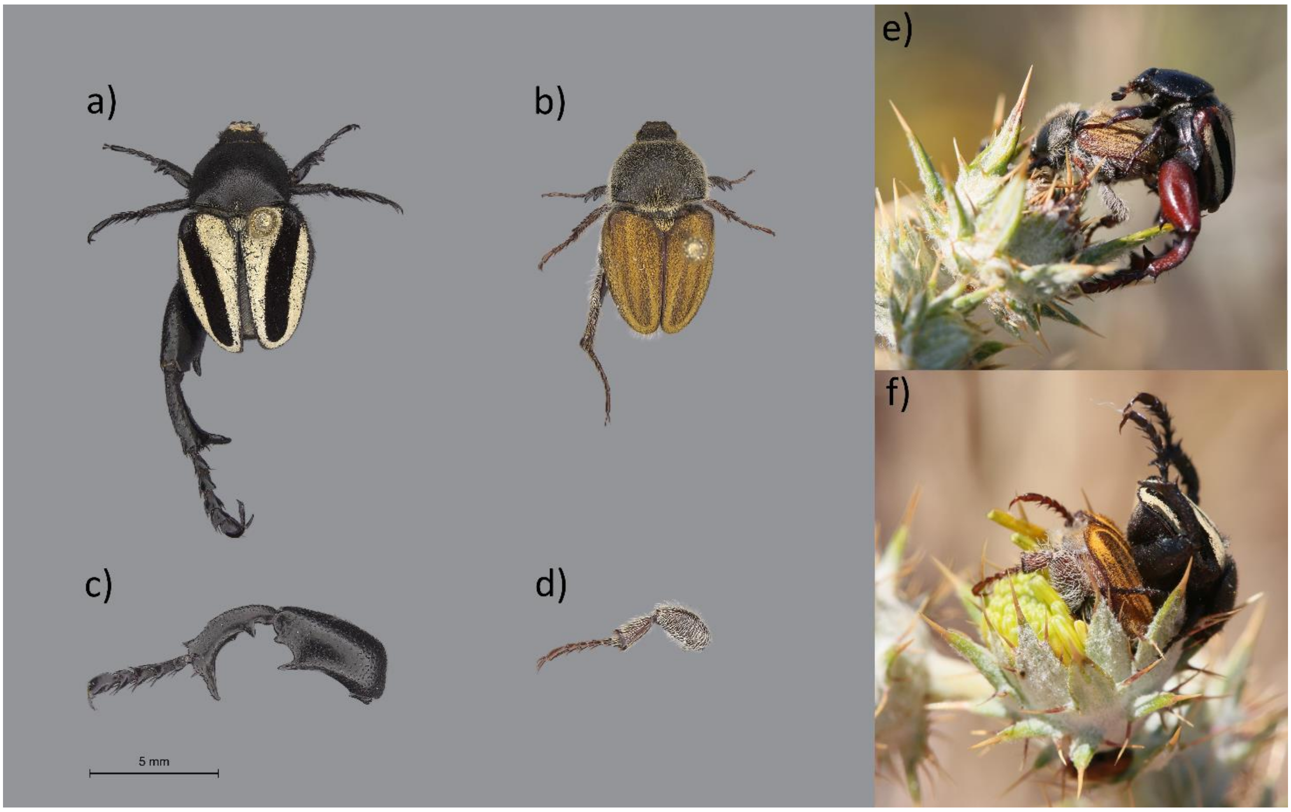

Figure 2.1. (a) Dorsal view of male (left) and (b) female (right) specimens of *Heterochelus chiragricus* showing hind leg and colour dimorphism; (c) lateral view of male and (d) female hind legs: note the greatly enlarged male hind legs with prominent inter-locking femoral and tibial spines. Densely packed pale-yellow and black scales form the striking patterns on the elytra of the male. Photographs (e) and (f) were taken *in situ* at the study site and illustrate mating, and male mate guarding of the female, respectively. Note the striking intersexual differences in colouration and contrast, the posturing of male hind legs, and also the intrasexual colour dimorphism present in the hind legs: deep red as in (e), or jet black as in (f).

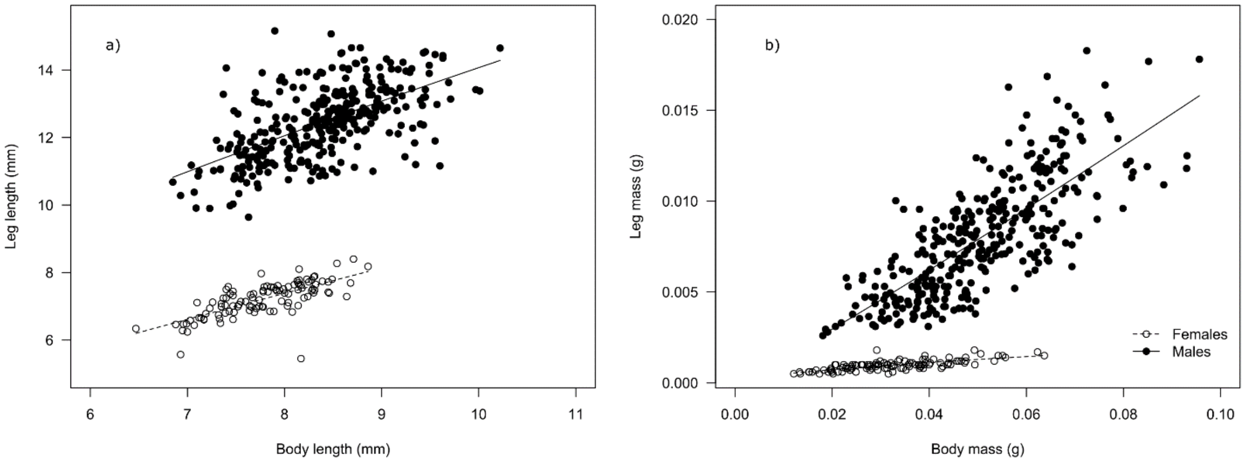

Figure 2.2. Fitted nonlinear regression showing the relationship between (a) leg and body length ($R^2 = 0.90$, $P < 0.001$) and (b) leg and body mass ($R^2 = 0.82$, $P < 0.001$) for females ($n = 106$) and males ($n = 326$).

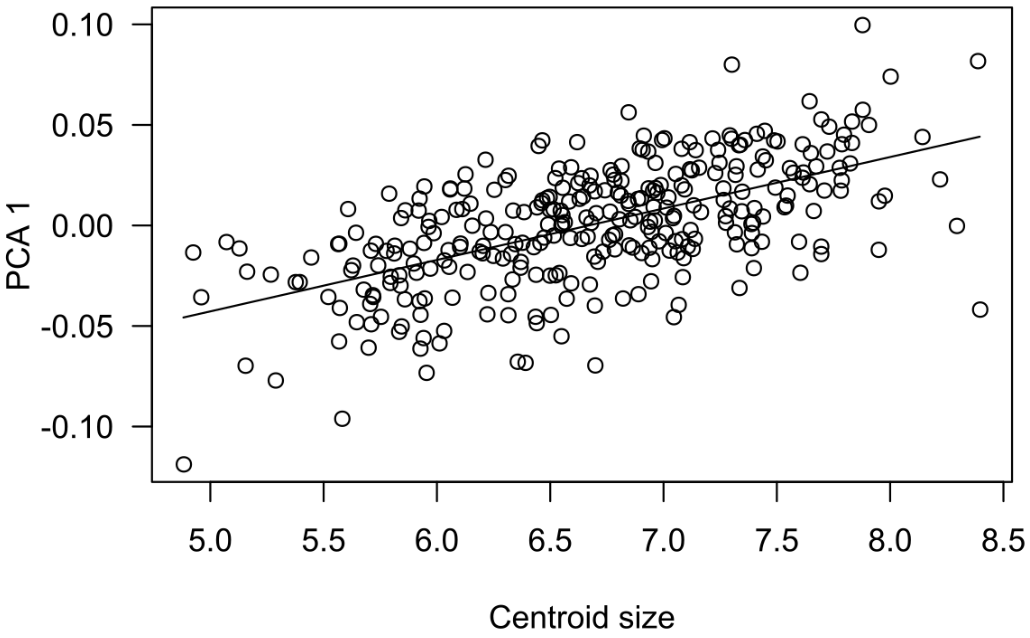

Figure 2.3. Major axis regression of femur shape (PC1 scores) on centroid size. Femur size accounts for approximately 35% of the variation in femur shape ($R^2 = 0.35$, $P < 0.001$).

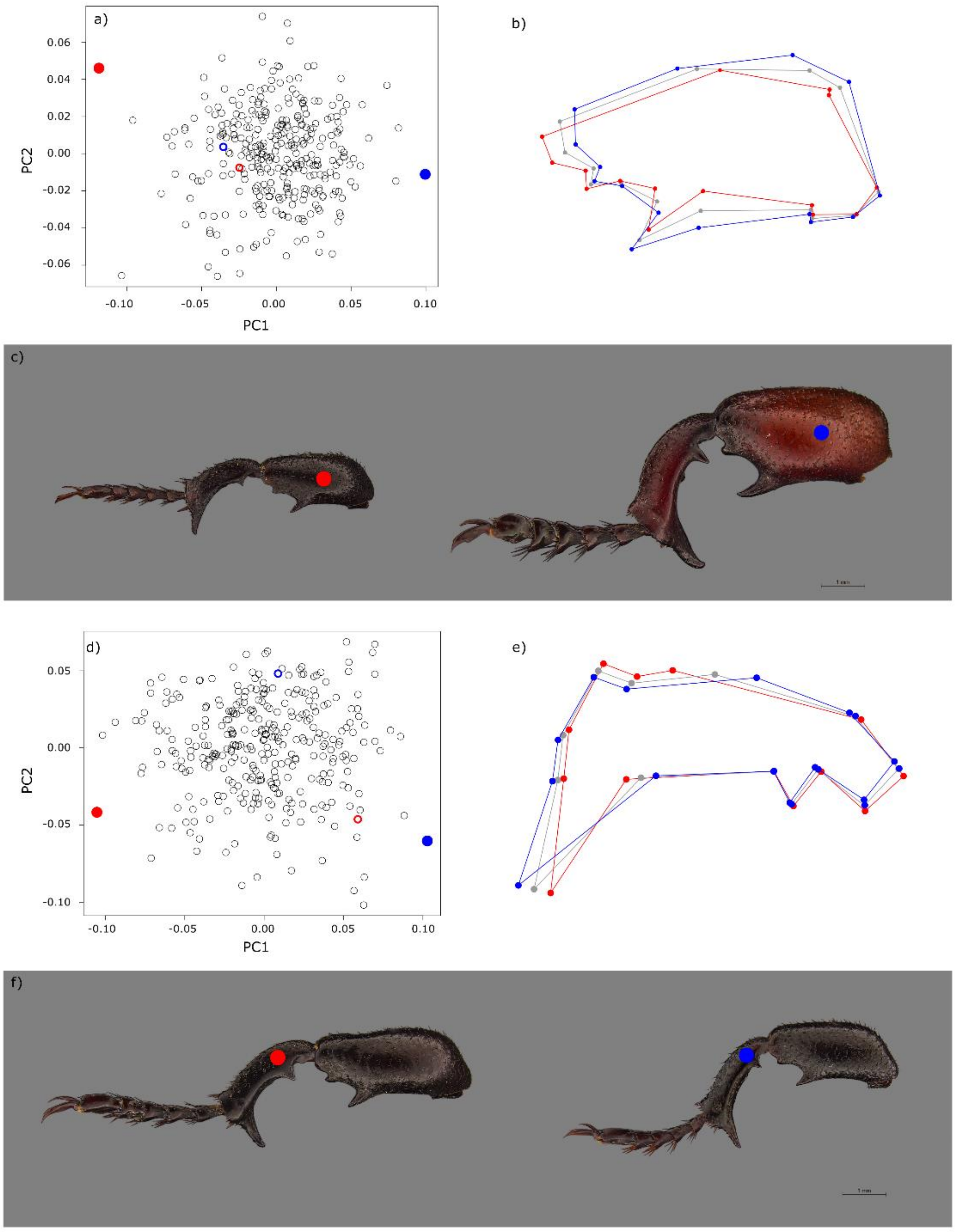

Figure 2.4. Shape variation in the femur and tibia of males as quantified by landmark-based geometric morphometrics. Panels a) and d) show variation in the PCA space of the femur and tibia, respectively. PC scores are computed from a covariance matrix derived from Procrustes-aligned landmark coordinates. Plots b) and e) are wireframes representing the range of shape variation along the PC1 axis of femur shape and tibia shape, respectively. Images c) and f) depict the legs bearing the femurs and tibias corresponding to the most extreme positive (red colouring) and negative (blue colouring) values along the first Principal axis of shape variation. The white points positioned along the edges of the femur and tibia of the leg on the right-hand side of image c) correspond to the positions of the landmarks used in geometric morphometric analysis of shape and size. The solid red/blue point in plot a) corresponds to the red/blue wireframe in panel b) and the femur of the leg on the left/right hand side of image c). Similarly, the solid red/blue point in plot d) corresponds to the red/blue wireframe in panel e) and the tibia of the leg on the left/right hand side of image f). The red/blue unfilled points in plot a) correspond to the scores of the femurs of the legs bearing the tibias depicted in image f), and the red/blue unfilled points in plot d) correspond to the scores of the tibias of the legs bearing the femurs depicted in image c).

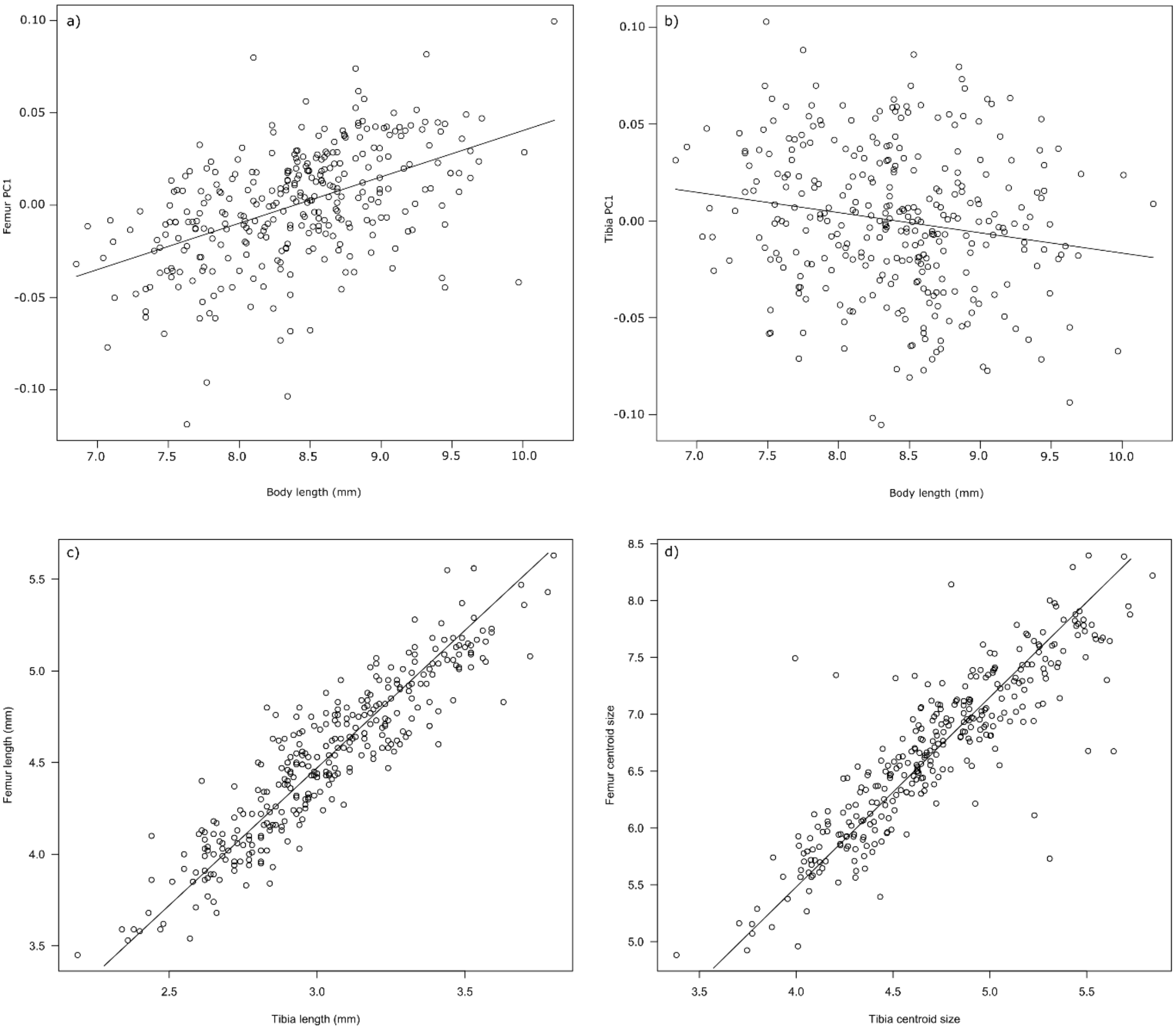

Figure 2.5. Nonlinear least squares regression for a sample of males for (a) femur centroid size on body length ($R^2 = 0.69$), and (b) tibia centroid size on body length ($R^2 = 0.69$). Major axis regression for a sample of males for (c) femur length on tibia length ($R^2 = 0.84$, $P < 0.001$), and (d) femur centroid size on tibia centroid size ($R^2 = 0.77$, $P < 0.001$).

Table 2.1. Set of candidate binomial generalised linear models with logit links.

Model	Number of parameters	Residual deviance	*df*	ΔAIC
Residency status of winner ~ (resident femur shape PC1-intruder femur shape PC1) + (resident femur shape PC2-intruder femur shape PC2) + (resident femur shape PC3-intruder femur shape PC3)	3	122.7	127	14.5
Residency status of winner ~ (resident tibia shape PC1-intruder tibia shape PC1) + (resident tibia shape PC2-intruder tibia shape PC2) + (resident tibia shape PC3-intruder tibia shape PC3)	3	141.9	127	33.7
Residency status of winner ~ (resident femur shape PC1-intruder femur shape PC1) + (resident femur shape PC2-intruder femur shape PC2) + (resident femur shape PC3-intruder femur shape PC3) + (resident tibia shape PC1-intruder tibia shape PC1) + (resident tibia shape PC2-intruder tibia shape PC2) + (resident tibia shape PC3-intruder tibia shape PC3)	6	115.2	124	13.1
Residency status of winner ~ log(resident femur centroid size – intruder femur centroid size)	1	112.2	129	0.0
Residency status of winner ~ log(resident femur centroid size – intruder femur centroid size)+ log(resident tibia centroid size – intruder tibia centroid size)	2	112.0	128	1.8
Residency status of winner ~ log(resident body length – intruder body length)	1	120.7	129	8.5

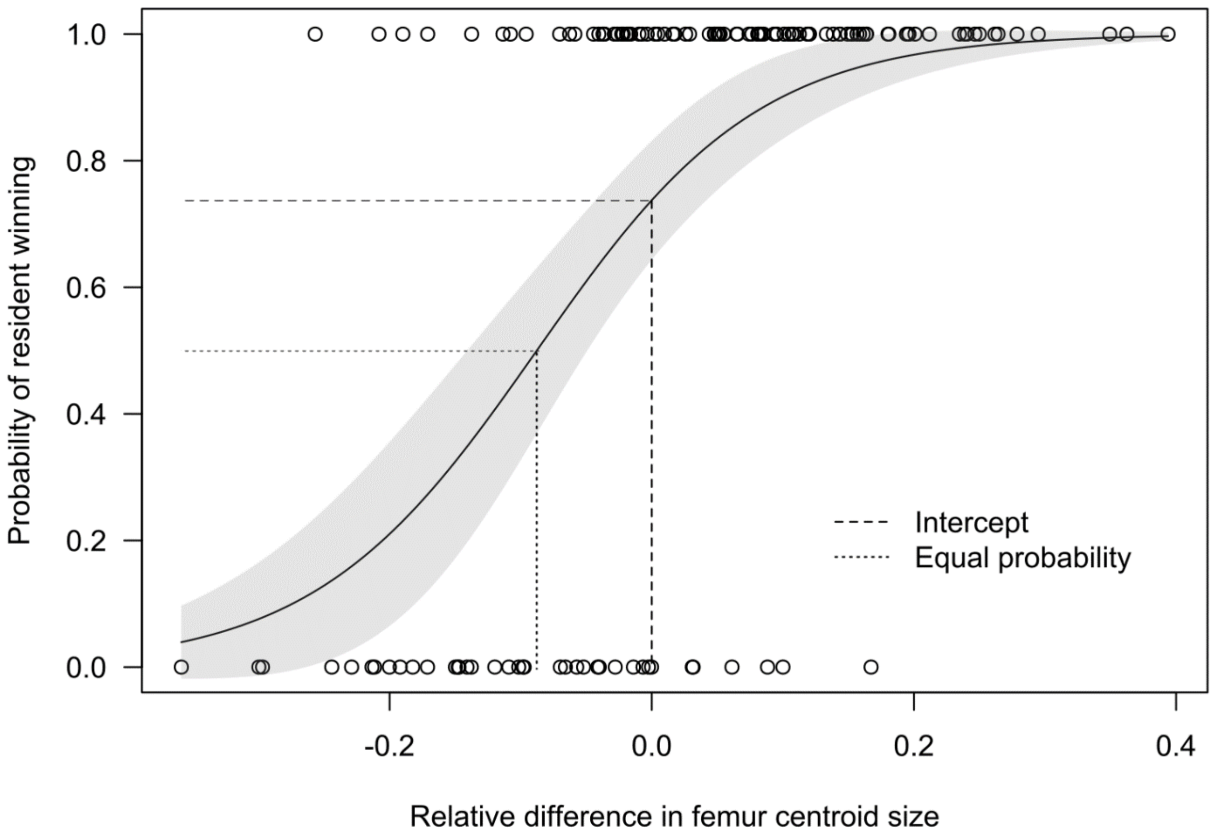

Figure 2.6. The best candidate model as ranked by AIC score. Binomial generalised linear model with logit link relating relative difference in femur centroid size to probability that a resident male is successful in an intrasexual contest ($Z = 5.21$, $P < 0.001$). Shaded region represents the 95% confidence interval.

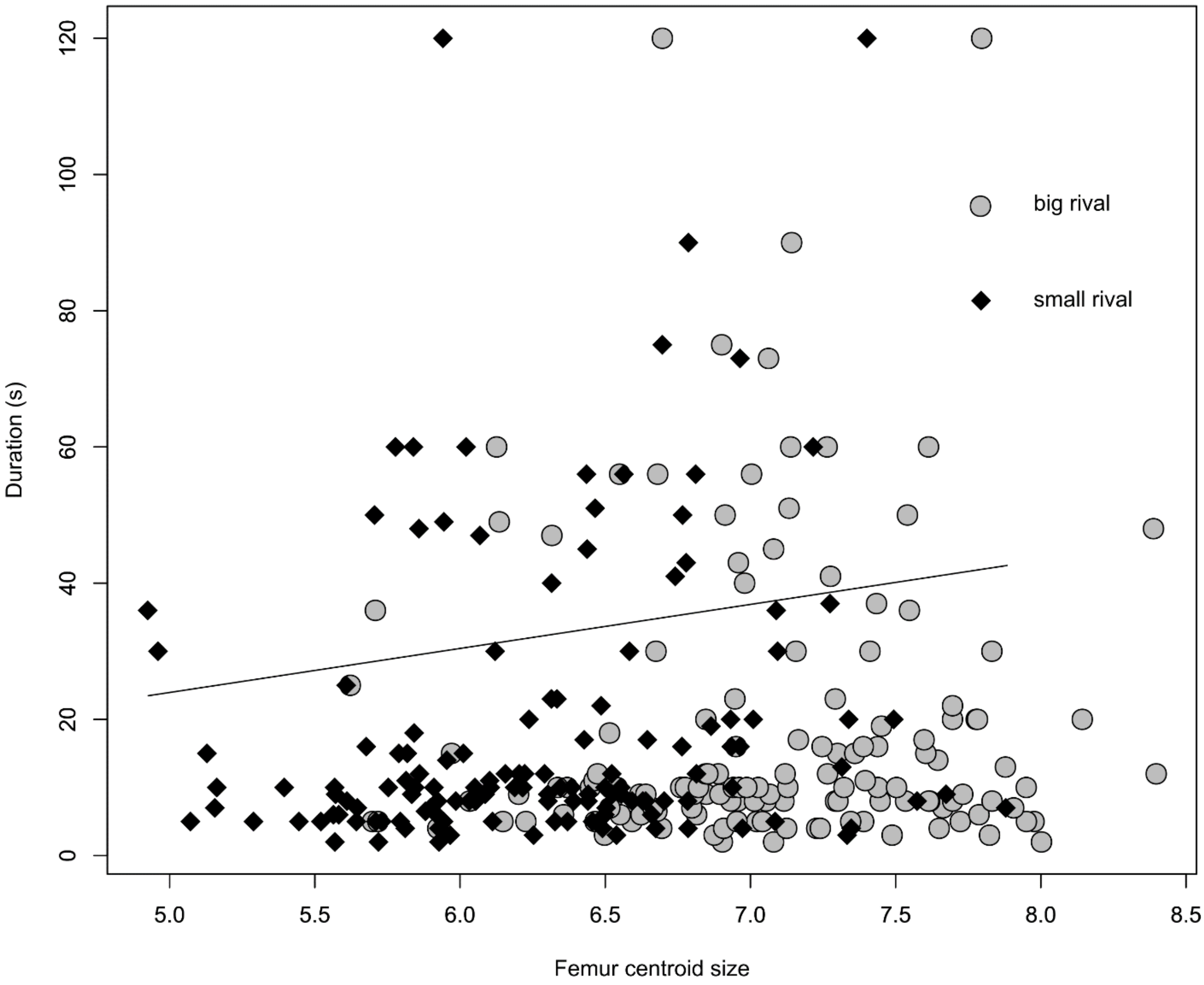

Figure 2.7. The relationship between bigger RHP rival size and duration, and smaller RHP rival size and duration for each dyad, controlling for residency status. Smaller RHP rival size was significantly correlated with contest duration ($\beta = 6.465$, SE = 2.109, $P < 0.01$; relationship represented by regression line), while bigger RHP rival size was not ($\beta = -1.846$, SE = 2.099, $P = 0.37905$).

2.5 DISCUSSION

We aimed to conduct a comprehensive study of intrasexual male contest behaviour in the monkey beetle *H. chiragricus*. We have verified that these contests are driven by competition between males for reproductive access to females. Our results suggest a significant role of weapon size in defining contest outcome, with individuals having larger weapons (hind femurs) more likely to win fights. Asymmetry in the subjective resource value of opponents, possibly due to residency effects, appears to be an important factor in determining contest outcomes. It must be noted that our results pertain only to contests that include physical contact, and so the conclusions drawn from our findings may not be informative of pre-contact contest behaviour in *H. chiragricus*, if any exists.

We found that the hind legs of males were significantly larger than those of females (>150% longer and >800% heavier) and bore large femoral and tibial spines and sculptured elements (Fig. 2.1). Male hind legs, particularly femur centroid size, scaled more steeply with body size than did female hind legs. These results support the presence of sexual dimorphism and a positive allometric scaling relationship between weapon and body size in males (Andersson 1994; Bonduriansky and Day 2003; Fromhage and Kokko 2014). Although there was high collinearity between male hind femur centroid size and hind tibia centroid size, the former exhibited a positive allometric scaling relationship while the latter scaled isometrically with body size (see also Colville et al. 2018). Opponents with relatively greater hind femur centroid sizes were significantly more likely to win contests. This pattern was not found when assessing relative difference in tibia centroid size within fighting pairs. However, our observations of naturally occurring contests showed that fighting males frequently used their femur and tibia together as levers and clamps to pry guarding males off females and toss rivals off flower capitula. An investigation of the forces exerted by rivals during battle and the biomechanical functioning of their hind legs would contribute insights into the relative importance of each leg component during physical contests (see McCullough 2014).

Hind legs as weapons are well-documented in several insect taxa, including leaf-footed bugs (Emberts et al. 2018), frog-legged leaf beetles (O'Brien et al. 2017), and wētās (Fea and Holwell 2018), but not within the Scarabaeidae, where horns are prevalent (Snell-Rood and Moczek 2013). Surprisingly, given their large spines and sculptured form, and variation in the shape of leg components (Fig. 2.1; Fig. 2.4), we found no support for a significant role of hind femur or hind tibia shape in contributing to contest outcome. Although the gracile, feathered tarsi of males were not significant predictors of contest success, we observed that mate-guarding or *in copula* males "waved" and "vibrated" their tarsi at intruding males. The compound eyes of insects are, overall, poor at discerning details at distance (Caves et al. 2018). Monkey beetles, however, appear to have high visual acuity for colours and patterns (Hutchinson 1946; Picker and Midgley 1996; Steiner 1998a; Steiner 1998b) and may be able to assess the hind leg size of other males as a proxy of their RHP through aerial patrolling before landing on an occupied flower. The densely scaled elytra of males are strikingly patterned with boldly contrasting colours (Fig. 2.1) and may compliment their hind legs as signals of their RHP, in a similar manner to how colour indicates fitness of male damselflies *Calopteryx maculata* to potential rivals (Fitzstephens and Getty 2000).

Our analyses of the correlates of contest costs produced results that provide conflicting support for two mutually exclusive assessment strategies. The significant positive correlation between smaller rival RHP and contest duration combined with the lack of correlation between larger rival RHP and contest duration, suggests that male monkey beetles persist in fights according to their own RHP only; that is, they utilize a self-assessment strategy to resolve contests (Taylor et al. 2001; Taylor and Elwood 2003; Arnott and Elwood 2009). Residency status of the rival with greater RHP had a significant impact on contest duration, suggesting that although persistence may ultimately be constrained by own RHP, contests in which more powerful individuals had an ownership advantage were resolved more quickly than contests in which intruders had greater RHP than residents. This finding concurs with past studies that have found a significant role for ownership in animal contests (Parker and Thompson 1980; Jennions and Backwell 1996), in some cases even overriding RHP in determining contest outcome (e.g., Kasumovic et al. 2011). The lack of correlation found between mean rival RHP and contest duration in size-matched contests, however, supports a mutual assessment strategy (Arnott and Elwood 2009; Green and Patek 2018). Although there is some variation in how different practitioners have defined mutual and self-assessment strategies (Green and Patek 2018), they are inarguably based on fundamentally different assumptions concerning decision-making and contest structure (Briffa and Elwood 2009; Briffa 2014). Briffa and Elwood (2009) and Green and Patek (2018) assert that self-assessment and mutual assessment strategies cannot be distinguished solely from analysis of RHP and contest cost, and that other aspects of the system's contest dynamics should be taken into account, such as patterns of escalation and de-escalation (Parker 1974; Briffa, Hardy, Gammell, et al. 2013; Mesterton-Gibbons and Heap 2014), whether or not contestants engage in behavioural matching (Payne 1998; Taylor and Elwood 2003), or whether there are asymmetries in resource ownership (e.g., Verrell 1986). Data for the above incorporated into game theory models may clarify our understanding of contest assessment strategies in animal systems.

In populations with male-biased operational sex ratios, as seen in our study species, each reproductively mature female has intrinsic value as a scarce resource (Kvarnemo and Ahnesjö 1996; Weir et al. 2011). Additionally, each male assigns a subjective value to winning a female, dependant on internal factors such as depletion of energetic resources or whether they have mated before, and on external factors, such as whether they are in initial possession of the female or are the intruder (Enquist and Leimar 1987; Hurd 2006; Arnott and Elwood 2008; Arnott and Elwood 2009; Stockermans and Hardy 2013). This 'motivation' can have a significant influence on contest dynamics, so that an intruder male may only win if he has a great enough RHP to overcomes the ownership advantage of a rival (Parker 1974; Hammerstein 1979; Leimar and Enquist 1984), such as seen in jumping spiders (Kasumovic et al. 2011). Our findings suggest that mate-guarding or *in copula* males place higher value in maintaining possession of the female than intruding males do in wresting her away. Parasitoid wasps *Eupelmus vuilleti* exhibit similar contest dynamics, albeit in female-female competition over hosts on which to oviposit. Females placed subjective resource value on a host depending on their own egg load and previous exposure to hosts (Mohamad et al. 2010), and females with higher egg loads were more likely to win agonistic interactions over oviposition sites. Although the aim is oviposition rather than mating, the conclusions to be drawn about the role of motivation and subjective resource value appear applicable to most animal contests. Other authors have postulated that resident males may have a 'positional' advantage because they are in physical possession of the female and may be difficult to dislodge from her, as found to be the case in the amphipod *Gammarus pulex* (Prenter et al. 2006), and for burrow owners during contests between male fiddler crabs (Fayed et al. 2008). This may also be the case with monkey beetles - we observed male *H. chiragricus* straddling females bodily whilst mate-guarding them and gripping them tightly with their mid and fore legs while fending off intruders with powerful kicks from their hind legs.

We have shown that the RHP of males is determined by weapon size, and that together with ownership effects, this determines contest outcome. Our results emphasize that identifying the mode of assessment in animal contests is challenging, even when the key variables of RHP and contest cost are interpreted in light of behavioural data that contextualises contest dynamics. Similar conclusions have been reached in several studies using diverse animal systems (Bridge et al. 2000; Elias et al. 2008; Judge and Bonanno 2008; Briffa and Elwood 2009). The emerging viewpoint is that the dynamics of animal contests vary greatly between systems and even throughout the lifespans of contestants, and that these sources of variability need to be explicitly accounted for in both theoretical and empirical studies. Promising avenues of research include sequential behavioural analysis (Green and Patek 2018), the quantification of ageing-associated physiological changes in contestants (Fawcett and Johnstone 2010; Fawcett and Mowles 2013; Ecology 2017), and quantification of the effects of past experience on the decision-making and fighting abilities of contestants (Fawcett and Johnstone 2010; Fawcett and Mowles 2013). There has, however, been a general lack of formal integration of these into evolutionary game theory models of contest behaviour. This disjuncture may explain the mixed support, as seen in our study and others, for different game theory-informed models of competitive assessment strategies.

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3. CORRELATED DIVERGENCE IN CONTEST ARENA, MALE WEAPONRY, AND SIGNALLING TRAITS AMONG ALLOPATRIC AND SYMPATRIC TAXA IN A CRYPTIC SPECIES COMPLEX

3.1 ABSTRACT

Much of the research on speciation by sexual selection has examined female choice. Male competition has been relatively overlooked, but in recent years several hypotheses for speciation by intrasexual male competition have been proposed. The ‘divergent fighting contexts’ hypothesis proposes that divergence in the contest substrate should necessitate adjustments in fighting style, in turn selecting for weapons best suited to new fighting conditions. Divergence of the substrate among populations may cause reproductive isolation if sufficient divergence in fighting style and weaponry occur to allow local males to consistently out-compete immigrants. However, this hypothesis has not been empirically studied and it has been recognised that there are considerable challenges to testing this hypothesis in wild populations. Here, I examine the evidence for the ‘divergent fighting contexts’ hypothesis, while accounting for abiotic gradients, and spatial and phylogenetic distance, in *Scelophysa trimeni*, a sexually dimorphic (hind leg, body colour) cryptic species complex of monkey beetle. My study system comprises allopatric and sympatric populations, occupying a large geographic range (>400 km) of mostly homogeneous abiotic environment with variable contest substrate. Male *S. trimeni*, using their enlarged hind legs as weapons, engage in intrasexual contests over reproductive access to females on flower heads. I quantified the amount of covariation between male sexually dimorphic traits (weapon shape and size, elytral colouration) and the contest environment (flower head size and colour, abiotic variables). I found that male colouration and weaponry varied significantly among

populations and that this variation exhibited phylogenetic autocorrelation. Variation in these traits among populations was associated both with variation in the abiotic environment and differences in the contest substrate (flower head). Weapon size covaried with flower size, and male colour covaried with flower colour and abiotic variables. Weapon shape also covaried with abiotic variables but did not exhibit covariation with the contest substrate. These findings suggest an important association between variation in the contest substrate and male competitive traits and highlight a need for future research to assess whether trait variation is adaptive, and to what extent it is condition-dependent and phenotypically plastic. I conclude that there is scope for sexual selection to drive diversification in sexually dimorphic monkey beetles competing on divergent contest substrates, most likely in combination with natural selection.

3.2 INTRODUCTION

Sexual selection is frequently invoked when sexually dimorphic taxa exhibit unexpectedly high rates of speciation in comparison to their monomorphic relatives (West-Eberhard 1983; Servedio and Boughman 2017; Janicke et al. 2018). In practice, it has proven difficult to demonstrate that sexual selection can act as the primary causal agent of speciation (Panhuis et al. 2001; Servedio and Boughman 2017). To drive speciation by reproductive isolation, sexual selection must be capable of driving phenotypic divergence in secondary sexual traits (weapons or ornaments) (McCullough et al. 2016; Tinghitella et al. 2018a). Additionally, this trait divergence must be able to enforce reproductive isolation to lead to speciation (Scordato et al. 2014). The ability of sexual selection, especially intrasexual contest competition (Burdfield-Steel and Shuker 2018), to cause reproductive isolation is highly contentious (Qvarnström et al. 2012; Servedio and Bürger 2014; Chenoweth et al. 2015). Rather, prevailing theoretical and empirical work suggests that sexual selection is most likely to drive speciation when operating in tandem with natural selection (Ritchie 2007; Miller and Svensson 2014; Tinghitella et al. 2018a).

Work on sexual selection and diversification has historically been dominated by a focus on mate choice, to the extent that sexual selection and mate choice have often been considered synonymous (Qvarnström et al. 2012; Møller and Alatalo 1999). We therefore have a richer body of theoretical and empirical work on mate choice (predominantly *female* choice) as a driver of ornament diversification and speciation than we do for male contest competition as a driver of weapon diversification and speciation (Qvarnström et al. 2012; McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018a). The paucity of research into the evolutionary implications of male competition partially results from a lack of clear mechanisms by which male competition could cause reproductive isolation (Tinghitella et al. 2018b). Reviews of the field (e.g., Qvarnström et al. 2012; Miller 2013; McCullough et al. 2016; Dijkstra and Border 2018; Lackey et al. 2018; McCullough and Emlen 2018; Tinghitella et al. 2018a) are beginning to address this imbalance by laying the theoretical framework on which future empirical work can build, specifically by constructing hypotheses of possible mechanisms (e.g., Dijkstra et al. 2007; Dijkstra and Groothuis 2011; Procter et al. 2012; Lackey and Boughman 2013; Keagy et al. 2016). Interactions between natural selection and sexual selection are key components in many of these theories, and their authors call for research into how environmental structure, complexity, and heterogeneity mediate sexual selection for exaggerated weaponry in natural populations (McCullough et al. 2016; Svensson 2019). In this regard, McCullough et al. (2016) discuss six hypotheses in depth. They emphasize the lack of work done to date on the processes by which male contest competition may drive trait diversification and speciation and highlight the need for field-based studies on natural populations to understand the contribution of natural selection to speciation by sexual selection (Gosden and Svensson 2008; Miller and Emlen 2010; Qvarnström et al. 2012). Working with wild populations in the field, in contrast to laboratory studies, allows researchers to assess how selection regimes operate *in situ* with minimal disturbance (Rodríguez-Muñoz et al. 2010).

The aim of the present research is to apply the “divergent fighting contexts” hypothesis discussed by McCullough et al. (2016), Lackey et al. (2018), and Tinghitella et al. (2018a) to a wild system in the field. This hypothesis proposes that armaments (weapon traits), ornaments (e.g., intrasexual signalling traits), and fighting style are adapted to the fighting substrate of different habitats. If the characteristics of the fighting arena vary between populations, these traits and fighting style should diverge accordingly (McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018a). If enough trait divergence between populations allows local males to consistently out-compete migrants, then prezygotic reproductive isolation should follow (McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018a). There are considerable challenges to testing this hypothesis in wild populations. Numerous extraneous environmental variables, such as abiotic gradients and/or historical geographic barriers, may obscure any relationships between covariation in contest substrate, weapon form, and signal traits, with patterns of phylogenetic divergence. Adaptation of weaponry, signals and fighting styles to contest substrates may also be constrained by phylogenetic signal or swamped by gene flow between geographically proximate populations locally adapted to different substrates (Felsenstein 1985; Ng et al. 2013; Kaldhusdal et al. 2015). Additionally, development × environment interactions should be considered due to the heightened phenotypic plasticity and condition-dependence of sexually selected traits relative to traits under natural selection (Moczek 2009). The phenotypic expression of male weaponry in adult holometabolous insects may therefore be impacted by development × environment interactions.

If intrasexual contest competition can drive diversification in the manner proposed by the divergent fighting contexts hypothesis, it may be key to understanding the extraordinary species richness and morphological disparity of groups such as the Scarabaeidae, which are notable for their male-male competition for females and their diversity of male weaponry (Spector 2006; Colville et al. 2018). Some scarabs, such as *Onthophagus* spp. (dung beetles) and Dynastinae (rhinoceros beetles), are renowned for ‘extreme’ male-borne horns that encompass diverse morphologies that are associated with diverse contest substrate type and fighting style (Eberhard 1980; Emlen et al. 2005; McCullough et al. 2014). Other scarab groups are less well known, but no less remarkable for their diverse male weaponry. The monkey beetles (Scarabaeidae: Hopliini), with over 1600 species, exhibit an impressive diversity of weapons with ~64% of species showing male-borne enlarged and weaponised hind legs (Colville et al. 2018). Monkey beetle genera with high proportions of leg-and colour dimorphic species tend to be more speciose, suggesting that sexual selection may have played a role in the taxonomic diversification of this group (Colville et al. 2018).

Here, I test the relationships between spatial divergence in contest arena characteristics and male weaponry and ornamentation (colour) among several spatially dispersed, wild populations of the sexually dimorphic monkey beetle *Scelophysa trimeni* Péringuey, 1885. Sexual dimorphism is present in hind leg size and colouration, females having gracile legs and multiple colour morphs, while males have robust, well-sclerotised, and armoured legs, and are varying shades of blue, from blue-black to cerulean (Fig. 3.1). In sexually dimorphic monkey beetles, males compete for females, wrestling with their hind legs on flower heads that serve as contest arenas, and hind femur size is important in determining contest outcome (Rink et al. 2019; Chapter 2).

I expect that if the divergent fighting contexts hypothesis is operational then between-population variation in hind leg size and shape will be correlated with variation in contest substrate (disc-shaped flower size). Assuming that male colouration serves as a visual signal of resource holding power (Wong and Candolin 2005; Arnott and Elwood 2008; Arnott and Elwood 2009) to other males, and in light of observations (Colville et al. 2018) that this colouration varies among populations, I expect that this variation in male *S. trimeni* should be inversely correlated with among population flower colour variation to maintain contrast between signalling males and their background (flower colour). I expect that these relationships may be mediated by gene flow between populations and exhibit phylogenetic autocorrelation. I supplement detailed field-collected data on allopatric and sympatric natural populations with double-digest restriction site associated data (ddRAD; Peterson et al. 2012) and analyse these data in a mixed-effects model framework to quantify the relative importance of the developmental environment, as measured by abiotic habitat variation, and micro-scale contest substrate variation on combative trait diversification in *S. trimeni*.

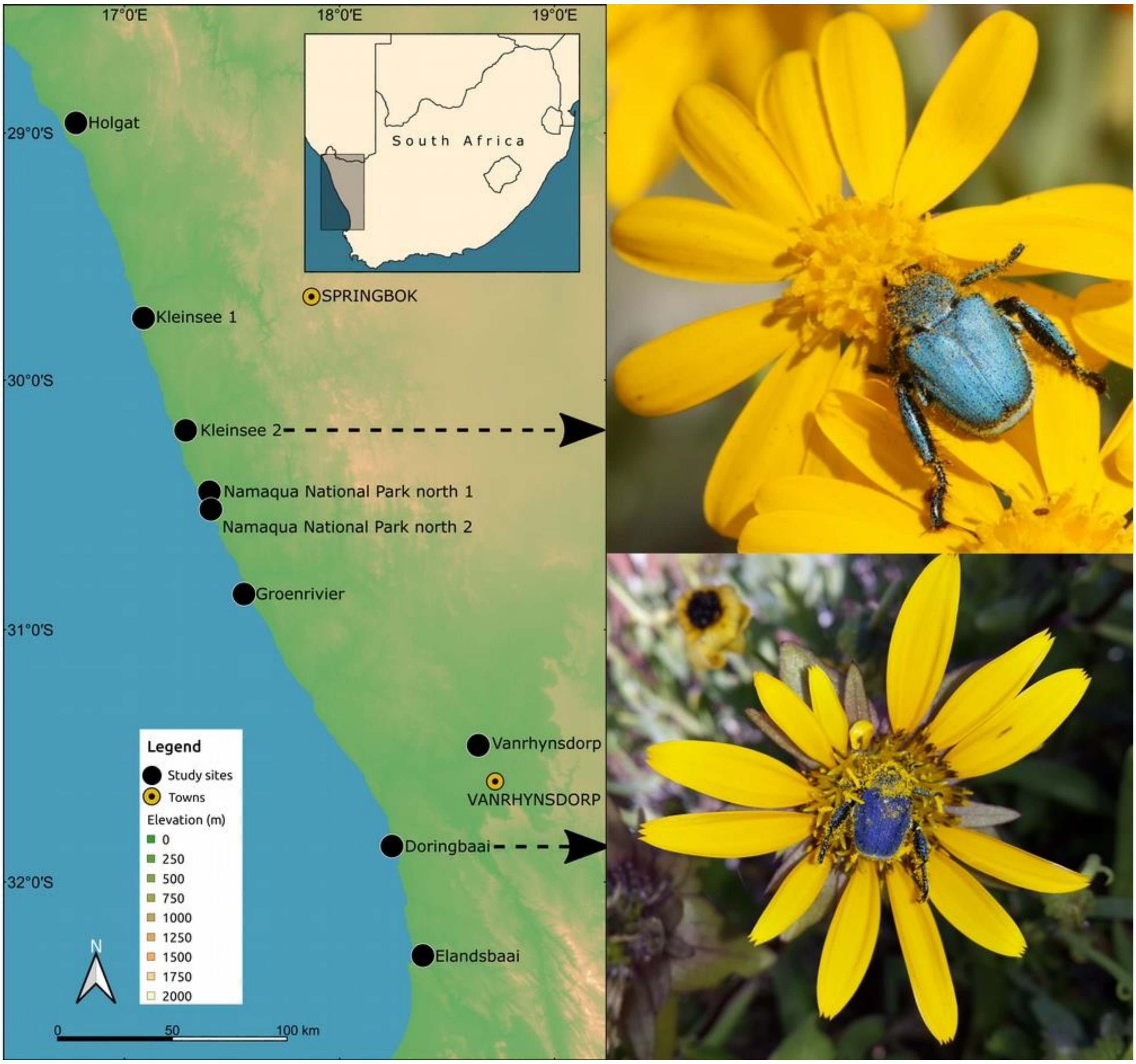

Figure 3.1. Left: *Scelophysa trimeni* is restricted to a narrow band along the west coastal region of South Africa, where it is associated with coastal vegetation types and nutrient-poor soils. It is also known from one inland locality, Vanrhynsdorp, where it has tracked an intrusion of coastal soils and vegetation. Namaqua National Park north 1 and Vanrhynsdorp each contain two genetically distinct sympatric populations of *S. trimeni* (Fig. 3.3). The study sites are referred to by the following abbreviations: Holgat (HLGT); Kleinsee 1 (KLS1); Kleinsee 2 (KLS2); Namaqua National Park north 1 (NNP1, containing a ‘robust’ population referred to as NP1R and a ‘gracile’ population referred to as NP1G); Namaqua National Park north 2 (NNP2); Groenrivier (NNPG); Vanrhynsdorp (VDRP, containing a ‘robust’ population referred to as VDPR and a ‘gracile’ population referred to as VDPG); Doringbaai (DBAY); and Elandsbaai (EBAY). Males exhibit striking variation in colour and contrast against the contest substrate among populations. Shown here is a KLS2 male on *Othonna cylindrica.* (top right) and a DBAY male on *Didelta carnosa* (bottom right).

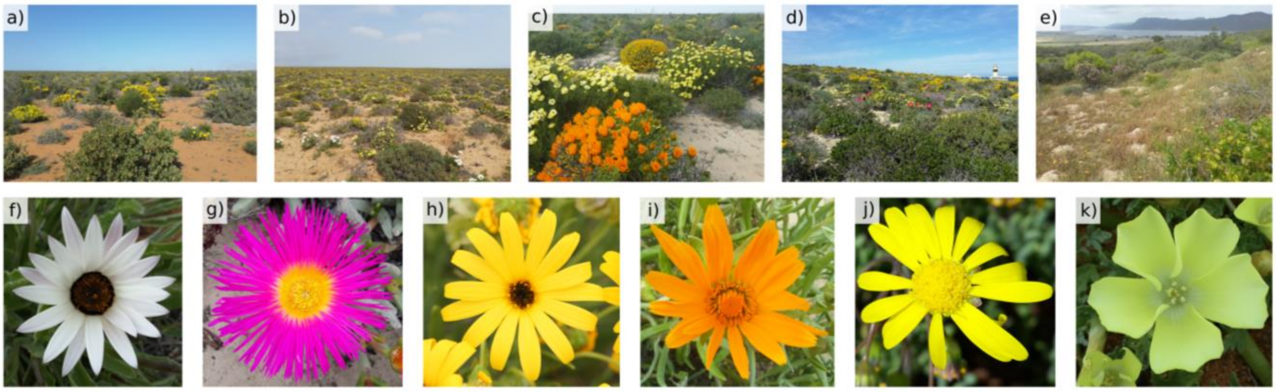

Figure 3.2. Top: vegetation structure at study sites a) HLGT, b) KLS1, c) NNP2, d) NNPG, and e) EBAY. Bottom: Flower disc and petal colour in f) *Arctotis scullyi*, g) *Jordaaniella cuprea*, h) *Tripteris oppositifolia*, i) *Didelta carnosa*, j) *Othonna cylindrica*, and k) *Grielum humifusum*.

Figure 3.3. Top: green-morph female (left) and male (right) *S. trimeni* feeding on flowers of *Ruschia sp.* in the EBAY population. Note the colour and hind leg dimorphism. Bottom: two males competing for a female (center) in the DBAY population. Note males' use of their hind legs to grapple with one another.

3.3 METHODS

Study system

Scelophysa trimeni is distributed along the west coastal region of the Greater Cape Floristic Region of South Africa (Fig. 3.1), a largely homogeneous habitat with little large-scale variation in abiotic factors or vegetation structure (Fig. 3.2; Mucina et al. 2006; Cowling et al. 2009). There are no isolating topographical barriers, such as mountains. While the Knersvlakte may present a barrier to gene flow in some vertebrates (Moodley and Harley 2005; Smit et al. 2007), this does not appear to be the case for monkey beetles, which are important pollinators of the region's Aizoaceae (Struck 1995). *Scelophysa trimeni* populations thus appear separated only by geographic distance with no apparent physical barriers to gene flow. This uniform coastal environment has likely experienced relative climatic stability since the beginning of the Miocene, approximately 23MYA (Cowling et al. 2009; Pickford et al. 2014; Dewar and Stewart 2016). The geographical extent over which *S. trimeni* is distributed (approx. 400 km north to south) may bring vicariance effects into play, meaning that gene flow may be limited by the distance between populations (i.e., isolation-by-distance) (Wiley, 1988).

Female *S. trimeni* are polymorphic with regards to body colour and multiple distinct colour morphs co-occur, often showing crypsis matching host flower colours. Males are always blue, although they vary from blue-black to cerulean across their distribution, and they often contrast brilliantly (to the human eye) with their host flowers (Fig. 3.3), predominantly disc-shaped Asteraceae and Aizoaceae, most of which are white, yellow, or orange (Fig. 3.2). Males compete on these disc-shaped flower heads, which serve as fighting platforms (Fig. 3.3). Fights consist of competitors wrestling with and kicking each other with their enlarged, well-sclerotized hind legs. In monkey beetle species contest success is correlated with hind femur size (see Chapter 2 for details on monkey beetle contest dynamics). The soil-dwelling larvae feed on organic matter through the hot, dry summer before undergoing pupation underground and emerging as adults in early Spring. The climatic and edaphic conditions under which the larval and pupal stages are completed thus may mediate the development of exaggerated hind legs in the adult males. Host flowers vary among *S. trimeni* populations with respect to species diversity and abundance, with some flower species recorded at multiple populations whereas others occurred at only a single site. Flowers also exhibit intraspecific colour and size variation both within and between field sites.

Field sites

I conducted my fieldwork during August – September in 2015 and again in August 2016, to coincide with the mass flowering of monkey beetles' host flowers and emergence of adult beetles in the Austral Spring (Cowling et al. 1999; Colville et al. 2018). I selected nine study sites based on the latitudinal range of *S. trimeni* (Figs. 3.1 and 3.2). At each site I conducted sampling and observations within a 100m x 200m plot with assistance from three field assistants. I sampled each site at least for one full day (~ 8 hours) of favourable weather: an ambient temperature of at least 18 °C and full sun, conditions when beetles are active, and flowers are open.

Environmental and contest arena data collection

Between four and six soil samples were taken at the corners and centre of each site. At each point approximately 730 cm^3 of soil to a depth of 14.5 cm was sampled using an auger and stored in airtight plastic bags. A 50g subsample of each soil sample (44 samples in total) was analysed for soil organic carbon ("soil carbon") content using the Walkley-Black method

(Walkley and Black 1934; Walkley 1947) by the Western Cape Government's soil, water, and plant diagnostics facility (Elsenburg; www.elsenburg.com). I obtained mean summer temperature ("temperature") by calculating the grand mean of the mean monthly temperatures for October to March 1950-1999 published by Schulze and Maharaj (2004) and mean annual precipitation from 1950-1999 ("precipitation"; Schulze and Lynch 2007), from data in the South African Atlas of Climatology and Agrohydrology (Schulze et al. 2007). I identified each flower from which I sampled male *S. trimeni* to species where possible, otherwise to genus. I measured petal length and capitulum diameter using digital callipers accurate to 0.01 mm and then calculated total flower diameter as 2 × petal length + capitulum diameter.

Flower spectrometry

I sampled five fresh flowers of each of the most abundantly flowering monkey beetle host species at each site (Table 3.2). I measured reflectance spectra of flower discs and petals in the wavelength range 300 nm to 700 nm (UV-VIS) using an Ocean Optics USB2000+ spectrometer with a Deuterium Tungsten Halogen light source (DT-MINI-2-GS; Ocean Optics, Dunedin, FL, USA), operated via OceanView software v1.5.2 (Ocean Optics, Dunedin, FL, USA). The spectrometer was calibrated against a PTFE diffuse white reflectance standard (WS-1, Ocean Optics, Dunedin, FL, USA) and a matt black film canister was used to block out ambient light during measurements and used as a dark standard between specimens. The probe was fixed in a reflection probe holder (RPH-1, Ocean Optics, Dunedin, FL, USA) at a 45° angle to the specimen to minimise specular glare (Ng et al. 2013). I took one reflectance measurement of each flower's disc and three measurements equidistant along the length of one petal. I prepared the spectra for analysis using the R package 'pavo' (Maia et al. 2019). I used the function *procspec*() to bin the spectra in 1 nm increments, applied Loess smoothing (Cleveland and Devlin 1988), corrected negative reflectance values (Montgomerie 2006), and then calculated the mean disc and petal reflectance spectra for each species at each site using the *aggspec*() function. I then extracted ultra-violet chroma (S1U) and blue chroma (S1B), which indicate the relative contributions of the UV (> 400 nm) and blue (400 nm – 500 nm) spectral ranges to total brightness (Montgomerie 2006). Each flower on which a male *S. trimeni* was recorded was assigned the mean S1U and S1B values for that species at that site.

Table 3.1. Number of male *S. trimeni* in each population recorded on host flowers.

Population	*Arctotis scullyi*	*Asteraceae sp. 1*	*Asteraceae sp. 2*	*Asteraceae sp. 3*	*Didelta carnosa*	*Dimorphotheca sinuata*	*Dimorphotheca sp.*	*Grielum humifusum*	*Jordaaniella spongiosa*	*Othonna cylindrica*	*Othonna sedifolia*	*Ruschia sp.*	*Senecio arenarius*	*Tripteris oppositifolia*	*Ursinia anthemoides*	Total
DBAY					71		2							27		100
EBAY			5			51		13		2		4	1			76
HLGT	14													47		61
KLS1										56	10			36		102
KLS2	13				87					1				7		108
NNP2	32				75				1					1		109
NNPG	49				11				10	1				13		84
NP1G	1				62						1					64
NP1R	6				22											28
VDPG				1						5			5	63	17	91
VDPR		1											1	5		7

Beetle data collection

At each site I sampled approximately 100 individuals of *S. trimeni*, from which I obtained phenotypic, genetic, and demographic data. Specimens were euthanised by freezing. Immediately following euthanisation, I preserved 10 – 17 randomly selected specimens per site in RNALater™ (Ambion) and stored them at -40 °C for molecular analysis. One hind leg from each specimen was stored in 99% EtOH. The pronotal width of each male was measured with digital callipers accurate to 0.01 mm.

Genetics

Genomic DNA was extracted using either a standard phenol:chloroform extraction protocol or with the Qiagen DNeasy Kit (Qiagen, Valencia, CA, USA). We prepared ddRAD libraries following the protocol described in (Peterson et al. 2012). Genomic DNA (500 ng) of each individual was digested using 0.5 µL of SbfI-HF (0.1 U/ µL) and 0.5 µL of EcoRI (0.1 U/ µL) at 37°C for three hours. Each sample was then ligated to one of 18 unique barcodes (P1 and P2). Pools of 9 samples were size selected for fragments between 450-550 base pairs (bp) using Pippen Prep (Sage Science, Beverly, MA, USA). After size selection, integrity and quantification of samples was assessed using the Agilent 2100 Bioanalyzer system (Agilent, Santa Clara, CA, USA). Each library was amplified using 8-10 PCR amplification cycles and dual-indexed using Illumina adapters (P5 and P7; Peterson et al. 2012). A final quantification was performed using the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Libraries were sequenced across one lane of the Illumina HiSeq 4000 platform at the Vincent J. Coates Genomics Sequencing Laboratory, University of California, Berkeley).

Raw sequence reads for all samples were de-multiplexed and then cleaned by trimming adaptors and low-quality reads following the protocols in Bi et al. (2012) and Singhal (2013).

Using a threshold of 30% missing data, 835 loci were retained across 113 individuals including outgroup taxa. These loci were aligned using MAFFT (Katoh et al. 2009) and a maximum likelihood phylogeny constructed in RAXML v8.2.12 (Stamatakis 2014) on the CIPRES portal (Miller et al. 2010). The tree was rooted on an undescribed, closely related *Scelophysa* species, and node support was evaluated using the rapid bootstrapping procedure implemented in RAXML. A GTR model of nucleotide substitution was applied to each locus independently.

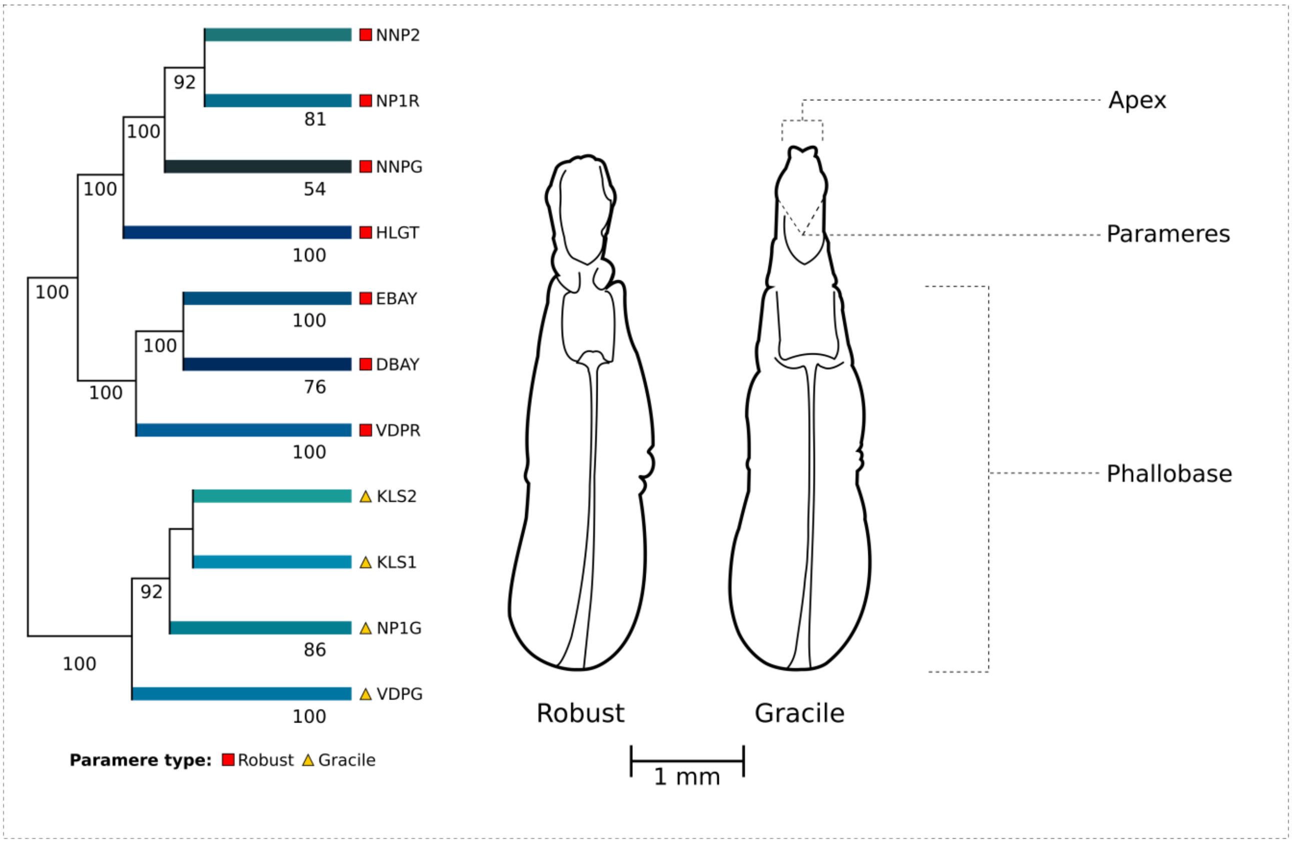

Figure 3.4. Maximum likelihood phylogeny of study populations based on ddRAD data (835 loci, 63174 bp), with terminal branches collapsed to reflect populations, alongside diagrams of typical ‘robust’ and ‘gracile’ male paramere types. Branch colour represents population mean male colour.

Male genitalia

Male genitalia are a key morphological character in delimiting monkey beetle species (Dombrow and Colville 2020). As it was not possible to sequence all males collected at each site, I dissected out the genitalia of both sequenced and non-sequenced males to ascertain whether non-sequenced males could be assigned to the same population as those that had been sequenced. A morphological match would add phenotypic support to the phylogeny and support the expansion of the phylogeny to all males at each site. A mismatch would suggest that greater genotypic diversity existed at each site than indicated by the phylogeny, and that non-sequenced males would need to be excluded from analyses considering genetic distance among populations. I dissected out the parameres of all male specimens using fine forceps under a dissecting microscope. I soaked parameres representative of the two morphotypes identified in the sampled individuals in a solution of 10% KOH overnight to clear the cuticle of soft tissue. Once cleared I rinsed them with H_20 and then imaged them with an Olympus SZ61 stereomicroscope fitted with an Olympus SC30 digital camera. Lines drawings which I made based on these images are depicted in Fig. 3.4.

Geometric morphometrics

I used a geometric morphometrics framework to quantify male hind leg form (shape and size) variation. Geometric morphometric methods have superseded traditional morphometric methods as the preferred means of quantifying form variation in anatomical traits (Adams et al. 2004; Zelditch et al. 2012; Gelvartes 2013). Unlike traditional linear morphometric methods, which conflate shape and size and thus preclude analyses of their variation as separate components of form (Zelditch et al. 2012), geometric morphometrics methods produce shape information, via Procrustes superimposition, that is independent of the relative size, position, and orientation of specimens (Zelditch et al. 2012). Geometric morphometrics analyses produce metrics of shape and size data that are independent of one another and can easily be utilised in downstream analyses (Mitteroecker and Gunz 2009) to quantify patterns of shape and size variation. I photographed the right hind legs of several males from each field site using an Olympus SZ61 stereomicroscope fitted with an Olympus SC30 digital camera, which I calibrated with a stage micrometer to ensure that relative size information would be accurately quantified during geometric morphometric analyses. I fitted sliding semi-landmarks to the legs using the 'tps' series of software (Rohlf 2015), treating the femur, tibia, tarsus, and tarsal claw as separate data sets so that I could examine their shape and size variation independently of one another. As some legs were damaged, I was not able to digitise all leg components of all males. Hence, sample sizes for each population differed between leg components, from 5 to 106 with a mean of ~72. I prepared these data for analysis using the R package 'geomorph' (Adams et al. 2020). I applied the Procrustes superimposition procedure (Gower 1975) to each of the four datasets using the function *gpagen*(), after which I plotted the Procrustes shape variables in tangent space (Revell 2009) using function *gm.prcomp*(). I extracted individuals' shape scores along all principal components that explained ≥ 5 % of total shape variation in each leg component for input into downstream analyses. I also retained the centroid size (CS) of each leg component of each male.

Beetle spectrometry

Reflectance spectra were obtained from 100 males from each study site. Preserved specimens were prepared for reflectance measurements by submergence and gentle agitation in isopropanol for 30 seconds before being dried at 40 °C for 30 minutes to improve their colour fidelity by removing excess moisture. I measured the reflectance spectra of male elytra in the wavelength range 300 nm to 700 nm (UV-VIS) using the same equipment as for flower reflectance spectra (see above). I took one spectral reading from each of four locations on the elytra: anterior right; anterior left; posterior right; and posterior left (Fig. 3.5). The spectra were imported into R and prepared for further analysis using the R package ‘pavo’ (Maia et al. 2019) using the same procedure as for flower spectra (see above). For each male I calculated a mean reflectance spectrum averaged from the four elytral readings. I then extracted S1U and S1B values from this mean reflectance spectrum (Montgomerie 2006).

Figure 3.5. Locations of reflectance measurements on male *S. trimeni*. Measurements were smoothed and averaged to obtain a mean reflectance spectrum for each male, from which UV and blue chroma values were extracted. Anterior right (AR); anterior left (AL); posterior right (PR); and posterior left (PL).

Data analysis

I conducted all statistical analyses in R version 3.6.3 (R Core Team 2020). I implemented linear mixed effects models to quantify the relationships between environmental variation and secondary sexual trait variation in male *S. trimeni*:

$$T_{ij} = \beta_0 + \beta_1 \times b_j + \mu_{oj} + \epsilon_{ij}$$

(1)

where T_{ij} is the value of the secondary sexual trait in male *i* in population *j*, β_0 is the intercept, β_1 is the regression coefficient, b_j is the mean of the environmental predictor variable in population *j*, μ_{oj} is the population random effect that allows the intercept to vary between populations, and ϵ_{ij} are the residuals. The random effect is assumed to follow a normal distribution with mean 0 and variance σ^2_{cj}. The residuals are assumed to either be independent or correlated according to the spatial Euclidean distance between populations, or correlated according to the phylogenetic distance between populations, measured as the distance from the root of the phylogeny to the most recent common ancestor of each pair of populations. The secondary sexual traits that I studied are shown in Table 3. Using linear mixed effects models allowed me to account for phylogenetic and spatial autocorrelation between populations. As the field of geometric morphometrics so far lacks suitable derivations of other models of trait evolution (e.g., Ornstein-Uhlenbeck; Hansen 1997; Beaulieu et al. 2012) for highly multivariate data (Adams and Collyer 2018; Adams and Collyer 2019), I was restricted to implementing a Brownian approach.

I began by assessing whether phylogenetic or spatial autocorrelation was present by constructing three intercept-only models for each trait, i.e., without the term b_j. Each of these models imposed a different correlation structure on ϵ_{ij}. In the first model, n1, ϵ_{ij} are uncorrelated; in the second model, n2, ϵ_{ij} exhibit correlation as a function of the phylogenetic distances between populations according to a Brownian motion model of evolution; and in the third model, n3, ϵ_{ij} exhibit correlation as a function of the spatial distance between populations, with their correlation decaying at an exponential rate.

I used AIC values (Akaike 1974) to compare the fits of models n1, n2, and n3. I utilized the covariance structure of the best-fitting intercept-only model in further linear mixed effects models quantifying the relationship between each trait and environmental and contest substrate variables.

Each model quantified between-population variance of a male secondary sexual trait as a function of a single predictor variable describing either variation in the abiotic environment (precipitation, temperature, and soil carbon) or variation in the contest substrate. All traits were modelled in relation to each of the abiotic environment variables. Hind leg traits were modelled as a function of flower diameter only, while beetle chroma traits were modelled as functions of the corresponding flower chroma traits (i.e., beetle UV chroma was modelled as a function of petal UV chroma and, separately, of disc UV chroma).

For each trait, I quantified the percentage of unexplained between-population variance in the intercept-only model that was explained by each covariate as:

$$100 * \frac{\sigma^2_{0j} - \sigma^2_{cj}}{\sigma^2_{0j}}$$

where σ^2_{0j} is the estimated unexplained between-population variance from the intercept-only model and σ^2_{cj} is the estimated unexplained between-population variance from the covariate model.

For each trait, I selected the best fitting model based on its AIC (Aikaike 1974) and AIC weighting (Wagenmakers and Farrell 2004). I fitted all models using the function *lme*() in the R package ‘nlme’ (Pinheiro et al. 2020). I fitted all models using maximum likelihood estimation. I prepared model selection tables using the R package ‘stargazer’ (Hlavac 2018).

I controlled for any effects of trait correlations with body size by running a second set of models that included pronotum width as a covariate in addition to the contest substrate and environmental variables analysed above. The intercept-only models were replaced by ones which modelled trait value in response to pronotum width (the “baseline” models). Subsequent models assessed the percentage of unexplained among-population variance in the baseline models were explained by the addition of an environmental covariate. Model fits were assessed as described above.

3.4 RESULTS

Differences in the developmental environment and contest substrate among populations

Precipitation ranged from 81 mm at HLGT to 233 mm at EBAY, and temperature ranged from 19.23 °C at NNP1 and NNP2 to 23.38 °C at VDRP (Table 3.2). Soil carbon differed significantly between sites (Range: 0.148 ± 0.066 (VDRP), 0.540 ± 0.144 (NNP1 and NNP2); ANOVA: $F_{8,39} = 2.4087$, $P = 0.032$; Table 3.2). The diameters of visited flowers differed significantly between populations (ANOVA: $F_{1,10} = 140.79$, $P < 0.0001$; Table 3.2; Fig. 3.6). Based on spectra obtained from the most abundantly flowering monkey beetle host species at each site, neither the UV chroma nor blue chroma of flowers differed significantly between localities (ANOVA: disc UV chroma: $F_{8,58} = 0.386$, $P = 0.924$; disc blue chroma: $F_{8,58} = 0.742$, $P = 0.655$; petal UV chroma: $F_{8,58} = 1.057$, $P = 0.405$; petal blue chroma: $F_{8,58} = 0.327$, $P = 0.952$; Table 3.2; Fig. 3.6).

Variation in weapon form

All leg components differed significantly in mean size and shape between populations (Table 3.3). Males in population NNP2 exhibited the largest femora (Fig. 3.7: CS ± SE = 8.870 ± 0.045) and tibiae (Fig. 3.8: CS ± SE = 9.097 ± 0.043), while NP1R males had the largest tarsi (Fig. 3.9: CS ± SE = 5.310 ± 0.059) and DBAY males bore the largest claws (Fig. 3.10: CS ± SE = 3.728 ± 0.025).

Femur shape

More than 80% of the variation in femur shape was encapsulated along PC1, which showed a gradient from more gracile hindlimb femora in KLS2 males to bulkier hindlimb femora in EBAY males, although with considerable variation within populations (Fig. 3.11).

Tibia shape

Less than 30% of the variation in tibia shape was described by PC1 (Fig. 3.12). More negative PC1 scores, such as obtained for males from NNPG, were associated with greater sculpturing of the tibia and more pronounced tibial spines. Populations showed some division along PC2, which accounted for almost 20% of the variation in tibia shape. Males from HLGT, NNPG, NNP2 and NP1R generally obtained negative scores along PC2 while males from KLS2, VDPG and NP1G obtained positive PC2 scores.

Tarsus shape

More than 40% of tarsus shape was associated with the first PC axis (Fig. 3.13). Populations appear to occupy distinctive regions of shape space. Males from EBAY, HLGT, NNP2, NNPG, and NP1R had PC1 scores < 0, which are associated with a trend towards bulkier tarsi. Males from KLS1 and KLS2, NP1G, and VDPG had scores > 0 on PC1, indicating more gracile tarsi in these populations. Males from DBAY and VDPR had intermediate scores on PC1.

Claw shape

PC1 (80.54%; Fig. 3.14) describes a shift from broader claws for PC1 scores < 0, as for males from HLGT, NP1G, and NNP2, to more strongly curved claws for PC1 scores > 0, as for males from DBAY and EBAY, although these populations show large scatter in shape space.

Beetle Chroma

Male UV chroma (Fig. 3.15) and blue chroma (Fig. 3.16) differed significantly between populations (UV: $F_{10,826} = 181.5$, $P < 0.0001$; blue: $F_{10.826} = 193.7$, $P < 0.0001$; Table 3.3). The most UV purity was exhibited by HLGT males (S1U ± SE = 0.323 ± 0.003; Fig. 3.15) while VDPG males exhibited the purest blue colouration (S1B ± SE = 0.402 ± 0.002; Fig. 3.16). Sympatric populations did not exhibit significant differences for most traits, but males from NP1G and NP1R did have significantly different tibia shapes and chroma values (Table 3.4; Fig. 3.12; Fig. 3.15; Fig. 3.16).

Covariance between competitive traits and the abiotic environment and contest substrate

Phylogenetic and spatial distances between populations were positively correlated with one another ($R^2 = 0.430$, 95% CI = 0.272−0.565, t = 5.193, DF = 119, $P < 0.0001$). Phylogenetic correlation structures significantly improved the fit of null models for most leg component traits, except for femur size, where the best fit was obtained with a spatial correlation structure, and tarsus size, where the best fit was obtained without specifying a correlation structure (Table 3.5). For all hind leg components, the most variation in size between populations (> 35%) was explained by a positive covariance with the mean diameter of the flower species visited by each male (Table 3.6, Table 3.7, and Fig. 3.17). Although flower diameter explained 36% of between-population variation in hind femur size, the null model obtained the lowest AIC (Table 3.6). Leg component shapes showed differing patterns of covariation with the response variables (Table 3.6). For femur shape variation, AIC favoured temperature, which explained 20% of the variation between populations (Table 3.6, Table 3.7, and Fig. 3.18). While soil carbon explained more than 40% of between population variation in claw shape (Table 3.6, Table 3.7, and Fig. 3.19); it explained less than 4% of tibia shape variation, and precipitation explained more than 27% of claw shape variation but less than 5% of shape variation for other hind leg components (Table 3.6). Tibia shape was poorly (<5%) explained by all the environmental (abiotic and contest substrate) variables considered, and the null model was best supported by the data (AIC = -14559.190; Table 3.6).

Phylogenetic correlation structures significantly improved null model fit for both aspects of male chroma (Table 3.5). Variations in UV and blue chroma between populations showed relationships with both abiotic and flower chroma variables (Table 3.6). Beetle UV chroma showed covariation with temperature, soil carbon, and a positive relationship with flower petal UV chroma, with the latter being best supported by the data. While beetle blue chroma variation was partially explained by variation in flower disc blue chroma (~15%), the most favoured explanatory factor by AIC score was precipitation, with which beetle blue chroma scaled positively across populations (Table 3.7; Fig. 3.20).

In general, accounting for relationships between trait values and body size did not substantively alter the importance of contest substrate and environmental variables in explaining trait variance among populations (but see tarsus shape and claw shape; Table A1).

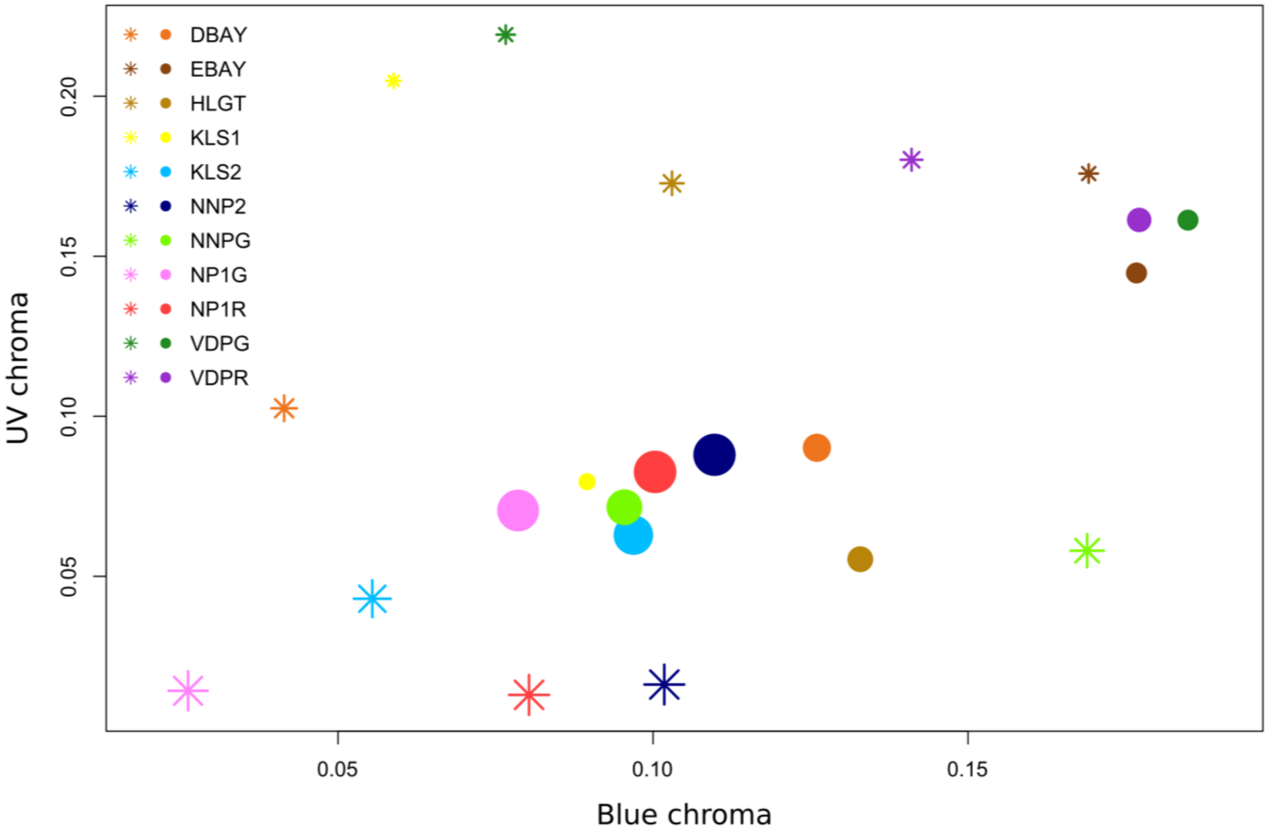

Figure 3.6. Mean disc and petal UV chroma and blue chroma of flowers visited by male *S. trimeni* in each population. Stars refer to petals and filled circles refer to flower discs. Symbol diameter is proportional to mean total diameter of flowers visited in each population.

Table 3.2. Variables relating to the abiotic and contest substrate to which the relationships of several competitive and signalling traits of male *S. trimeni* were quantified in a linear mixed model framework. MAP = Mean Annual Precipitation, MST = Mean Summer Temperature, SOC = Soil Organic Carbon, S1U = UV chroma, S1B = Blue chroma.

	Abiotic environment			Contest substrate				
Population	MAP (mm)	MST (°C)	SOC (%)	Flower diameter (mm)	Disc S1U	Disc S1B	Petal S1U	Petal S1B
DBAY	177	20.00	0.326±0.058	56.889±0.949	0.085±0.036	0.168±0.027	0.107±0.037	0.136±0.053
EBAY	233	19.45	0.238±0.056	41.546±1.128	0.073±0.031	0.126±0.031	0.130±0.032	0.178±0.047
HLGT	81	20.17	0.413±0.069	51.990±1.501	0.060±0.019	0.142±0.015	0.079±0.030	0.134±0.043
KLS1	104	19.56	0.520±0.139	33.402±1.281	0.102±0.033	0.138±0.026	0.121±0.034	0.161±0.052
KLS2	126	19.50	0.327±0.040	80.344±1.646	0.066±0.016	0.126±0.018	0.112±0.029	0.181±0.04
NP1G	93	19.23	0.540±0.144	85.758±1.682	0.085±0.030	0.157±0.041	0.197±0.030	0.200±0.086
NP1R				87.339±4.234				
NNP2				86.891±1.470	0.067±0.022	0.133±0.023	0.112±0.028	0.141±0.037
NNPG	82	20.23	0.305±0.044	73.060±2.708	0.046±0.016	0.082±0.014	0.100±0.036	0.097±0.040
VDPG	170	23.38	0.148±0.066	41.755±1.276	0.089±0.019	0.143±0.020	0.171±0.029	0.163±0.045
VDPR				48.986±2.757				

Table 3.3. ANOVA tables for differences in competitive response traits between populations. CS = centroid size.

Trait	DF	Sum of squares	Mean squares	F	*P*
Femur CS					
Site	10	100.30	10.035	72.46	<0.0001
Residuals	785	108.7	0.138		
Tibia CS					
Site	10	54.64	5.464	36.47	<0.0001
Residuals	809	121.22	0.150		
Tarsus CS					
Site	10	34.49	3.449	50.92	<0.0001
Residuals	798	54.04	0.068		
Claw CS					
Site	10	23.78	2.3783	50.36	<0.0001
Residuals	779	36.79	0.0472		
Femur shape					
Site	10	0.855	0.08552	16.27	<0.0001
Residuals	1581	8.309	0.00526		
Tibia shape					
Site	10	0.224	0.02239	21.12	<0.0001
Residuals	3269	3.466	0.00106		
Tarsus shape					
Site	10	0.099	0.009928	8.918	<0.0001
Residuals	4034	4.491	0.001113		
Claw shape					
Site	10	0.832	0.08324	21.000	<0.0001
Residuals	1569	0.220	0.00396		
UV chroma					
Site	10	1.2470	0.12470	181.5	<0.0001
Residuals	826	0.5674	0.00069		
Blue chroma					
Site	10	1.0495	0.10495	193.7	<0.0001
Residuals	826	0.4475	0.00054		

Table 3.4. Differences in mean trait values between sympatric populations at sites NNP1 and VDRP. Tukey honest significant differences test. Values in brackets are lower and upper limits of 95% confidence intervals around the trait difference.

	NP1R – NP1G		VDPR – VDPG	
Trait	Difference	*P*	Difference	*P*
Femur CS	0.279 (-0.013; 0.570)	0.075	0.549 (-0.003; 1.102)	0.052
Tibia CS	0.057 (-0.243; 0.357)	1.000	0.174 (-0.400; 0.749)	0.996
Tarsus CS	0.182 (-0.020; 0.384)	0.122	0.157 (-0.229; 0.543)	0.966
Claw CS	0.098 (-0.070; 0.268)	0.729	0.007 (-0.316; 0.330)	1.000
Femur shape	0.036 (-0.004; 0.076)	0.121	0.025 (-0.051; 0.101)	0.994
Tibia shape	-0.017 (-0.03; -0.005)	<0.001	-0.011 (-0.035; 0.013)	0.939
Tarsus shape	-0.006 (-0.018; 0.005)	0.812	-0.008 (-0.030; 0.014)	0.988
Claw shape	-0.005 (-0.039; 0.030)	1.000	-0.018 (-0.084; 0.048)	0.998
UV chroma	-0.066 (-0.086; -0.046)	<0.0001	0.030 (-0.003; 0.063)	0.114
Blue chroma	-0.62 (-0.079; -0.044)	<0.0001	-0.017 (-0.047; 0.012)	0.733

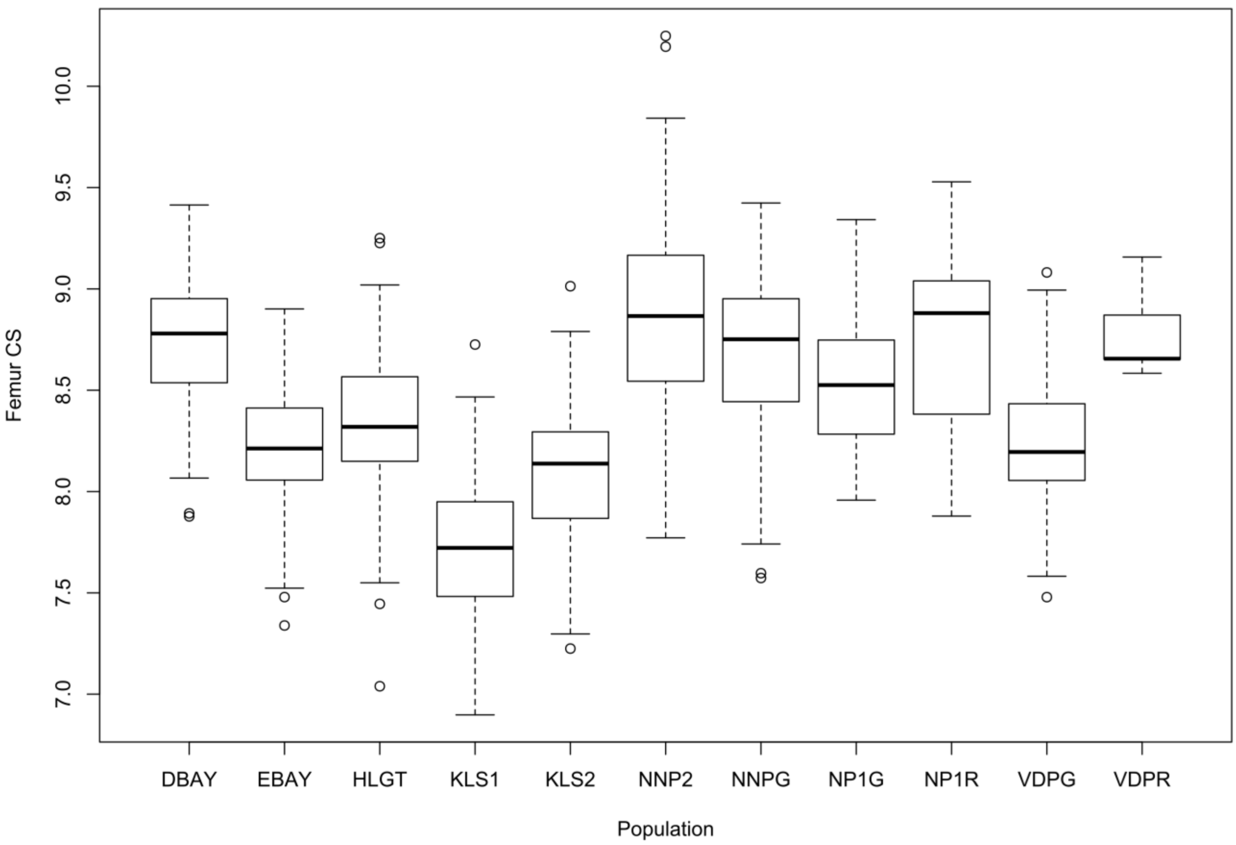

Figure 3.7: Distribution of male hind femur centroid size (CS) in 11 populations of *S. trimeni* along the West Coast of South Africa. Horizontal lines indicate median values and outlined points are outliers. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

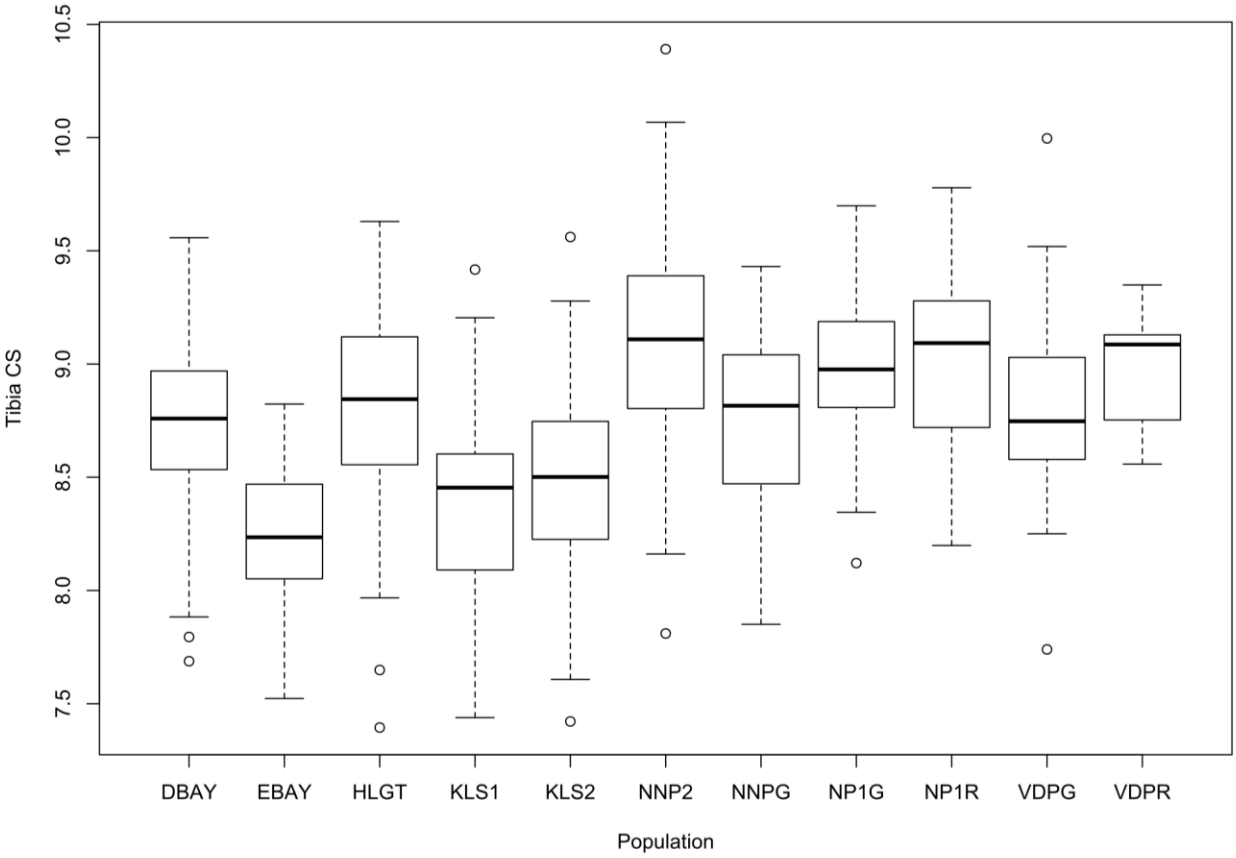

Figure 3.8: Distribution of hind tibia centroid size (CS) in 11 populations of *S. trimeni* along the West Coast of South Africa. Horizontal lines indicate median values and outlined points are outliers. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

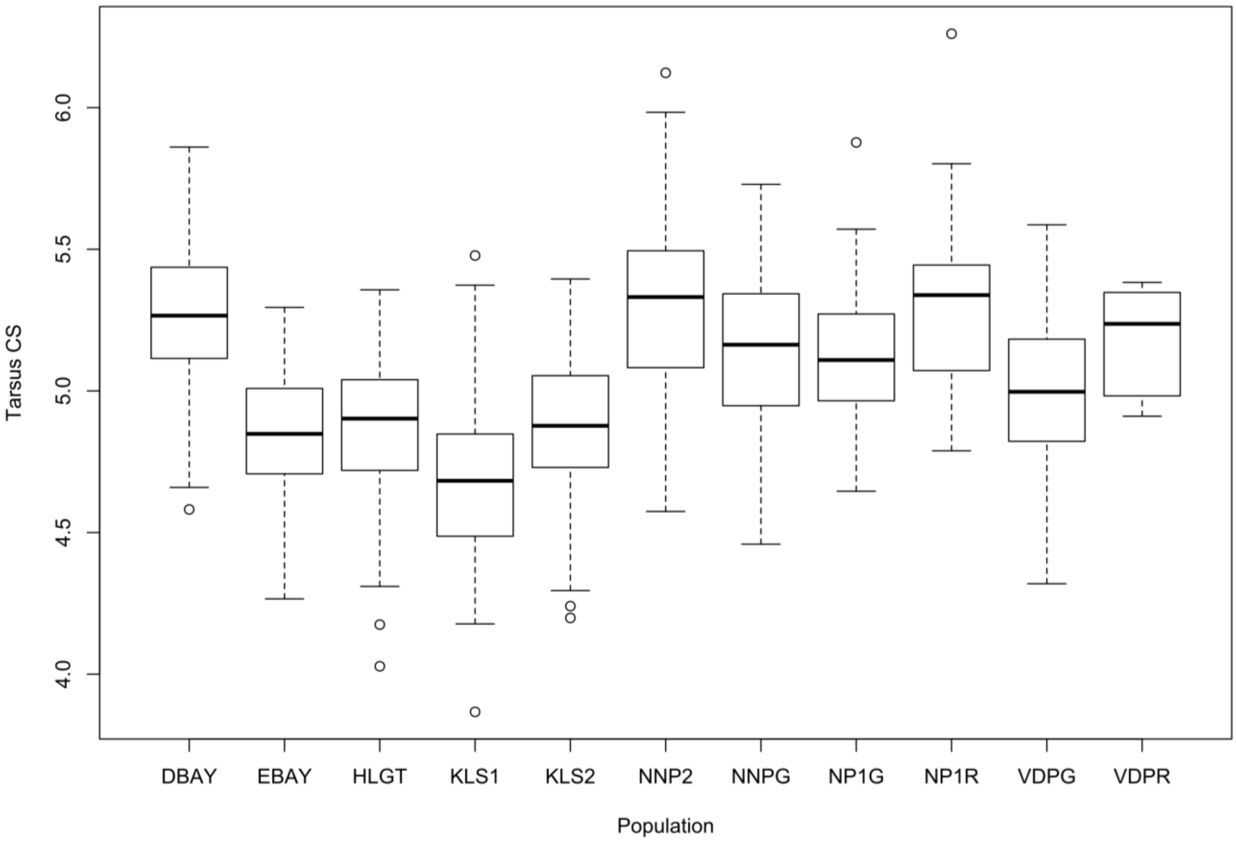

Figure 3.9: Distribution of male hind tarsus centroid size (CS) in 11 populations of *S. trimeni* along the West Coast of South Africa. Horizontal lines indicate median values and outlined points are outliers. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

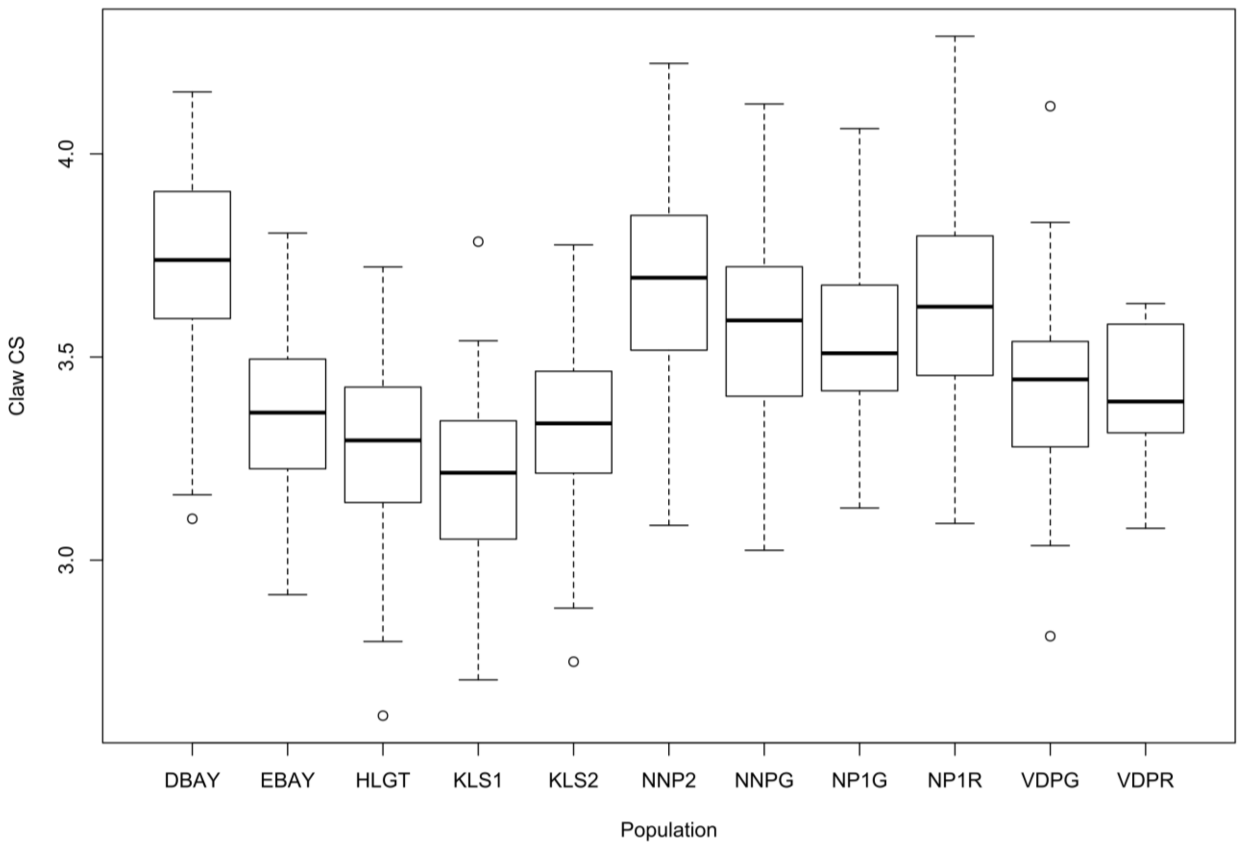

Figure 3.10: Distribution of male hind claw centroid size (CS) in 11 populations of *S. trimeni* along the West Coast of South Africa. Horizontal lines indicate median values and outlined points are outliers. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

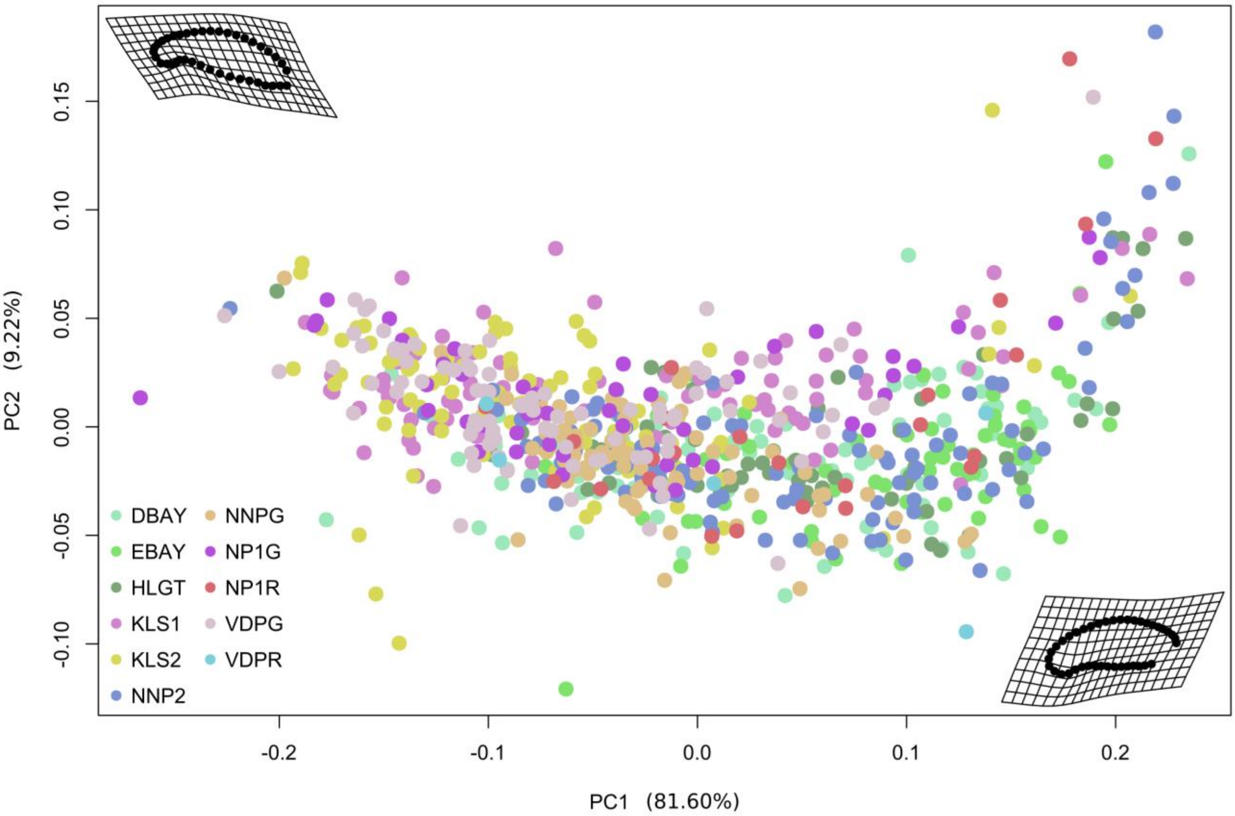

Figure 3.11. Principal component (PC) analysis of male hind femur shape variation within and between populations of *S. trimeni*. The two thin plate spline deformation grids illustrate the extremes of shape variation captured by PC1. Each point represents an individual male belonging to the population associated with the point colour. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

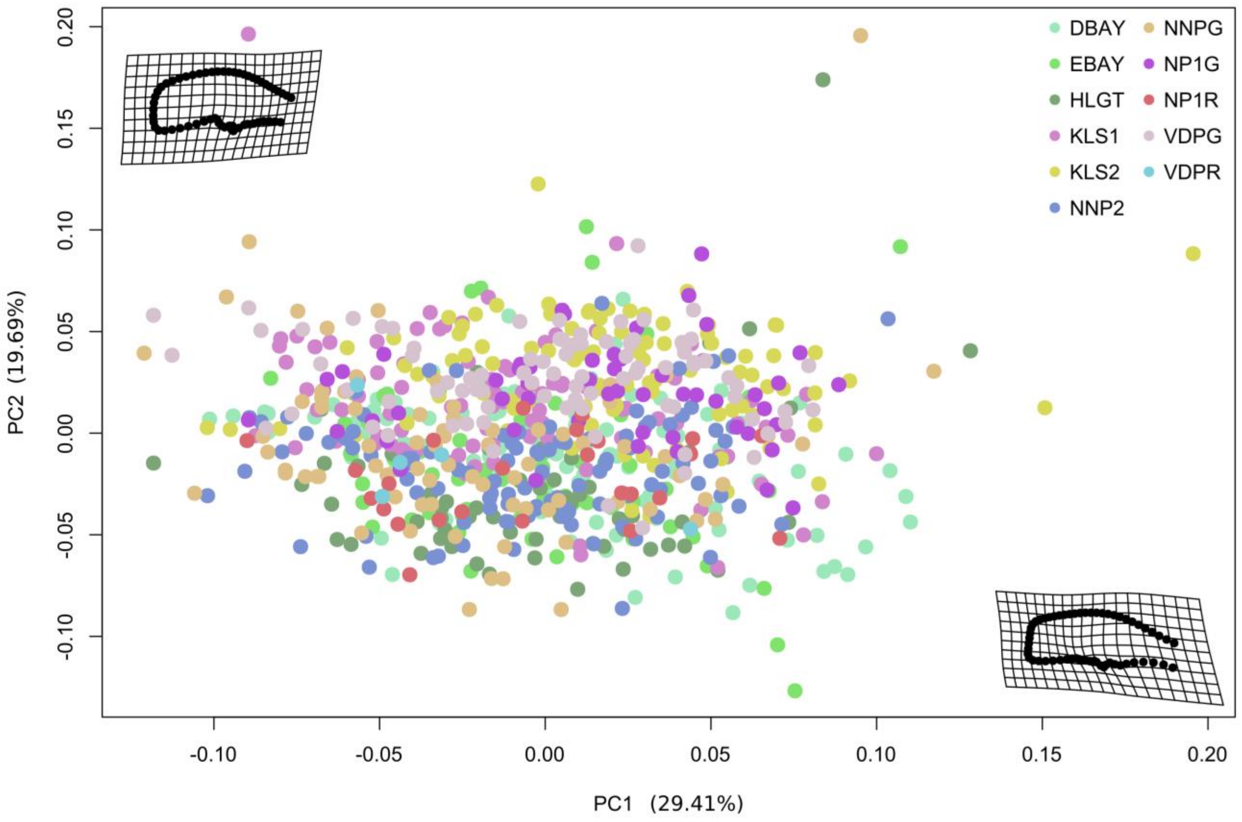

Figure 3.12. Principal component (PC) analysis of male hind tibia shape variation within and between populations of *S. trimeni*. The two thin plate spline deformation grids illustrate the extremes of shape variation captured by PC1. Each point represents an individual male belonging to the population associated with the point colour. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

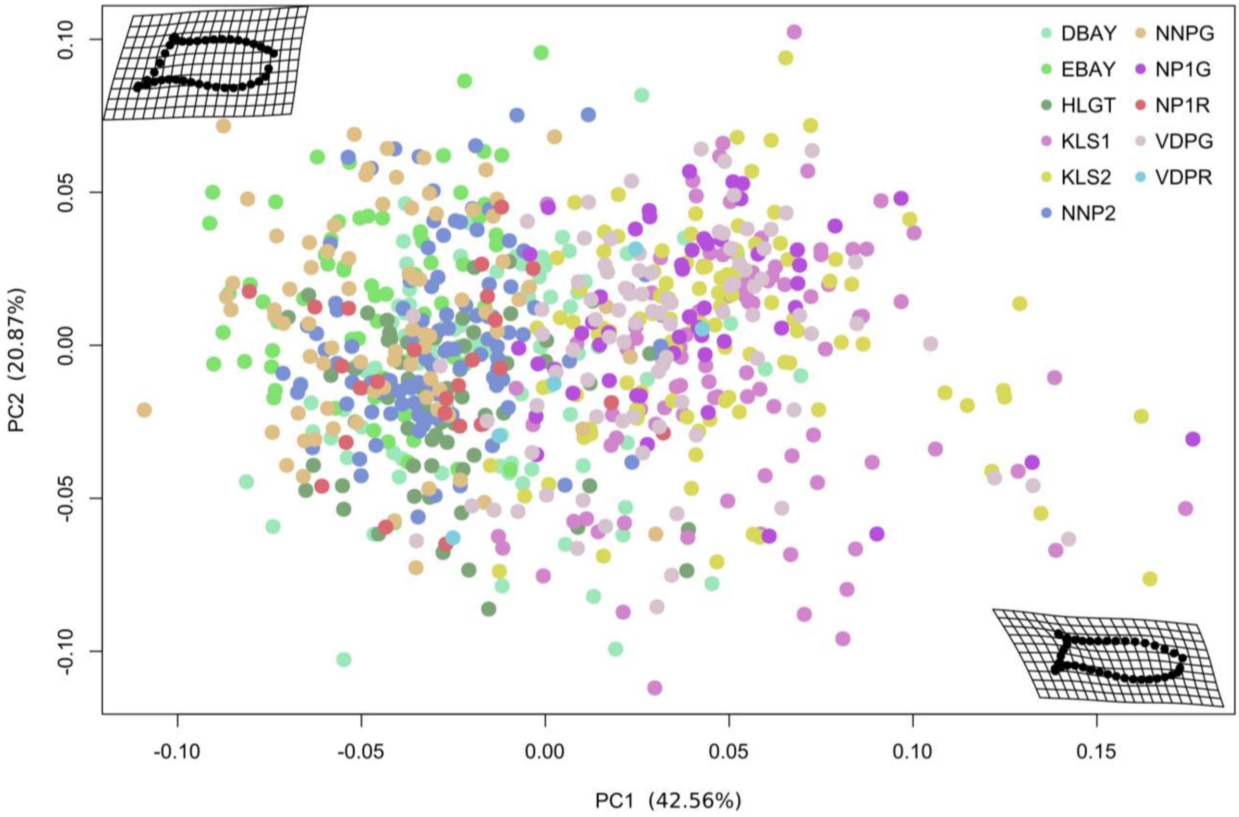

Figure 3.13. Principal component (PC) analysis of male hind tarsus shape variation within and between populations of *S. trimeni*. The two thin plate spline deformation grids illustrate the extremes of shape variation captured by PC1. Each point represents an individual male belonging to the population associated with the point colour. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

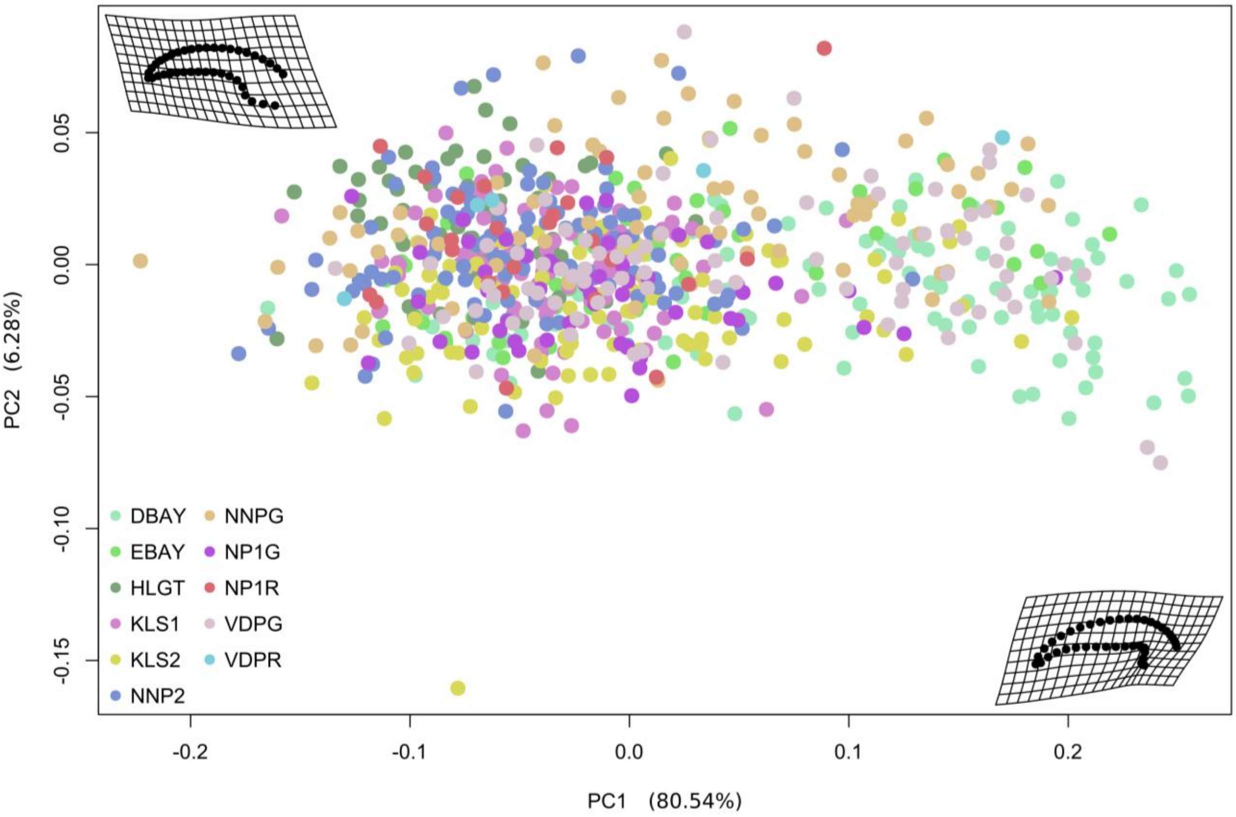

Figure 3.14. Principal component (PC) analysis of male hind claw shape variation within and between populations of *S. trimeni*. The two thin plate spline deformation grids illustrate the extremes of shape variation captured by PC1. Each point represents an individual male belonging to the population associated with the point colour. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

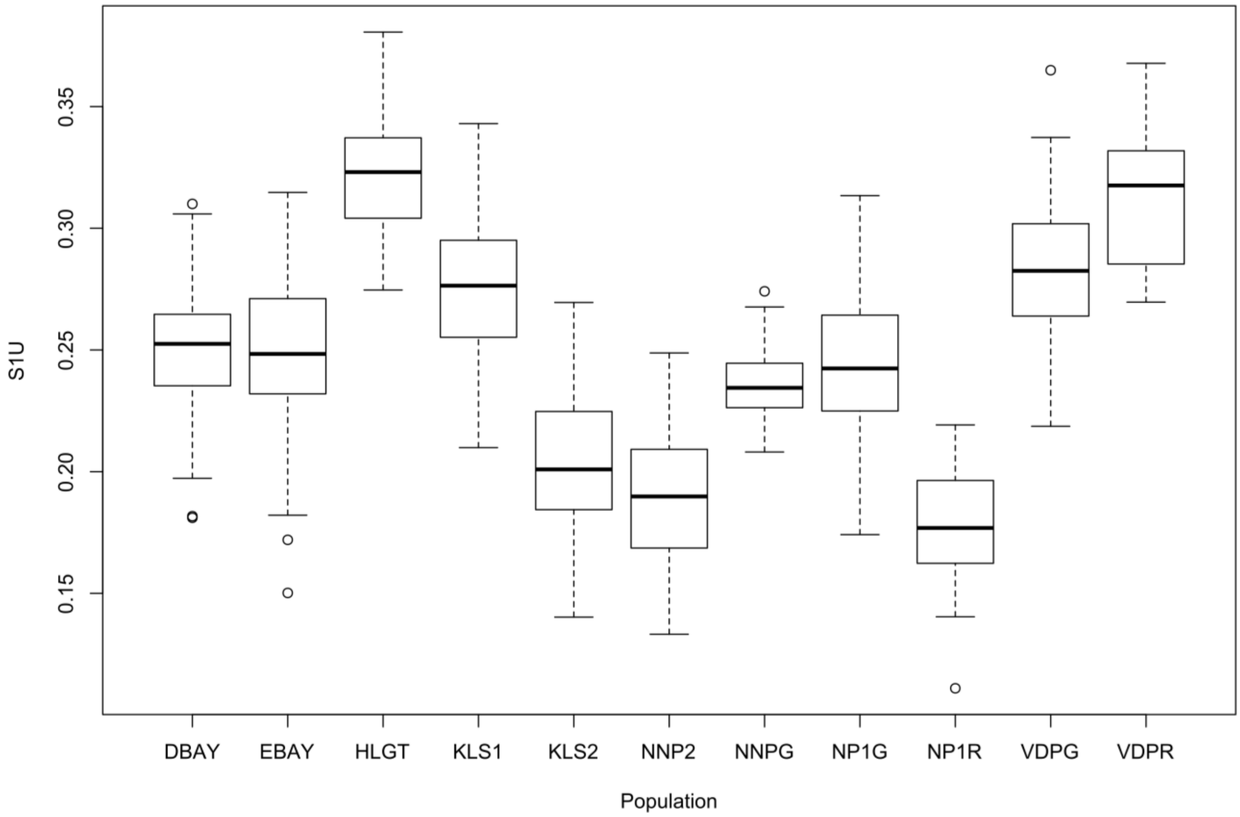

Figure 3.15: Distribution of male UV chroma (S1U) in 11 populations of *S. trimeni* along the West Coast of South Africa. Horizontal lines indicate median values and outlined points are outliers. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

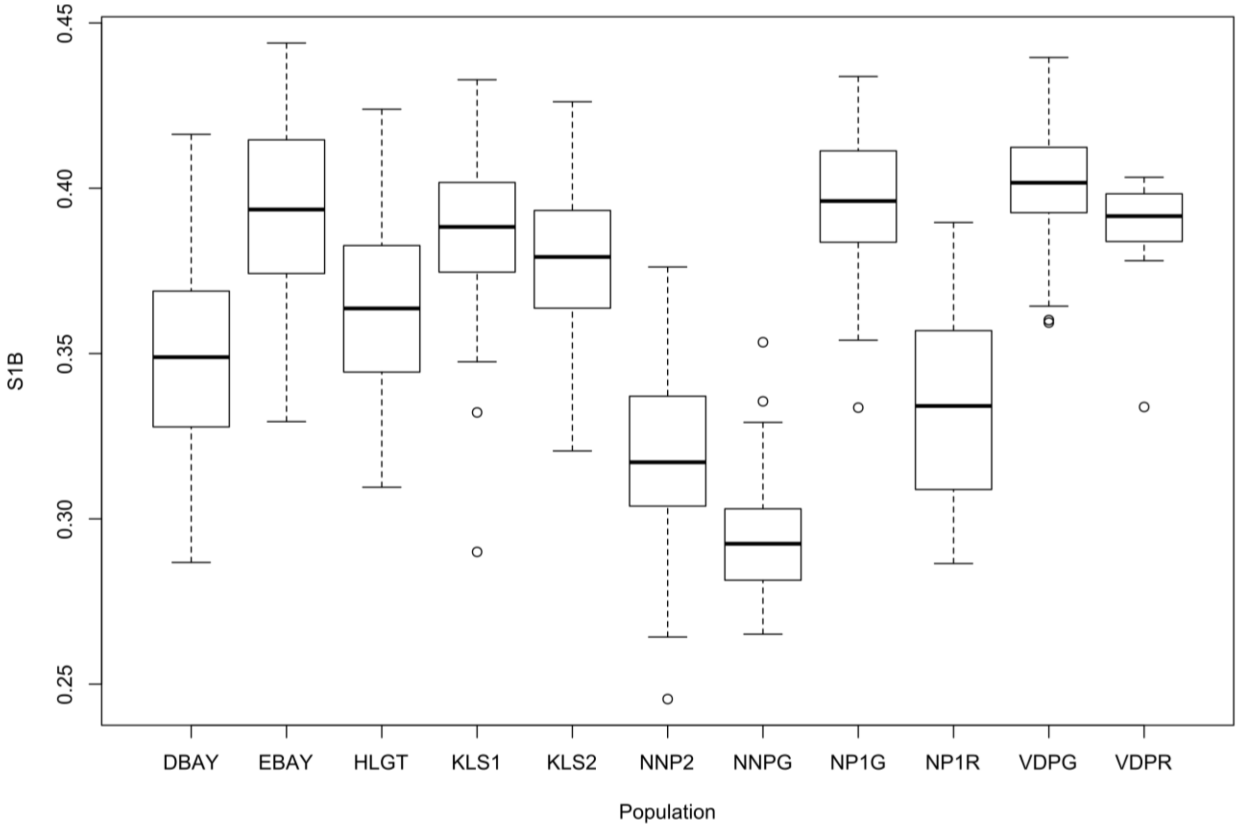

Figure 3.16: Distribution of male UV chroma (S1U) in 11 populations of *S. trimeni* along the West Coast of South Africa. Horizontal lines indicate median values and outlined points are outliers. DBAY = Doringbaai, EBAY = Elandsbaai, HLGT = Holgat, KLS1 = Kleinsee 1, KLS2 = Kleinsee 2, NNP2 = Namaqua National Park north 2, NNPG = Namaqua National Park Groenrivier, NP1G = Namaqua National Park north 1 gracile population, NP1R = Namaqua National Park north 1 robust population, VDPG = Vanrhynsdorp gracile population, VDPR = Vanrhynsdorp robust population. See Fig. 3.1 for map of population localities.

Table 3.5. Covariance structure selection table. For each trait of interest, three intercept-only linear mixed effects models were constructed with a) no covariance structure, b) phylogenetic (Brownian covariance structure), and c) spatial (exponential) covariance structure. The appropriate covariance structure for further modelling of each trait was selected based on the intercept-only model that best fit the data as indicated by its AIC score. CS = centroid size. *K* corresponds to the number of parameters estimated in each model, deviance is a measure of goodness-of-fit with smaller values indicating better fit of model to data, and AIC is the Akaike Information Criterion. ΔAIC is the difference in AIC between the lowest-scoring model and all others, while *w*AIC is the AIC weight of each model and indicates the relative plausibility of all models relative to the lowest-scoring model.

Trait	*K*	Deviance	AIC	ΔAIC	*w*AIC
Femur CS					
No covariance structure	3	727.958	733.958	5.846	0.051
Phylogenetic covariance structure	3	816.982	822.982	94.871	0.000
Spatial covariance structure	4	720.111	728.111	0.000	0.949
Tibia CS					
No covariance structure	3	806.074	812.074	385.119	0.000
Phylogenetic covariance structure	3	420.955	426.955	0.000	1.000
Spatial covariance structure	4	806.067	814.067	387.112	0.000
Tarsus CS					
No covariance structure	3	156.826	162.826	0.000	0.716
Phylogenetic covariance structure	3	728.819	734.819	571.992	0.000
Spatial covariance structure	4	156.671	164.671	1.845	0.284
Claw CS					
No covariance structure	3	-131.289	-125.288	369.913	0.000
Phylogenetic covariance structure	3	-501.201	-495.201	0.000	1.000
Spatial covariance structure	4	-131.288	-123.288	371.913	0.000
Femur shape					
No covariance structure	3	-3810.640	-3804.640	777.774	0.000
Phylogenetic covariance structure	3	-4588.414	-4582.414	0.000	1.000
Spatial covariance structure	4	-3811.275	-3803.275	779.138	0.000
Tibia shape					
No covariance structure	3	-13127.330	-13121.330	1437.853	0.000
Phylogenetic covariance structure	3	-14565.190	-14559.190	0.000	1.000
Spatial covariance structure	4	-13127.660	-13119.660	1439.530	0.000
Tarsus shape					
No covariance structure	3	-16009.150	-16003.150	2037.846	0.000
Phylogenetic covariance structure	3	-18046.990	-18040.990	0.000	1.000
Spatial covariance structure	4	-16009.220	-16001.220	2039.770	0.000
Claw shape					
No covariance structure	3	-4225.4770	-4219.4770	771.618	0.000
Phylogenetic covariance structure	3	-4997.095	-4991.095	0.000	1.000
Spatial covariance structure	4	-4225.562	-4217.562	773.533	0.000
UV chroma					
No covariance structure	3	-3663.540	-3657.540	393.447	0.000

Phylogenetic covariance structure	3	-4056.986	-4050.986	0.000	1.000
Spatial covariance structure	4	-3663.540	-3655.540	395.447	0.000
Blue chroma					
No covariance structure	3	-3865.925	-3859.925	393.447	0.000
Phylogenetic covariance structure	3	-4259.371	-4253.371	0.000	1.000
Spatial covariance structure	4	-3865.925	-3857.925	395.447	0.000

Table 3.6. Selection table for linear mixed effects models of male trait variation in relation to environmental and contest substrate variation between populations. CS = centroid size. Null models = intercept-only models. For null models, unexplained among-population variance refers to variance in traits between populations unexplained by the random effect, while for covariate models it refers to between-population variance in the trait unexplained by the random effect and covariation of trait and covariate. Variance explained (%) is the reduction of unexplained between-population variance by the covariate model over that of the null model. *K* corresponds to the number of parameters estimated in each model, deviance is a measure of goodness-of-fit with smaller values indicating better fit of model to data, and AIC is the Akaike Information Criterion. ΔAIC is the difference in AIC between the lowest-scoring model and all others, while *w*AIC is the AIC weight of each model and indicates the relative plausibility of all models relative to the lowest-scoring model. See Table A1 for among-population trait variance explained by environmental and contest substrate variation after accounting for trait relationships with body size.

Model	Unexplained between-population variance	Variance explained (%)	*K*	Deviance	AIC	ΔAIC	*w*AIC
Femur CS							
Null	0.112	NA	4	720.111	728.111	0.000	0.881
MAP	0.110	2.348	5	726.873	736.873	8.762	0.011
MST	0.112	0.450	5	727.025	737.025	8.914	0.010
SOC	0.111	1.140	5	726.999	736.999	8.888	0.010
Flower diameter	0.072	35.796	5	722.737	732.737	4.626	0.087
Tibia CS							
Null	0.060	NA	3	420.955	426.955	2.600	0.135
MAP	0.043	28.183	4	417.862	425.862	1.508	0.232
MST	0.059	2.215	4	420.620	428.620	4.266	0.058
SOC	0.054	10.867	4	419.999	427.999	3.645	0.080
Flower diameter	0.037	38.296	4	416.354	424.354	0.000	0.494
Tarsus CS							
Null	0.039	NA	3	156.826	162.826	3.471	0.127
MAP	0.038	2.858	4	156.545	164.545	5.190	0.054
MST	0.039	-0.019	4	156.818	164.818	5.462	0.047
SOC	0.039	2.458	4	156.607	164.607	5.251	0.052
Flower diameter	0.022	41.965	4	151.356	159.356	0.000	0.720
Claw CS							
Null	0.026	NA	3	-501.201	-495.201	3.269	0.133
MAP	0.026	1.149	4	-501.343	-493.344	5.126	0.053
MST	0.025	4.263	4	-501.712	-493.711	4.759	0.063
SOC	0.025	5.313	4	-501.839	-493.839	4.632	0.067
Flower diameter	0.016	38.627	4	-506.471	-498.471	0.000	0.683
Femur shape							
Null	4.797×10^{-4}	NA	3	-3810.640	-3804.640	777.742	0.000
MAP	4.730×10^{-4}	1.399	4	-4588.520	-4580.520	1.862	0.164

MST	3.882×10^{-4}	19.080	4	-4590.381	-4582.381	0.000	0.417
SOC	4.340×10^{-4}	9.516	4	-4589.451	-4581.451	0.931	0.262
Flower diameter	4.789×10^{-4}	0.168	4	-4588.422	-4580.422	1.959	0.156
Tibia shape							
Null	9.278×10^{-5}	NA	3	-14565.190	-14559.190	0.000	0.328
MAP	8.831×10^{-5}	4.816	4	-14565.860	-14557.860	1.324	0.169
MST	9.260×10^{-5}	0.192	4	-14565.890	-14557.890	1.301	0.171
SOC	8.948×10^{-5}	3.561	4	-14566.030	-14558.030	1.155	0.184
Flower diameter	9.018×10^{-5}	2.796	4	-14565.600	-14557.600	1.585	0.148
Tarsus shape							
Null	1.902×10^{-5}	NA	3	-18046.990	-18040.990	0.000	0.356
MAP	1.817×10^{-5}	4.438	4	-18047.370	-18039.370	1.618	0.158
MST	1.840×10^{-5}	3.207	4	-18047.180	-18039.180	1.817	0.143
SOC	1.700×10^{-5}	10.582	4	-18047.810	-18039.810	1.182	0.197
Flower diameter	1.856×10^{-5}	2.399	4	-18047.210	-18039.210	1.782	0.146
Claw shape							
Null	4.410×10^{-4}	NA	3	-4997.095	-4991.095	3.159	0.104
MAP	3.206×10^{-4}	27.306	4	-5000.389	-4992.389	1.865	0.198
MST	3.352×10^{-4}	24.004	4	-4999.747	-4991.747	2.507	0.143
SOC	2.610×10^{-4}	40.830	4	-5002.254	-4994.254	0.000	0.503
Flower diameter	4.164×10^{-4}	5.589	4	-4997.733	-4989.733	4.521	0.052
UV chroma							
Null	1.974×10^{-3}	NA	3	-4056.986	-4050.986	10.112	0.006
MAP	1.876×10^{-3}	4.964	4	-4057.560	-4049.560	11.539	0.003
MST	1.243×10^{-3}	37.031	4	-4062.116	-4054.116	6.983	0.029
SOC	1.532×10^{-3}	22.367	4	-4059.813	-4051.813	9.286	0.009
Disc S1U	1.761×10^{-3}	10.799	4	-4058.280	-4050.280	10.819	0.004
Petal S1U	6.446×10^{-4}	67.341	4	-4069.099	-4061.099	0.000	0.949
Blue chroma							
Null	1.121×10^{-3}	NA	3	-4259.371	-4253.371	0.939	0.183
MAP	8.582×10^{-4}	23.471	4	-4262.310	-4254.310	0.000	0.292
MST	1.008×10^{-3}	10.142	4	-4260.563	-4252.563	1.747	0.122
SOC	1.037×10^{-3}	7.559	4	-4260.252	-4252.252	2.058	0.104
Disc S1B	9.461×10^{-4}	15.634	4	-4261.249	-4253.249	1.061	0.172
Petal S1B	9.950×10^{-4}	11.268	4	-4260.633	-4252.633	1.678	0.126

Table 3.7. Summaries of linear mixed effects models explaining the most among-population variance in each secondary sexual trait of interest in male *S. trimeni*. SE = standard error, DF = degrees of freedom, CS = centroid size.

Trait	Parameter	Value	SE	DF	t-value	*P*
Femur CS	Intercept	7.829	0.278	785	28.147	<0.0001
	Flower diameter	0.010	0.004	9	2.330	0.045
Tibia CS	Intercept	8.271	0.204	809	40.565	<0.0001
	Flower diameter	0.008	0.003	9	2.433	0.038
Tarsus CS	Intercept	4.648	0.157	798	29.588	<0.0001
	Flower diameter	0.006	0.002	9	2.692	0.0247
Claw CS	Intercept	3.141	0.132	779	23.689	<0.0001
	Flower diameter	0.005	0.002	9	2.585	0.0294
Femur shape	Intercept	0.142	0.095	1581	1.492	0.136
	MST	-0.007	0.005	9	-1.477	0.174
Tibia shape	Intercept	0.004	0.008	3269	0.540	0.589
	MAP	-5.042×10^{-5}	6.088×10^{-5}	9	-0.828	0.429
Tarsus shape	Intercept	0.003	0.004	4034	0.800	0.423
	SOC	-0.009	0.010	9	-0.932	0.375
Claw shape	Intercept	0.035	0.015	1569	2.320	0.020
	SOC	-0.097	0.038	9	-2.582	0.030
UV chroma	Intercept	0.199	0.013	826	15.100	<0.0001
	Petal UV chroma	0.459	0.098	9	4.672	0.001
Blue chroma	Intercept	0.320	0.025	826	12.573	<0.0001
	MAP	0.0003	0.0002	9	1.831	0.100

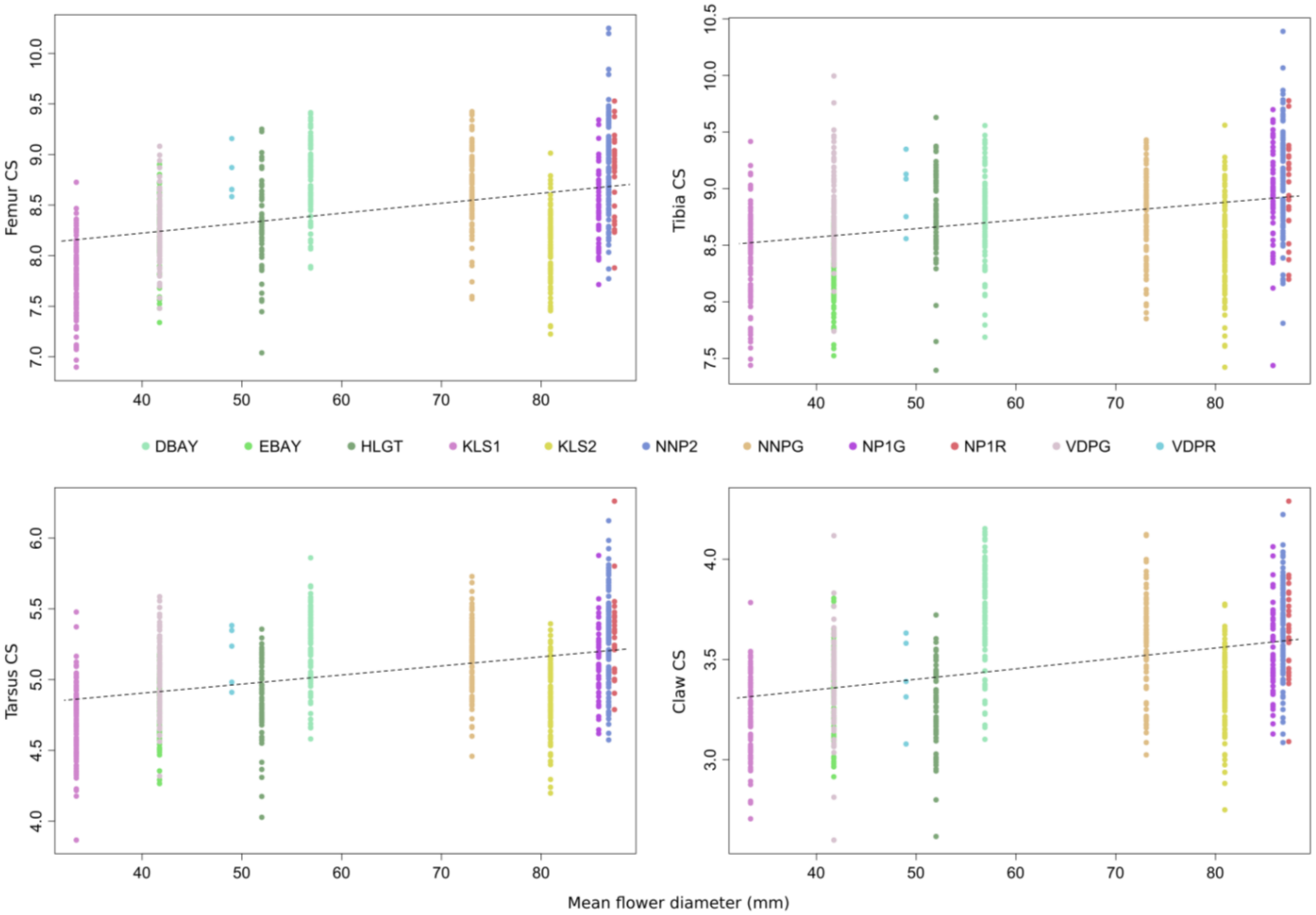

Figure 3.17. Variation in leg component sizes as a function of mean flower diameter in each population. Regression line slope and intercept values extracted from models explaining the most variation in leg component sizes between sites (Table 3.6). Each point represents an individual male belonging to the population associated with the point colour.

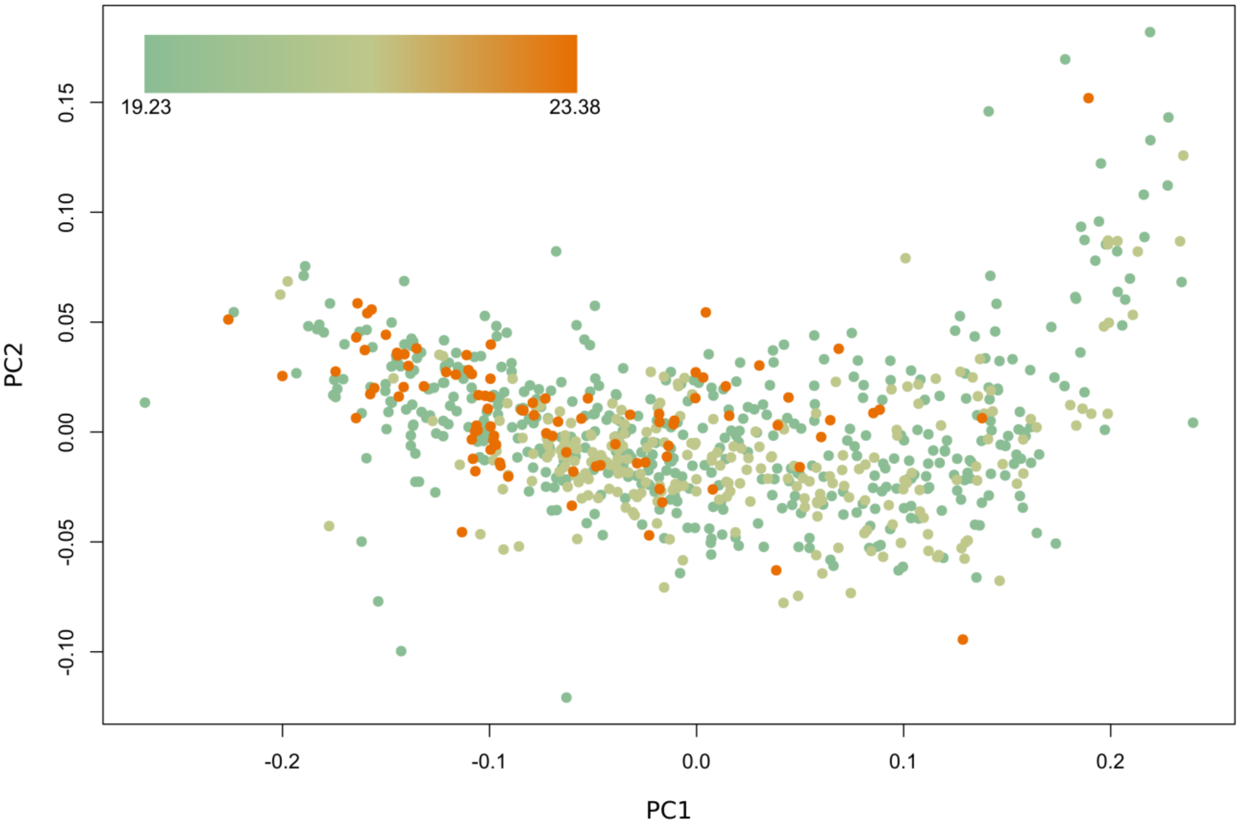

Figure 3.18. Principal component analysis (PCA) of femur shape. Colour indicates MST (°C) at the locality of each population. See Figure 3.8 for warp grids illustrating extremes of femur shape represented along PC1.

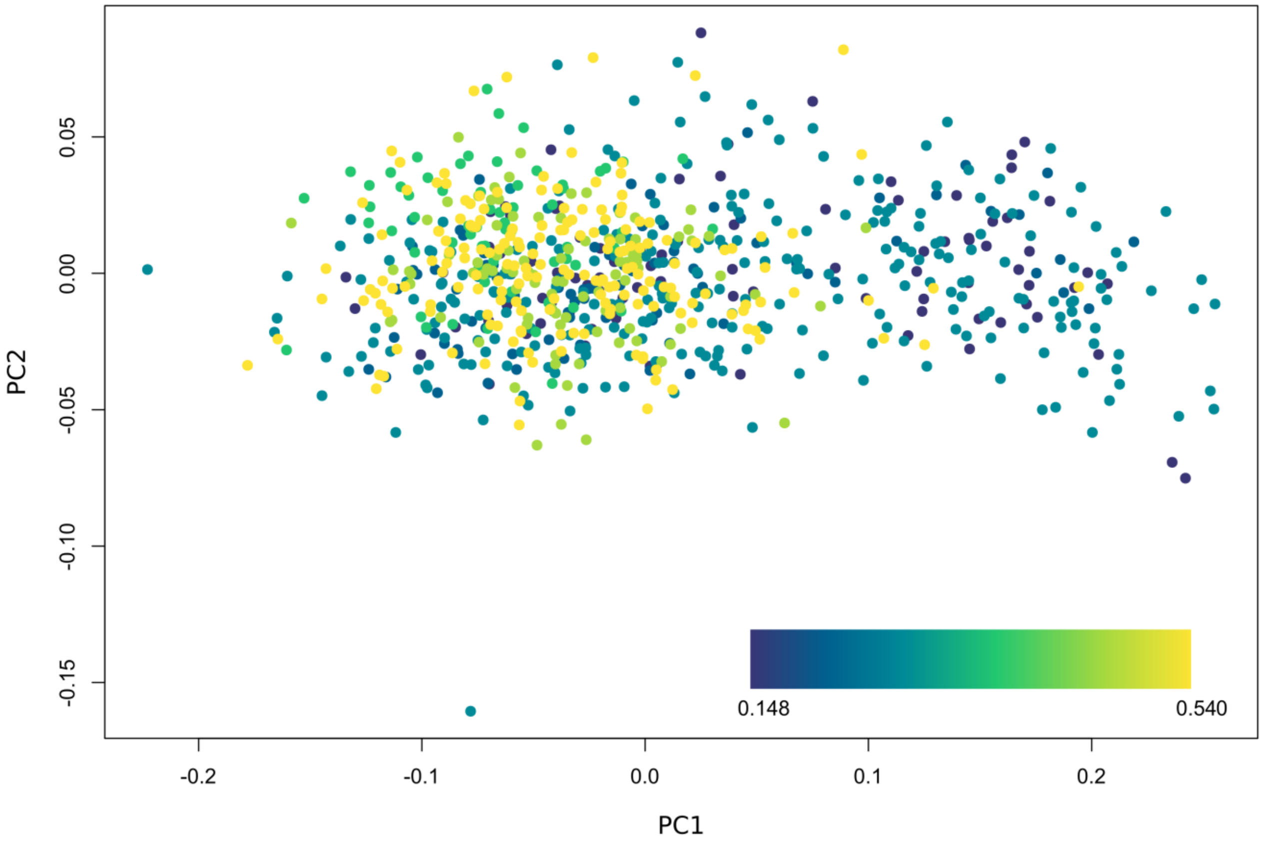

Figure 3.19. Principal component analysis (PCA) of claw shape. Colour indicates SOC (% organic carbon) at the locality of each population. See Fig. 3.8 for warp grids illustrating extremes of claw shape represented along PC1.

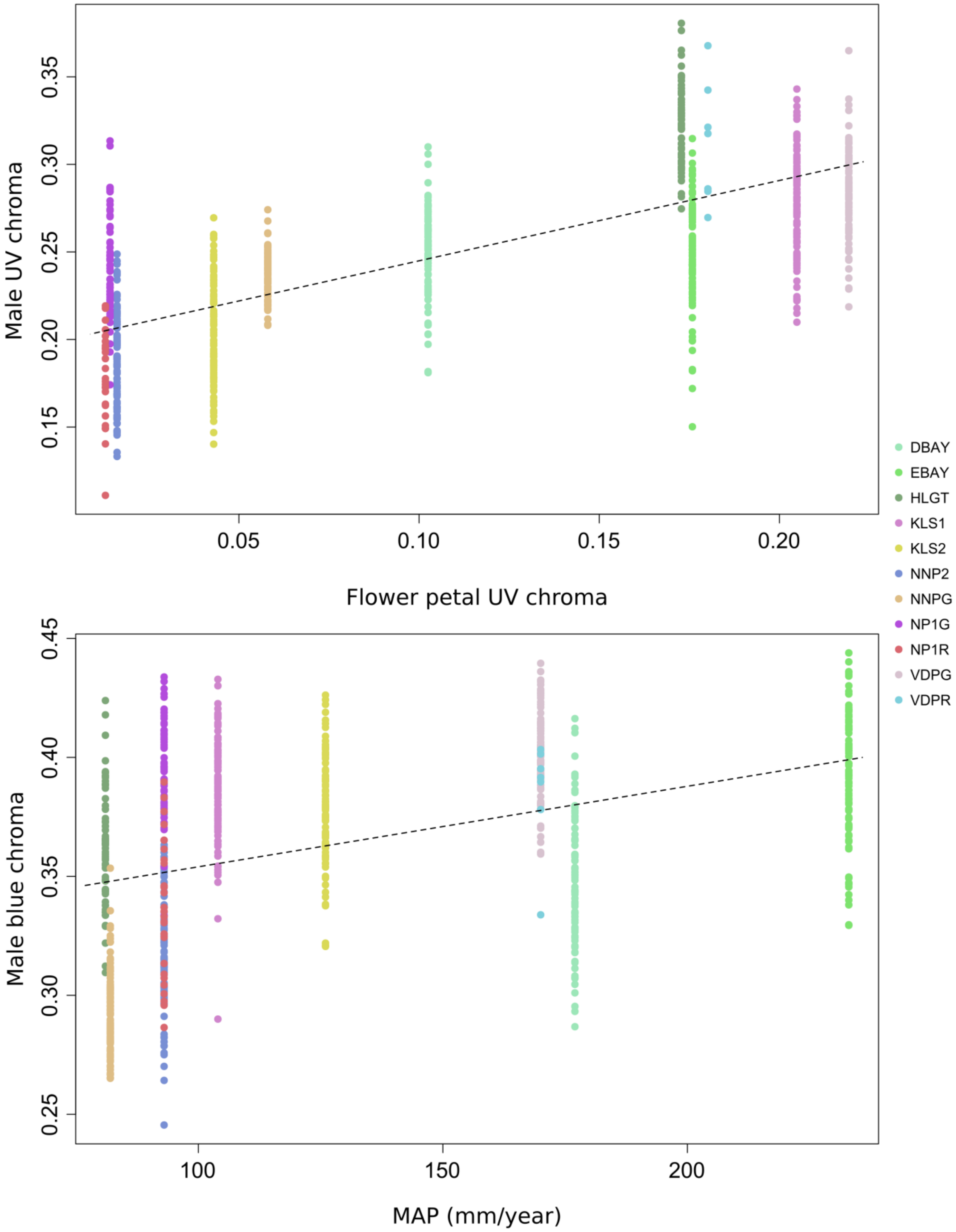

Figure 3.20. Between-population variation in UV and blue chroma in male *S. trimeni* as a function of flower petal UV chroma and MAP, respectively. Each point represents an individual male belonging to the population associated with the point colour. Regression line slope and intercept values extracted from models explaining the most variation in chroma values between sites (Table 3.6).

3.5 DISCUSSION

In this chapter, I have attempted to assess empirical support for one of the most promising recent hypotheses linking divergence in traits in intrasexual contest systems to speciation. I have obtained strong support for key aspects of the divergent contest substrates hypothesis, having found that male *S. trimeni* weapon form and colour differ significantly among populations, and that divergence in weapon size and colour have occurred along gradients of contest substrate size and colour variation. I have also found evidence that some aspects of male competition traits, namely leg component shapes and blue chroma, vary along abiotic environmental gradients, including precipitation and temperature. I found that patterns of covariation generally reflected the phylogenetic structure of the populations. I did not find evidence in support of spatial autocorrelation between populations' trait values. However, I did find that phylogenetic distance was correlated with spatial distance, suggesting that isolation-by-distance may play a role in contributing to trait divergence among populations.

There has been a surge of theoretical and review work published over the last decade seeking to revive research interest in the speciation process in intrasexual contest systems (e.g., Qvarnström et al. 2012; McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018; Lindsay et al. 2019). This work has synthesized knowledge in the field, situated it within the broader fields of sexual selection and speciation, developed numerous hypotheses concerning causal links between intrasexual contest competition and speciation, and has urged empirical work to be undertaken to further our understanding of whether intrasexual contest competition may lead to speciation, and if so, how. Despite this solid theoretical foundation, no studies to date have empirically examined whether divergent fighting contexts are associated with divergent weaponry.

Here, I have quantified variation in two key aspects of weapon morphology, namely size and shape, with respect to variation in the abiotic environment and of the substrate on which contests occur. A previous study (Rink et al. 2019) in the monkey beetle *H. chiragricus* showed that male hind leg (weapon) size was a key determinant of contest success. I have found in this chapter that the size of four major components of the male hind leg, namely femur, tibia, tarsus, and claw, differ significantly among populations of *S. trimeni*, as does size of the contest substrate (mean flower diameter). Moreover, I found that approximately 35% of the variation in the sizes of each leg component between populations was explained by variation in flower size. Leg component size exhibited a positive correlation with flower size, meaning that populations in localities with larger flowers had males with larger hind legs than did populations with access to smaller flowers. Given that larger hind legs offer a competitive advantage to combatant insects (Snell-Rood and Moczek 2013; Fea and Holwell 2018), including monkey beetles (Rink et al. 2019), the finding that smaller flowers support males with smaller hind legs suggests that flower size constrains the practical size of male monkey beetle weaponry, perhaps due to mobility or manoeuvrability costs. Such cost-benefit trade-offs appear to be common in animal weaponry and other phenotypically plastic traits (Emlen 1997; Whitman and Agrawal 2014; Giery and Layman 2019). Sexual selection drives exaggerated traits (e.g., Miller and Emlen 2010; Painting and Holwell 2014; Lavine et al. 2015), but the increasing functional, metabolic, and/or fitness costs of these structures means that weapon size reflects the opposing selective pressures of sexual and natural selection (Emlen 2001; Knell et al. 2004; Somjee et al. 2018). The huge jaws of male *Cyclommatus metallifer* stag beetles impact their locomotion (Goyens et al. 2015), and while male rhinoceros beetles with larger horns are more likely to win contests, larger horns are also more likely to break during battle, placing an upper limit on their practical size (McCullough

2014). The cost to male *S. trimeni* of decreased mobility on a small flower surface may thus counteract sexual selection for larger hind legs.

While variation in weapon size was well explained by flower size variation, I found that variation in weapon shape among populations was uncorrelated with variation in flower size. Weapon shape in other insect systems is known to reflect the mode of fighting (McCullough et al. 2014; McCullough et al. 2015; Goyens et al. 2016), which in turn is expected to vary according to the topology of the fighting substrate (McCullough et al. 2016). However, leg shape in male *H. chiragricus* monkey beetles does not influence contest success (Rink et al. 2019). Here, I found that approximately 20% of femur shape variation among populations occurred along a temperature gradient (MST). Claw shape varied most according to soil organic carbon, but also covaried with precipitation (MAP) and temperature (MST). Tibia and tarsus shape exhibited some variation along abiotic gradients, but these models were poorly supported. Sexual selection may be acting more strongly on hind leg size than on hind leg shape in male monkey beetles, or it may be that hind leg shape is especially sensitive to the developmental environment as captured in the abiotic variables considered in my analyses. The relationships of temperature with various aspects of development and phenology, such as development time and adult body size, are complex and vary for different taxa (Kingsolver and Huey 2008). For insects, higher temperatures during development are generally associated with shorter development time and smaller adult body size (Kingsolver and Huey 2008), as found for *Onthophagus taurus* (Rohner and Moczek 2020). Developmental conditions could possibly impact adult trait shape by biasing the phenotypic expression of the genotype and influencing the relative developmental rates of different traits (Frankino et al. 2009; Moczek 2015; Casasa and Moczek 2019; Hu et al. 2020). These findings cannot be conclusively interpreted as evidence for phenotypic plasticity and/or condition dependence. However, they do warrant the experimental manipulation of *S. trimeni* populations to pinpoint whether among-population trait variation arises from genotype × environment effects (i.e., reflecting phenotypic plasticity), genetic differences among populations, or both. In addition to phenotypic plasticity being the result of adaptation, it may also facilitate adaptation to novel environments if genetic canalisation occurs (Casasa and Moczek 2018). Quantifying the phenotypic plasticity and genetic basis of competitive traits in monkey beetles may thus allow us to understand the relationship between phenotypic and genotypic divergence in monkey beetles and provide insights into the mechanisms that have facilitated their evolutionary diversification.

Both UV and blue chroma differed significantly among populations and exhibited covariation with abiotic environmental variables and with the chroma of the contest substrate, despite flower chroma not exhibiting significant differences among populations. Variation in male UV chroma among populations was particularly well explained (variance explained = ~67%, AIC = -4061.099) by among-population variation in mean petal UV chroma, with which it scaled positively (Table 3.6 and Fig. 3.20). Mean male UV chroma was consistently greater than petal UV chroma in all populations (Fig. 3.20). This is consistent with the patterns expected for traits that function as signals of fitness or quality to conspecifics and thus need to be visible against the signalling background (Miller and Svensson 2014; Wellenreuther et al. 2014), as in female mantids (White et al. 2014), male dewlap colouration in male West Indian *Anolis* lizards (Leal and Fleishman 2004), and male visual signalling in the wolf spider *Schizocosa ocreata* (Clark et al. 2011). In intrasexual contest competition, displaying vivid colouration may ward off would-be opponents by signalling the bearer’s resource-holding ability by virtue of their physical condition (Whiting et al. 2003; Senar 2006; Vedder et al. 2010; Hamilton et al. 2013). Little of the variation in male blue chroma between populations was explained by the abiotic and contest substrate variables examined. Most blue chroma variation occurred along the precipitation gradient (MAP; variance explained = ~23%, AIC = -4254.310), with smaller portions explained by the other abiotic variables and substrate characteristics (Table 3.6). Monkey beetle colouration is structural, arising from nanoscale sculpturing of the scales that densely coat their cuticle (Colville et al. 2018). Extensive work on phenotypic variation in ultraviolet and blue structural colouration in butterflies (Papke et al. 2007; Kemp 2006; Pecháček et al. 2014), birds (Keyser and Hill 1999; McGraw et al. 2002; Vedder et al. 2010), fish (Maan and Sefc 2013; Zhou and Fuller 2016), and lacertid lizards (Bajer et al. 2012; de Lanuza et al. 2014) suggests that vivid male colouration that serves as a fitness signal, either to choosy females or to rival males, and exhibits greater phenotypic variation and sensitivity to physical condition than do non-sexually selected colour traits. In male *S. trimeni* a relatively large proportion of variance in blue chroma is explained by environmental variables in comparison to contest substrate chroma (Table 3.6). The correlation of male blue chroma with rainfall may indicate a function of the elytral scales to mitigate the adverse effects of raindrop impacts on beetle mobility (Kim et al. 2020). Male UV chroma also exhibited variation along temperature and larval nutrient availability gradients (Table 3.6), but these explained relatively less variance than petal UV chroma. The high proportion of male UV chroma variation tied to variance in petal UV chroma, despite petal UV chroma not differing significantly among populations, indicates that male UV chroma may be an important aspect of signalling colouration. These patterns suggest that sexual selection may be acting more strongly on UV chroma whereas the structural features of the elytral scales that give rise to blue chroma serve to mitigate raindrop effects, as mentioned above. Overall, structural colouration in male *S. trimeni* varies in response to both abiotic and contest substrate variation between populations, and variation in male colour likely reflects a dynamic interplay between natural selection and sexual selection pressures.

In accounting for scaling relationships between competitive traits and body size, I assumed that such relationships were linear and thus could be captured in a mixed effects linear model framework. However, work in other groups, most notably beetle horns, suggests that nonlinear relationships, such as sigmoidal, may be common in weapon-body size allometric scaling relationships (Emlen 1996; Moczek 2005). Weapon shape may exhibit a multivariate allometric scaling relationship with weapon size (Eldred et al. 2016; Mills et al. 2016). Lastly, weapon shape appears to covary with body size (McCullough et al. 2015). Correlations between body size and weapon shape are documented in rhinoceros beetles *Oryctes nasicornis* (Goczał et al. 2019). Complex factors such as these may account for disparities between the results presented in Table 3.6 with those in Table A1, specifically for tarsus and claw shape. Future study of competitive trait variation across environmental gradients should aim firstly to quantify the shape of the allometric relationship between body size and trait values, and secondly to assess whether competitive traits are responding directly to environmental variation through selection for e.g., optimal fighting style, or indirectly through natural selection acting on body size variation.

At sites NNP1 and VDRP, I found that beetles belonged to two sympatric populations. These sympatric populations occupy the same physical space and thus can be expected to experience identical natural selection regimes in terms of the variables I measured; however, this was a limited set of environmental variables and it is possible that these did not accurately capture the selection environment that these beetles experience. Populations NP1G and NP1R differed significantly in one component of weapon shape, as well as both UV and blue chroma. In contrast, VDPG and VDPR males lacked significant differences in any of the traits studied. Each of the sympatric population pairs (NP1G and NP1R, and VDPG and VDPR) are more distantly related to one another than they are to other populations within their clades (Fig. 3.4); the evolutionary divergence between ‘robust’ and ‘gracile’ male genitalia forms being the most basal split in the phylogeny. Incompatible genitalia morphotypes may enable these sympatric populations to remain reproductively isolated from one another, a lock-and-key mechanism that may have arisen through hybridisation avoidance or variation in males’ paternity success (Rentz 1972; Arnqvist 1998; Masly 2012). In the genus *Onthophagus*, the sympatric sister species *O. taurus* and *O. illyricus* exhibit divergent tunnelling behaviour and concomitant divergent adaptations in digging appendages, which enables them to minimise interspecific resource competition (Macagno et al. 2016). VDPG and VDPR males’ similarity in male competitive traits, despite being relatively distantly related to each other, likely resemble one another since they have evolved under the same set of selective pressures as a result of sharing the same contest substrate and abiotic environment. If monkey beetle genitalia do follow the ‘lock-and-key’ Principal, males should be able to ‘pick their battles’ by relying on some form of species recognition to assess which females are worth fighting for and be able to recognise possible competitors (West-Eberhard 1983; Grether et al. 2009). Female *S. trimeni* are colour polymorphic, and perhaps those in sympatric populations are of different colour morphs and thus distinguishable by males. Female polymorphisms have been extensively studied in damselflies (e.g., Svensson and Abbott 2005; Hammers and van Gossum 2008; Gosden and Svensson 2008; Sánchez-Guillén et al. 2011), where they are associated with sexual conflict (van Gossum and Sherratt 2008; Gering 2017) and interspecific harassment of females by non-conspecific males (Forbes

1991). Understanding their function in *S. trimeni* would further elucidate this system's evolutionary ecology.

These patterns of covariation between male competitive traits and their contest environment provide compelling support for animal weaponry to diverge among populations in response to spatial variation in the contest substrate and abiotic environment. Several of the traits that I have investigated here exhibited both phylogenetic autocorrelation (Table 3.5) and correlations with the environment occupied by each population. These findings may indicate that stabilizing selection has acted to conserve traits that are adaptive to each environment (Hansen 1997). However, without experimental work examining fitness benefits conferred by different trait values within and across populations occupying different environments, the covariance of trait values with environmental gradients cannot be inferred as adaptation. High levels of molecular divergence among the study populations and phenotypic divergence in terms of primary and secondary sexual traits in males support these as distinct taxa. *Scelophysa trimeni* is a species complex that has arisen in a mostly homogenous habitat without clear allopatric boundaries or historical vicariant-type barriers (Irwin 2002). Taxonomic revisions (Dombrow and Colville 2020) indicate that many other monkey beetle species may be harbouring cryptic diversity, highlighting that sexual selection may be an important driver of monkey beetle diversification in the GCFR.

Conclusions

While more work is required to assess whether divergence in weapon and signalling traits are associated with reproductive isolation between populations, the present findings do suggest that secondary sexual traits in male *S. trimeni* have diversified along gradients of contest substrate variation and abiotic environmental variation. These findings support a role of sexual selection as a driver of evolutionary diversification when there are differences in the contest substrate and abiotic environment. Phylogenetic distance, but not spatial distance, was important in structuring trait variation among populations. However, the correlation of spatial distance with phylogenetic distance suggests that isolation-by-distance may have contributed to evolutionary divergence among populations. Future work could also examine the extent of reproductive isolation present in this system, both among allopatric and sympatric populations. Specifically, gene flow should be measured, and the advantage of local males in obtaining mating opportunities. Further study of both male and female genitalia should be pursued to assess support for or against the 'lock-and-key' hypothesis, as incompatible genitalia forms may be key in maintaining reproductive isolation in sympatry in this system. Secondly, the role of phylogeny should be explored, as well as other potential sources of constraints influencing male traits' evolutionary lability in response to variation in the contest substrate, as strong evolutionary constraints may account for deviations in the expected covariances among the male traits studied here, and the contest substrate. I explore these aspects in the following chapter.

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3.7 APPENDIX

Table A1. Selection table for linear mixed effects models of male trait variation in relation to environmental and contest substrate variation between populations, accounting for trait relationship with body size (pronotum width). CS = centroid size. Baseline models assess covariation of trait with body size only. For baseline models, unexplained among-population variance refers to variance in traits between populations unexplained by both the random effect and body size, while for covariate models it refers to between-population variance in the trait unexplained by the random effect, covariation with body size, and covariation of trait with environmental or contest substrate covariate. Variance explained (%) is the reduction of unexplained between-population variance by the environmental or contest substrate covariate model over that of the baseline model. *K* corresponds to the number of parameters estimated in each model, deviance is a measure of goodness-of-fit with smaller values indicating better fit of model to data, and AIC is the Akaike Information Criterion. ΔAIC is the difference in AIC between the lowest-scoring model and all others, while *w*AIC is the AIC weight of each model and indicates the relative plausibility of all models relative to the lowest-scoring model.

Model	Unexplained between-population variance	Variance explained (%)	*K*	Deviance	AIC	ΔAIC	*w*AIC
Femur CS							
Baseline	0.013	NA	5	292.548	302.548	1.234	0.231
MAP	0.013	1.945	6	292.449	304.449	3.135	0.090
MST	0.012	8.637	6	291.243	303.243	1.929	0.163
SOC	0.013	-0.333	6	292.502	304.502	3.187	0.087
Flower diameter	8.818 × 10-3	33.884	6	289.314	301.314	0	0.429
Tibia CS							
Baseline	5.402 × 10^{-9}	NA	4	450.278	458.278	0	0.316
MAP	5.138 × 10^{-9}	4.882	5	448.988	458.988	0.710	0.222
MST	5.717 × 10^{-9}	-5.832	5	449.058	459.058	0.781	0.214
SOC	4.453 × 10^{-9}	17.570	5	450.034	460.034	1.756	0.131
Flower diameter	5.300 × 10^{-9}	1.875	5	450.259	460.259	1.981	0.117
Tarsus CS							
Baseline	0.013	NA	4	-123.281	-115.281	0	0.305
MAP	0.012	1.820	5	-123.444	-113.444	1.837	0.122
MST	0.012	6.224	5	-123.943	-113.943	1.338	0.156
SOC	0.013	0.941	5	-123.355	-113.355	1.926	0.117
Flower diameter	0.010	18.128	5	-125.243	-115.243	0.038	0.300
Claw CS							
Baseline	1.480 × 10^{-9}	NA	4	-355.177	-347.177	0	0.369
MAP	1.481 × 10^{-9}	-0.081	5	-355.183	-345.183	1.993	0.136
MST	1.463 × 10^{-9}	1.151	5	-355.311	-345.311	1.866	0.145
SOC	1.409 × 10^{-9}	4.798	5	-355.434	-345.434	1.743	0.154
Flower diameter	1.212 × 10^{-9}	18.075	5	-355.905	-345.905	1.272	0.195

Femur shape							
Baseline	1.375×10^{-10}	NA	4	-3,666.470	-3,658.470	0	0.393
MAP	1.350×10^{-10}	1.846	5	-3,666.485	-3,656.485	1.984	0.146
MST	1.168×10^{-10}	15.074	5	-3,666.694	-3,656.694	1.776	0.162
SOC	1.287×10^{-10}	6.420	5	-3,666.597	-3,656.597	1.872	0.154
Flower diameter	1.360×10^{-10}	1.179	5	-3,666.472	-3,656.472	1.998	0.145
Tibia shape							
Baseline	1.990×10^{-11}	NA	4	-13,076.924	-13,068.924	0	0.393
MAP	1.828×10^{-11}	8.152	5	-13,076.974	-13,066.974	1.949	0.148
MST	1.989×10^{-11}	0.054	5	-13,076.924	-13,066.924	2.000	0.145
SOC	1.942×10^{-11}	2.424	5	-13,076.936	-13,066.936	1.987	0.146
Flower diameter	1.468×10^{-11}	26.197	5	-13,077.228	-13,067.228	1.694	0.168
Tarsus shape							
Baseline	4.127×10^{-11}	NA	4	-4,060.682	-4,052.682	0	0.351
MAP	5.056×10^{-11}	-45.185	5	-4,061.202	-4,051.202	1.479	0.167
MST	4.148×10^{-11}	-50.573	5	-4,061.077	-4,051.077	1.605	0.157
SOC	5.020×10^{-11}	-20.391	5	-4,061.388	-4,051.388	1.294	0.184
Flower diameter	3.352×10^{-11}	-76.395	5	-4,060.864	-4,050.864	1.818	0.141
Claw shape							
Baseline	6.585×10^{-11}	NA	4	-4,060.682	-4,052.682	0	0.351
MAP	9.561×10^{-11}	-45.185	5	-4,061.202	-4,051.202	1.479	0.167
MST	9.916×10^{-11}	-50.573	5	-4,061.077	-4,051.077	1.605	0.157
SOC	7.928×10^{-11}	-20.391	5	-4,061.388	-4,051.388	1.294	0.184
Flower diameter	1.162×10^{-10}	-76.395	5	-4,060.864	-4,050.864	1.818	0.141
UV chroma							
Baseline	1.389×10^{-3}	NA	4	-3,452.645	-3,444.645	9.529	7.938×10^{-3}
MAP	1.284×10^{-3}	7.568	5	-3,453.327	-3,443.327	10.847	4.107×10^{-3}
MST	7.178×10^{-4}	48.334	5	-3,457.746	-3,447.746	6.429	0.037
SOC	9.583×10^{-4}	31.026	5	-3,455.740	-3,445.740	8.435	0.014
Disc S1U	1.182×10^{-3}	14.917	5	-3,453.999	-3,443.999	10.175	5.747×10^{-3}
Petal S1U	1.911×10^{-4}	86.248	5	-3,464.175	-3,454.175	0	0.931
Blue chroma							
Baseline	7.800×10^{-4}	NA	4	-3,616.177	-3,608.177	1.260	0.175
MAP	4.834×10^{-4}	38.023	5	-3,619.437	-3,607.144	2.293	0.104
MST	6.839×10^{-4}	12.328	5	-3,617.22	-3,609.437	0	0.329
SOC	6.853×10^{-4}	12.141	5	-3,617.144	-3,607.220	2.217	0.108
Disc S1B	5.945×10^{-4}	23.790	5	-3,618.142	-3,607.256	2.182	0.110
Petal S1B	6.776×10^{-4}	13.131	5	-3,617.256	-3,608.142	1.295	0.172

4. EVOLUTIONARY CONSTRAINTS ON MALE BEETLE WEAPON DIVERSIFICATION

4.1 ABSTRACT

One hypothesis of speciation by intrasexual contest competition suggests that speciation may occur if divergent, incompatible weapon morphologies and fighting styles arise under divergent fighting contexts in allopatric populations. To understand how intrasexual contest competition may influence evolutionary processes, we need to identify and quantify the evolutionary constraints on weapon diversification. Here, I assess the roles of possible evolutionary constraints on weapon variation across diverse developmental environments and fighting contexts in the morphologically complex hind leg weaponry of male *Scelophysa trimeni* monkey beetles. To achieve this aim, I quantify and compare among-individual patterns of weapon shape and size variation and covariation within and among eleven populations and compare these trends to those quantified in an evolutionary context. My findings support a two-module pattern of modularity at both static and evolutionary levels and consistently low covariation between hind leg segments' size and shape hind leg shape varies generally independently of size. These findings indicate that monkey beetle weapons are relatively unconstrained in their evolutionary diversification across divergent fighting substrates and under varying developmental environments. At a broader level, these findings suggest that insects may be capable of exhibiting an evolutionarily labile response in male weaponry to variation in the contest substrate, which has important implications for hypotheses concerning the role of sexual selection in driving taxonomic diversification and speciation in heterogeneous environments, even at the microevolutionary scale.

4.2 INTRODUCTION

While mate choice is accepted as being capable of driving evolutionary diversification, the role of male contest competition in speciation has received disparately little research attention (Qvarnström et al. 2012; McCullough et al. 2016; Bierbach et al. 2018; Lackey et al. 2018) and its ability to drive speciation has long been a contentious topic (Eberhard 1980; Qvarnström et al. 2012; Burdfield-Steel and Shuker 2018). One of the causal routes to speciation by intrasexual contest competition is theorized to occur via incompatible weapon forms and fighting styles arising under divergent fighting contexts in allopatric populations (Qvarnström et al. 2012; McCullough et al. 2016; Dijkstra and Border 2018; Lackey et al. 2018; Tinghitella et al. 2018). Since larger weaponry generally confers greater competitive success and access to females (Simmons and Scheepers 1996; Karino et al. 2005; Snell-Rood and Moczek 2013; Goyens et al. 2015; McCullough and Simmons 2016; Rink et al. 2019), sexual selection should drive strong directional evolutionary change in weaponry (Kingsolver et al. 2001). This would favour ever increasingly large and elaborate weapons over evolutionary time and erode genetic variation (West-Eberhard 1983; Kodric-Brown and Brown 1984; Kingsolver et al. 2001; Emlen 2008). In practice, we observe extreme variability in weapon size and shape within populations (Miller and Emlen 2010; Miller et al. 2016), reflecting their phenotypic plasticity (Nur and Hasson 1984; Griffith and Sheldon 2001; Moczek 2010). Weapons are highly condition-dependent, and their sensitivity to the developmental environment, physiological, energetic, and biomechanical costs, and trade-offs with other traits, all impose constraints on their phenotypic expression (Emlen 2001; Warren et al. 2013; Johns et al. 2014; McCullough 2014; Sasson et al. 2016; Zinna et al. 2018; O'Brien et al. 2019). In order to understand how these costs constrain weapon evolution (Emlen 2001; Miller and Emlen 2010; McCullough and Emlen 2013), several studies have sought to identify and quantify the costs of sexually selected traits and link these to trait variation within and among taxa (e.g., Kotiaho 2001; Goyens et al. 2016; O'Brien et al. 2019).

Evolutionary constraints on animal weapons

Costs, trade-offs, and other limitations on animal weaponry have almost exclusively been studied at the population or species level ('static' constraints *sensu* Klingenberg 2014). However, to understand how intrasexual contest competition may drive phylogenetic divergence or speciation, we need to identify and quantify evolutionary constraints of weapon diversification. How free are weapon traits to evolutionarily respond to variation in selection regimes? Evolutionary constraints at the microevolutionary level may impart inertia to traits' capacities to respond to variations in selection regimes or may make some evolutionary trajectories and phenotypes more 'accessible' than others (Frankino et al. 2009). Constraints on trait evolution can include developmental (Eberhard and Gutiérrez 1991; Emlen et al. 2005) and functional factors (Okada et al. 2012; McCullough 2014), allometric scaling relationships (Klingenberg and Zimmermann 1992; Knell et al. 2004; Voje and Hansen 2013; Pélabon et al. 2014; Voje et al. 2014), and phylogenetic effects (Kappeler 1996). However, little of the extensive body of work on evolutionary constraints has examined their role in influencing the evolutionary diversification of animal weapons.

Modularity and integration of traits

Traits that share underlying developmental processes (e.g., pleiotropy) and/or contribute to a shared function (e.g., leg components need to interact to give rise to effective locomotion), are expected to covary more strongly amongst themselves than with other such sets of traits (Klingenberg 2014). These sets of strongly covarying (highly integrated) traits are termed modules. Modularity is quantified with the covariance ratio (CR; Adams 2016), which is a ratio of the amount of covariation (integration) between modules relative to the amount of covariation within them; the expected value under the null hypothesis of no modularity is one, as each trait is expected to covary equally with every other trait (Adams 2016). The degree to which modules can evolve independently of one another is constrained by the integration among them (Klingenberg 2005).

Body segmentation

Insects and other arthropods have capitalized on modularity with their segmented *bauplan*, a blueprint that is deemed to have been essential in enabling their cosmopolitan success (Savriama et al. 2017; Chipman and Edgecombe 2019; Clark et al. 2019). However, high integration between modules may constrain morphological diversification (Williams and Nagy 2001; Hansen 2003; Adams and Felice 2014). The less integration between modules, the more able they are to respond autonomously to selection (Wagner 1988; Hansen 2003; Hansen and Houle 2008). Studying patterns of modularity and integration is therefore essential in understanding how weapons have diversified in response to divergent selective regimes. However, modularity and integration have scarcely been studied in the context of sexually selected traits, and the extent to which covariance of traits within segments (modularity) and covariance of traits between segments (integration) have either constrained or facilitated the evolutionary diversification of male weaponry is largely unknown. Such work may be especially relevant in the case of complex weapons i.e., weapons composed of multiple anatomical segments, such as the enlarged hind legs present in several beetles including *Sagra femorata* (Katsuki et al. 2014; O'Brien et al. 2017) and *Megalopus armatus* (Eberhard and Marin 1996), and several coreid bugs (Miyatake 1997; Eberhard 1998).

Allometric constraints

Morphological allometry is the scaling of trait size with body size (Huxley 1924; Huxley and Teissier 1936). It may be examined at either ontogenic (within individuals through their development), static (across individuals of the same taxon at the same life stage), or evolutionary (across individuals at the same life stage between taxa) scales (Klingenberg 1992). As allometric slopes appear to require macroevolutionary timescales to evolve, microevolutionary variation in the scaling of trait size relative to body size may be allometrically constrained (Voje et al. 2014). The concept of allometry has expanded from the original definition developed by Huxley to include the scaling relationships of many other trait properties, such as mass and shape, with body size. Geometric morphometric studies of trait variation often quantify the allometric scaling relationships of trait shape with trait size. If this form of allometry ("multivariate" allometry; Klingenberg 1996; Klingenberg 2016) exhibits stable slopes over microevolutionary time, as narrow sense allometry does, then conservation of the scaling relationships between trait size and trait shape may limit weapon diversification within taxa (Pélabon et al. 2014). Quantifying evolutionary allometric slopes at the microevolutionary level may therefore provide insights into the rate at which a taxon is likely to diversify (Okie et al. 2013; Sansalone et al. 2018; Simons et al. 2020).

Phylogenetic constraints

Phylogenetic signal is produced by the statistical nonindependence of taxa due to their shared evolutionary history (Felsenstein 1985; Blomberg and Garland 2002; Losos 2008; Revell et al. 2008). Whether phylogenetic signal contains information on the evolutionary processes that have led to the present phylogeny, and if so, how much can be inferred, is debated (Revell et al. 2008; Ackerly 2009). Low and high estimates of phylogenetic signal are often considered suggestive of evolutionary lability and phylogenetic constraint, respectively (Revell et al. 2008; Adams 2014a; Gómez et al. 2016). For example, phylogenetic constraint may arise due to a historic erosion of genetic diversity, or low mutation rate (Wiens and Graham 2005; Ashman and Majetic 2006; Losos 2008; Chenoweth and McGuigan 2010). Phylogenetic effects may be key in determining weapon trait diversity because of their influence on trait responses to variation in selection pressures (Long et al. 2012). For example, taxa lacking the genetic diversity to exhibit evolutionary responses to diverging selective regimes should exhibit limited weapon diversification in response to variation in the physical substrate on which contests occur (Qvarnström et al. 2012; Murren et al. 2015; McCullough et al. 2016).

Evolutionary constraints on weapon diversification in divergent fighting contexts

As discussed above, each of these classes of evolutionary constraint may manifest as patterns in the covariation amongst traits. Developmental and functional constraints may be reflected in patterns of modularity and integration, allometric constraints as limited trait shape variation independent of variation in trait size, and phylogenetic constraints in less divergence of trait values among taxa than expected given the genetic distances between these taxa. Quantifying patterns of trait variation can thus provide insights into which forms of evolutionary constraint may be present, and the strength of each in influencing patterns of evolutionary diversification in animal weapons. In the ‘divergent fighting contexts’ hypothesis of weapon diversification (McCullough et al. 2016), divergence of the substrate or physical situation in which intrasexual contest competition occurs selects for weapon forms and fighting styles that perform optimally in different contest contexts. Highly constrained weapons would be expected to show limited evolutionary responses to contest substrate variation, both in terms of the range of accessible phenotypes (Braendle et al. 2010; Vermeij

2015; Wessinger and Hileman 2016) and in terms of their rate of evolution (Streisfeld and Rausher 2007; Rausher et al. 2008; Voje and Hansen 2013; Pélabon et al. 2014).

In this chapter, I focus on quantifying patterns of trait variation due to developmental, functional, and phylogenetic constraints on weapon-form evolution in the sexually dimorphic monkey beetle *Scelophysa trimeni*. *Scelophysa trimeni* is a putative cryptic species complex of monkey beetles distributed along a >400 km stretch of the West Coast of South Africa (Fig. 3.1, Chapter 3). Males compete on flower heads for mating access to females with their enlarged hind legs. Findings reported in my previous chapter (Chapter 3) suggest that male hind leg size and shape, and elytral colouration differ significantly among the study populations, suggesting that these traits are evolutionarily labile at the microevolutionary scale. These populations are clearly resolved phylogenetically and can be distinguished based on differences in male primary sexual traits. Male genitalia are dimorphic, either being 'gracile' or 'robust' in form (Fig. 3.4, Chapter 3). Two pairs of the study populations are sympatric, and within each pair, one population belongs to the 'gracile' clade and one to the 'robust' clade, suggesting that reproductive isolation is being maintained in sympatry, possibly by means of a 'lock-and-key' mechanism (Shapiro and Porter 1989).

As a group, monkey beetles have undergone considerable taxonomic and phenotypic radiations that have been most pronounced in the sexually dimorphic taxa of this tribe (Colville et al. 2018). Females' hind legs are relatively similar to their fore- and mid-legs, in terms of overall size and leg segments' shapes. In contrast, males' hind legs are considerably enlarged relative to their fore- and mid-legs, and one or more of the leg segments may be spiny and exhibit pronounced sculpturing and exaggeration (Colville et al. 2018). This morphological diversity, seen across a range of flower micro-habitats, suggests that these taxa utilise diverse fighting styles (Tinghitella et al. 2018). Because fine-scale patterns of monkey beetle taxonomic and phenotypic diversity are not fully explained by variation in their abiotic and floristic environment (Colville 2009), other drivers of the group's diversification, such as sexual selection, may help explain the group's diversity.

As male monkey beetle weaponry is multivariate (i.e., composed of multiple leg segments each varying in shape and size), its ability to respond to variation in the selective environment is expected to be mediated by evolutionary constraints arising at the level of each anatomical leg segment, as well as additional constraints arising from covariation between these leg segments. Such constraints may include shape-size allometry, morphological modularity and integration, and shared phylogenetic history between populations (Monteiro et al. 2005; Klingenberg et al. 2013; Stansbury and Moczek 2013). The extent to which shape and size variation and covariation in the male hind leg are constrained by these (and other) factors is expected to determine the extent to which weapon form in *S. trimeni* has been able to diversify in response to sexual selection in the context of divergent fighting habitats.

Here, I assess the roles of possible constraints on weapon diversification, in response to divergent fighting contexts, in the morphologically complex hind leg weaponry of male *S. trimeni.* I quantify and compare static patterns of weapon form (shape and size) variation and covariation within and among eleven populations and compare these trends to those quantified in an evolutionary context, i.e., taking the phylogenetic relationships among these populations into account. I examine: i) morphological constraints, which limit the independence of weaponised leg segments in their responses to selective pressures; ii) allometric constraints, which limit variation in shape independently of size; and iii) phylogenetic constraints, which limit phenotypic variation due to historical gains and losses of genetic diversity and phenotypic divergence between taxa in proportion to the time since their genetic divergence. Quantifying the extent of these constraints on weapon evolution in monkey beetles will increase our understanding of the ability of male monkey beetle weapons to respond to variation in the flower habitat, thus providing insights into the drivers of their evolutionary diversification in South Africa.

4.3 METHODS

Study system

I used the same study system as that for Chapter 3, namely the putative cryptic species complex, *Scelophysa trimeni*, which is distributed along the west coastal region of South Africa (Fig. 3.1, Chapter 3). The same specimens from which data was collected for Chapter 3 are examined here, with the exclusion of those which had missing leg segments. See Chapter 3 methods for a detailed description of the study system, field data collection methods, and geometric morphometric and genetic methods.

Preparation of geometric morphometric data

After trimming the hind leg landmark files to exclude individuals for whom one or more leg segments were missing, I subjected the dataset for each hind leg segment (i.e., femur, tibia, tarsus, and claw) to separate Procrustes superimpositions using the bending energy criterion for optimising the positions of sliding semi-landmarks (Bookstein 1997, Gunz and Mitteroecker 2013).

Shape and size variation among populations

I constructed phylomorphospaces to visualise differences in shape among populations for each leg segment (Sidlauskas 2008; Polly et al. 2013). These plots consist of a phylogeny superimposed on a Principal components analysis of population mean shape and are useful for the visual detection of phylogenetic patterns of shape variation (Adams and Collyer 2019a). I tested for significant differences among populations and between clades in leg segment shape and size in two separate ANOVAs.

Static modularity and integration

First, I constructed several alternative hypotheses of hind leg modularity, each describing different patterns of shape covariation among each of the four leg segments (femur, tibia, tarsus, and claw; Fig. 4.1, Table 4.1). Pooling all populations, I assessed how much the patterns of covariation among hind leg segments in this dataset aligned with the expected patterns of modularity described in each alternative hypothesis. To do so, I calculated the CR coefficient (an estimate of the observed modular signal), Z-score (a standardised multivariate effect size that enables comparison amongst data sets; Adams and Collyer 2016) and P-value for each hypothesis using the function *modularity.test*() in the R package ‘geomorph’ (Adams et al. 2020). The expected value of CR under the null hypothesis is obtained by the random assignment of shape variables into subsets (Adams 2016). A CR value significantly lower than that produced under the null hypothesis indicates that the modules described by the alternative hypothesis are significantly more independent (i.e., they covary significantly less) than expected under the null hypothesis, indicating strong support for the alternative hypothesis of modularity (Adams 2016). The calculation of a standardised effect size, i.e., an effect size that is independent of sample size and trait number, allows one to compare the support for each alternative hypothesis to identify the best-supported hypothesis of modularity (Adams and Collyer 2019b). Comparisons were conducted using the ‘geomorph’ function *compare.CR*(), and the best-supported model of modularity was used in downstream analyses to quantify differences in static modularity and integration among populations, and to quantify evolutionary modularity and integration.

Second, I conducted an among-population comparison of the strength of modular signal to assess whether some populations showed greater independence between the modules supported by the analyses described above. I used the *modularity.test*() function to quantify the CR coefficient, Z-score, and P-value for each population, separately, and then compared their modular signals using the *compare.CR*() function.

Third, I assessed whether each population exhibited significant integration between the modules described in the best-supported hypothesis of modularity by using the 'geomorph' function *integration.test*() to calculate the level of integration between modules for each population. This function estimates the amount of integration between modules using two-block partial least squares (PLS) regression and returns the r-PLS coefficient (an estimate of PLS correlation between two modules, or the mean correlation if more than two modules are input) and Z-score (multivariate effect size). The observed correlation coefficient (r-PLS) is compared to the distribution of correlation coefficients generated from random permutations of individuals within modules, which represents the null hypothesis of no integration between modules. The observed correlation coefficient is significant if it is larger than expected under this null hypothesis.

Last, I compared integration levels among populations and quantified pairwise population differences in integration using the *compare.pls*() function in 'geomorph'. This function compares effect sizes among populations using two-sample z-tests.

Static allometry

There are several approaches to the analysis of multivariate phenotypic allometry (reviewed in Klingenberg 2016), the most widely adopted of which defines allometry as covariation between shape and size (Klingenberg 2016). Under this definition, allometry is quantified as the multivariate regression of shape variables on the logarithm of centroid size (Klingenberg 2016). The value of the regression slope measures the change in shape per unit change in log centroid size, while the intercept value is equal to mean shape at mean size (i.e., least-squares mean; LS mean). An important component of morphometric analyses is the comparison of allometry among groups (Klingenberg and Froese 1991; Klingenberg 1996; Klingenberg 2016). Groups may differ in terms of mean shape at mean size (allometric intercept), rate of change of shape with size (allometric scaling slope), or both (Klingenberg 2016). Here I test for differences in allometric intercept and slope among males from different populations of *S. trimeni* using a workflow based on the 'geomorph' R package methods demonstrated by Collyer (2020). Similar approaches have been implemented by Cariveau et al. (2016) to compare interspecific allometry of bee proboscis length and by Kawano (2000) to assess among-genera differences in male mandible allometry in the Lucanidae.

Populations may share both intercept and slope (simple allometry):

$$y_{ij} = c + bx_{ij} + \varepsilon_{ij} \qquad (1)$$

where y_{ij} is shape of individual *i* in population *j*, in the form of a vector of Procrustes-aligned coordinates, *c* is a parameter (intercept), *b* is also a parameter (regression slope), x_{ij} is the log-transformed centroid size of that individual, and e_{ij} is the error term.

Alternatively, the intercept varies among populations, but the scaling slope is shared (common allometry):

$$y_{ij} = (c+a_j p_j) + bx_{ij} + \varepsilon_{ij} \qquad (2)$$

where in addition to the parameter in equation (1), an additional parameter a_j estimates population p_j differences in the intercept.

Lastly, populations may exhibit unique allometries, and differ in both allometric slope and intercept:

$$y_{ij} = (c+a_j p_{ij}) + (b+k_j p_j)x_{ij} + \varepsilon_{ij} \qquad (3)$$

where equation (2) is modified by allowing for a population effect *k* on slopes.

For each leg segment, I constructed models of simple, common, and unique allometry using the function *procD.lm*() in R package ‘geomorph’. All models used ordinary least squares (OLS), Type I sums of squares, and a randomised residual permutation procedure (RRPP; Collyer et al. 2015). Centroid size was log-transformed prior to input into the models.

To test for significant differences in LS means among populations, I conducted an ANOVA using *anova.lm.rrpp*() in R package ‘RRPP’ (Collyer and Adams 2018) to compare the model describing simple allometry to the model of common allometry. To test for homogeneity of allometric scaling slopes among populations I used *anova.lm.rrpp*() to compare the model of unique allometry to the model of common allometry. This procedure was performed for each leg segment separately.

Evolutionary modularity and integration

I quantified evolutionary modularity using the ‘geomorph’ function *phylo.modularity*(). Using this function, I tested the strength of evolutionary patterns of modularity among populations, assuming the best-supported hypothesis of static modularity and accounting for phylogenetic relatedness among populations. I then tested evolutionary integration among modules using the function *phylo.integration*(), again assuming the best-supported hypothesis of static modularity. These analyses were based on the population mean shape of each leg segment so that evolutionary (among-population) patterns were not conflated with static (within-population) patterns.

Evolutionary allometry

I quantified evolutionary covariation of shape with size using a phylogenetic ANOVA implemented by the function *procD.pgls()* (Adams 2014b). This phylogenetic Procrustes ANOVA assumes a Brownian motion model of trait evolution along the phylogeny. Brownian motion assumes that trait values change randomly over time (Felsenstein 1985; Harmon 2019). This assumption may have limited utility in modelling trait evolution under strong directional selection (Harano and Kutsukake 2018), including sexual selection (Hoekstra et al. 2001; Kelly 2008; O’Brien et al. 2017). Many authors prefer the Ornstein-Uhlenbeck (OU) model which allows traits to evolve toward an optimum (Hansen and Martins 1996; Hansen 1997; Butler and King 2004). However, to date there is no generalization of OU that displays appropriate Type I error rates with the highly multivariate data that are typical of geometric morphometric studies (Adams and Collyer 2018). I calculated evolutionary shape-size allometry separately for each leg segment using population mean trait values.

Phylogenetic signal

I tested for phylogenetic signal in patterns of among-population shape and size variation using population means and the *physignal*() function in the R package 'geomorph' (Adams et al. 2020). For multivariate data, such as shape, this function calculates a multivariate generalisation of Blomberg's *K* (Blomberg et al. 2003) developed by Adams (2014a) for use with geometric morphometric data. For univariate data, such as centroid size, it returns the standard Blomberg's *K.* In both cases, the function returns a *P*-value obtained by permutation of the data across the phylogeny and a multivariate effect size (Adams and Collyer 2019a). All statistical analyses were performed in R v3. 6. 3 (R Core Team 2020).

4.4 RESULTS

Shape and size variation among populations

For all leg segments, shape and size differed significantly among populations and between clades (Fig. 4.2; Table 4.2). Hind femora, tibiae, and tarsi exhibited clear differences in shape between gracile clade populations and robust clade populations (Fig. 4.2A-C). These clades separated out along PC1 (accounting for 86.70% of hind femur shape variation, 85.60% of hind tibia shape variation, and 69.27% of hind tarsus shape variation; Fig. 4.2A-C). Specifically, gracile clade populations (KLS1, KLS2, NP1G, and VDPG) tended to have relatively elongated hind leg components, whereas populations in the robust clade (DBAY, EBAY, HLGT, NP1R, NNP2, NNPG, and VDPR) tended to have thicker and more sculptured hind femora, tibia, and tarsi (Fig. 4.2A-C). Differences in hind claw shape between clades were less striking (Fig. 4.2D), with no separation of clades along PC1 in phylomorphospace. Rather, clade phylomorphospace was partitioned between clades along the second principal component of phylomorphospace, which accounted for < 10% of claw shape variation.

Population differences in claw shape varied from strongly hooked, thin claws in DBAY males, to broader, less strongly curved claws in HLGT. Mean centroid sizes of all leg segments varied by less than two orders of magnitude among populations, with males from NNP2 having, on average, the largest hind femora, tibiae, and tarsi, and DBAY males having the largest hind claws (Figs. 3.7 – 9, Chapter 3).

Static modularity and integration

The best supported hypothesis of modularity defined two modules: one consisting of femur and tibia, and a second consisting of tarsus and claw (Table 4.1; Fig. 4.1: H2, Fig. 4.3A; Table 4.3; Table A1). All populations exhibited strong modular signal for this hypothesis (Fig. 4.3B; Table 4.3), with KLS1 males exhibiting the strongest modular signal of all populations ($CR = 0.216$, $Z_{CR} = -29.654$, $P < 0.001$; Fig. 4.3B; Table 4.3). Overall, populations exhibited low, nonsignificant levels of integration between these two modules, implying that they vary in shape relatively independently of each other (Table 4.3; Fig. 4.4A; Tables A6-7).

Static allometry

Procrustes ANOVAs comparing the fit of common allometry versus simple allometry supported significant differences in LS means (mean shape at mean size) among populations, for all leg segments (Table 4.4; Figs. A1-4). Models of unique allometry did not fit the data significantly better than models of common allometry (Fig. 4.5). These results indicate that while males from different populations exhibit differences in mean shape at mean size (Table 4.5), they share a common allometric slope. Although size was a significant covariate in the common allometry model for each leg segment, it explained < 5% of shape variation (Table 4.5). A much larger proportion of shape variation in each leg segment was explained by population effects (> 25%; Table 4.5).

Evolutionary modularity and integration

There was significant support at the phylogenetic level for the morphological modules that were supported at the static level i.e., femur+tibia and tarsus+claw (CR = 0.7234, Z_{CR} = -15.3278, $P < 0.001$). Evolutionary integration between these modules was not significant (r-PLS = 0.508, $P = 0.567$, Z-score = -0.2268; Fig. 4.3B).

Evolutionary allometry

Evolutionary allometry accounted for < 5% of among-population shape variation for the femur, tibia, and tarsal leg segments, and > 26% of among-population claw shape variation (Fig. 4.6). None of these relationships were statistically significant; however, tibia and claw allometries were associated with large effect sizes ($Z > 1$; Table 4.6).

Phylogenetic signal

Femur and tibia shape variation among populations each exhibited stronger than expected phylogenetic signal. Tarsus and claw shape exhibited as much phylogenetic signal as expected due to Brownian motion (Table 4.7). When shape variation was analysed at the level of modules, both module 1 (femur + tibia) and module 2 (tarsus + claw) shape variation exhibited as much phylogenetic signal as expected under Brownian motion (module 1: K = 0.820; $P = 0.436$, Z-score = -0.080; module 2: K = 0.758; $P = 0.707$; Z-score = -0.653). All leg segment sizes exhibited levels of phylogenetic signal concordant with Brownian motion which were associated with small effect sizes (Table 4.7).

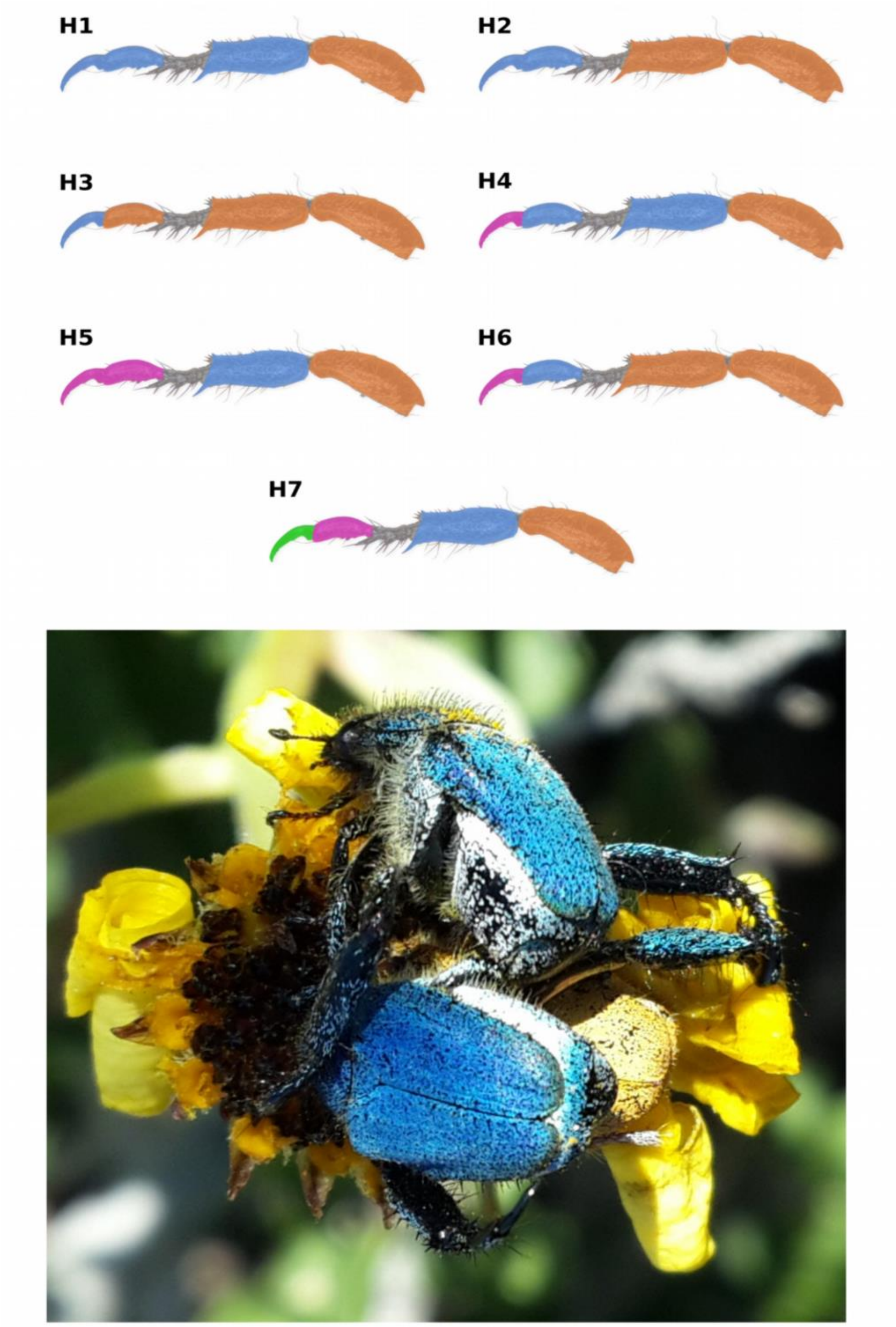

Figure 4.1. Above: hind leg of male *S. trimeni* illustrating the seven alternative hypotheses of modularity that were tested against the null hypothesis of no modularity. Leg segments, from right to left: femur, tibia, tarsus, and claw. Each module is indicated by a different colour. Distal tarsomeres were not digitised and thus are depicted in greyscale throughout. See Table 4.1 for full description of each alternative hypotheses and Tables A2-3 for pairwise comparisons. Below: two males (blue) competing for a female (green); note clasping of hind claws and extension of hind leg to push opponent.

Table 4.1. Alternative hypotheses of modularity in the hind legs of male *S. trimeni*. The null hypothesis, which is a hypothesis of no modularity, is not shown. '+' signifies leg components that are hypothesized to covary as parts within a module. '|' indicates module boundaries.

Hypothesis	Number of modules	Modular structure
H1	2	Femur | tibia + tarsus + claw
H2	2	Femur + tibia | tarsus + claw
H3	2	Femur + tibia + tarsus | claw
H4	3	Femur | tibia + tarsus | claw
H5	3	Femur | tibia | tarsus + claw
H6	3	Femur + tibia | tarsus | claw
H7	4	Femur | tibia | tarsus | claw

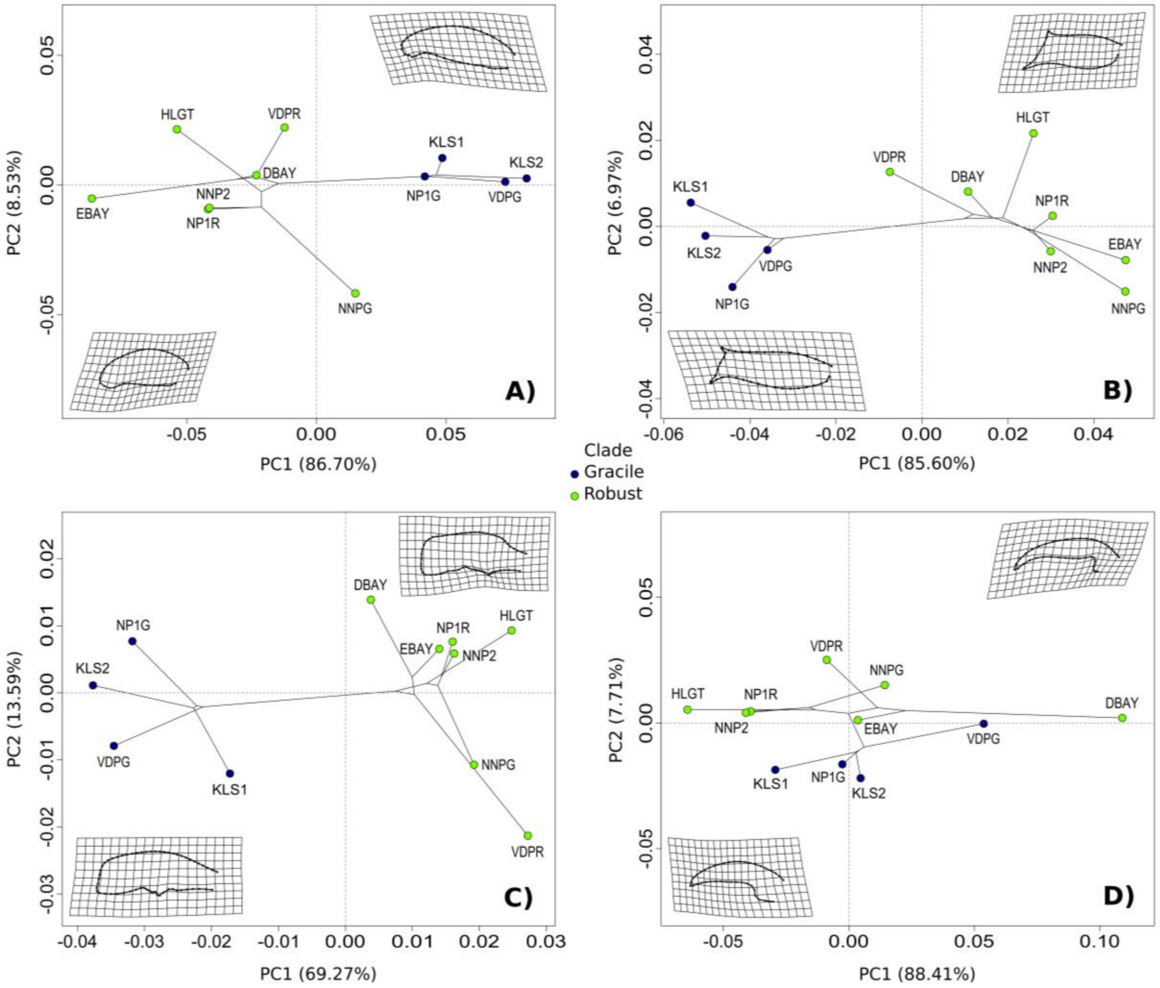

Figure 4.2. Phylomorphospace plots showing population mean shapes for: hind leg A) femur, B) tibia, C) tarsus, and D) claw. Clade refers to the sister taxa emerging from the basal split in the phylogeny (Fig. 3.4, Chapter 3). Clades are also distinguished by male genitalia form. ‘Gracile’ clade males have ‘gracile’ parameres, while ‘robust’ clade males have ‘robust’ parameres (Fig. 3.4, Chapter 3), indicated here by blue and green branch tips, respectively. The wireframes depict 2× magnifications of the mean shapes for populations situated on the extremes of Principal Component 1.

Table 4.2. Procrustes ANOVA results of difference in male hind leg shape and size among populations and clades. See Fig. 3.4 in Chapter 3 for phylogenetic relationships among populations and differences in paramere morphology between clades. Procrustes ANOVA is a generalization of univariate ANOVA to multidimensional data, such as shape data, that can also work with univariate response variables, such as centroid size data. Procrustes ANOVAs were performed with Type 1 sums of squares. Standardised effect size (Z-score) was calculated with RRPP over 1000 permutations to build the empirical sampling distribution of F-values with which the observed F-values were compared. R^2 is the coefficient of determination; F is the observed F-value; Z is the Z-score, calculated as $Z = (F-\hat{\mu}_F)/\hat{\sigma}_F$, where F is the observed F-value, $\hat{\mu}_F$ is the expected F-value under the null hypothesis of no difference in response variable among populations or clades, calculated as the mean value of the empirically-obtained sampling distribution, and $\hat{\sigma}_F$ is the standard deviation of the empirically-obtained sampling distribution. *P* is the P-value and is the probability of obtaining a larger F-value from the sampling distribution than the value observed in the test.

		Populations				Clades			
Leg segment	Variable	R^2	$F_{10,746}$	Z	*P*	R^2	$F_{1,755}$	Z	*P*
Femur	Shape	0.274	28.207	9.0981	0.001	0.19407	181.8	5.2436	0.001
	Size	0.496	73.444	9.127	0.001	0.25685	260.94	3.1277	0.001
Tibia	Shape	0.333	37.314	14.349	0.001	0.26691	274.88	8.6812	0.001
	Size	0.322	25.392	7.768	0.001	0.03101	24.162	2.0037	0.001
Tarsus	Shape	0.0732	5.895	9.0537	0.001	0..03106	24.202	5.8102	0.001
	Size	0.373	44.442	8.228	0.001	0.10063	84.476	2.6023	0 .001
Claw	Shape	0.265	26.876	8.460	0.001	0.01138	8.6909	2.5522	0.005
	Size	0.404	50.473	8.324	0.001	0.12951	112.33	2.811	0.001

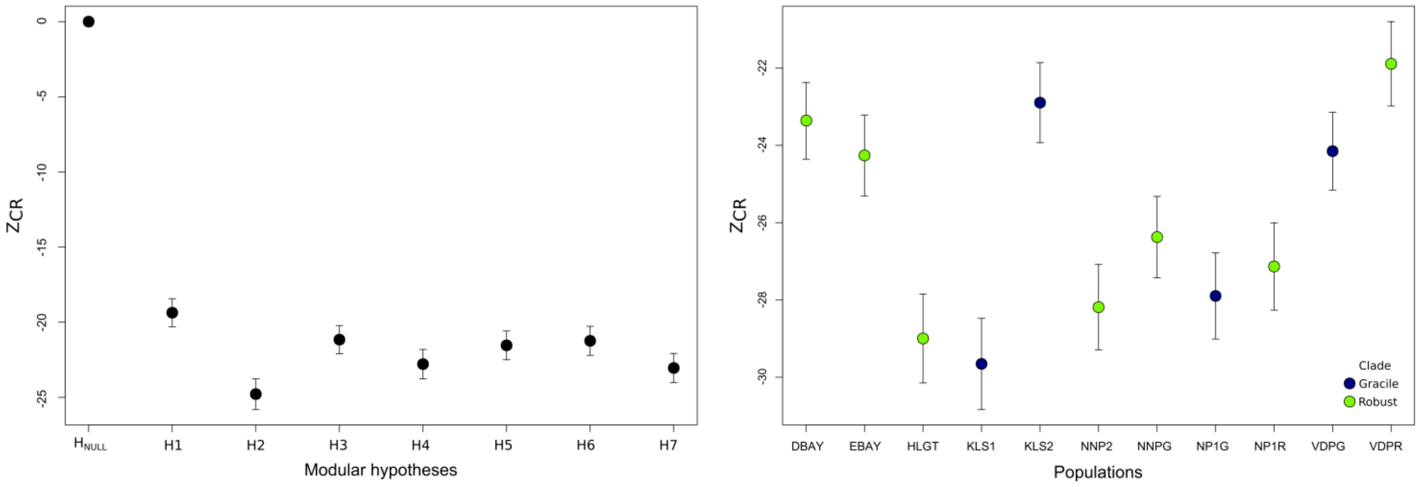

Figure 4.3. A) Comparison of the seven alternative hypotheses of modularity (H1-7) to the null hypothesis (H_{NULL}) of no modularity. The test statistic (CR; covariance ratio) is the ratio of mean covariance among the modules described in each hypothesis to the mean covariance within each module. The vertical axis is the standardised effect size (Z_{CR}) associated with each hypothesis; under the null hypothesis the effect size is 0 as all traits are expected to covary equally with all other traits. This standardised effect size allows for the comparison of modularity under different hypotheses and for different datasets. More negative effect size indicates stronger modularity signal. H2, having the most negative effect size of all hypotheses assessed ($Z_{CR} = -24.808$; Table A1), best fit the pattern of modularity present in the pooled hind leg data (CR = 0.117; $P = 0.001$). B) Comparison of levels of modularity, as defined by H2, among populations; population KLS1 had the strongest modular signal (CR = 0.216, ZCR = -29.654, $P < 0.001$; Table 4.3), indicating that the hind legs of males in this population exhibited the lowest ratio of covariance between modules to covariance within modules. Bars represent 95% confidence intervals.

Table 4.3. Modularity and integration in male hind legs within 11 populations of *S. trimeni*. CR = correlation ratio (ratio of between-module covariation to within-module covariation, where module 1 is comprised of femur and tibia, and module 2 is comprised of tarsus and claw; Fig. 4.1); Z_{CR} = standardised effect size of modular signal; *P* = p-value. Significance of the observed CR value is calculated over 1000 permutations in which traits are randomly shuffled between modules. Integration is a measure of the amount of covariation between module 1 and module 2. r-PLS = partial least squares correlation coefficient; Z-score = standardised effect size of integration signal; *P* = p-value. Under the null hypothesis of no integration between modules, r-PLS has an expected value of zero. Significance of the observed r-PLS value is calculated over 1000 permutations in which traits are randomly shuffled between modules. Standardised effect sizes (Z_{CR} and Z-scores) allow for the comparison of modularity and integration among different datasets even if they have different expected CR and r-PLS values, respectively.

Population	Modularity			Integration		
	CR	Z_{CR}	*P*	r-PLS	Z-score	*P*
DBAY	0.182	-23.367	<0.001	0.201	-0.385	0.614
EBAY	0.318	-24.260	<0.001	0.354	1.477	0.094
HLGT	0.232	-28.996	<0.001	0.388	-0.167	0.554
KLS1	0.216	-29.654	<0.001	0.376	1.001	0.160
KLS2	0.364	-22.895	<0.001	0.407	2.120	0.020
NNP2	0.164	-28.187	<0.001	0.236	-0.987	0.832
NNPG	0.165	-26.373	<0.001	0.182	-1.754	0.995
NP1G	0.245	-27.896	<0.001	0.305	-0.983	0.828
NP1R	0.371	-27.134	<0.001	0.501	-0.308	0.603
VDPG	0.174	-24.150	<0.001	0.244	-0.264	0.569
VDPR	0.568	-21.890	<0.001	0.666	-0.162	0.514

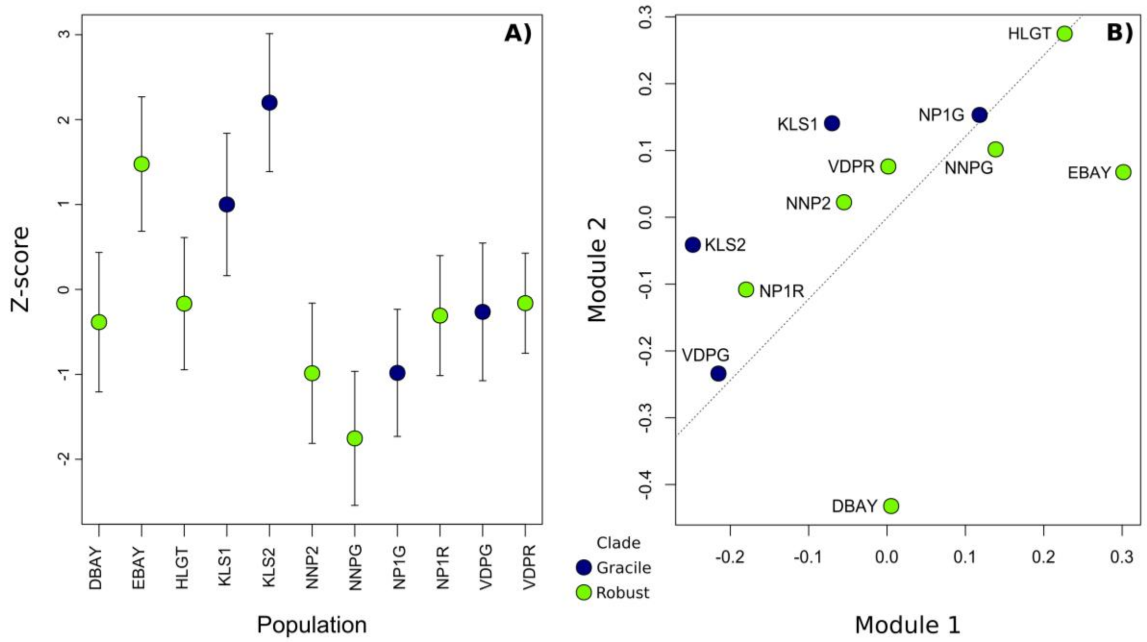

Figure 4.4. A) Level of integration between modules described in H2, for each population, shown as effect size (Z-score; see Fig. 4.1 and Table 4.1 for description of H2 modules). See Table 4.4 for associated r-PLS, Z-score and *P* values. B) Plot showing results of two-block partial least squares regression of evolutionary integration between module 1 and module 2 in H2. This analysis uses a Brownian motion model of evolution to account for phylogeny (r-PLS = 0.508, *P* = 0.567, Z-score = -0.227).

Table 4.4. Results of Procrustes ANOVA analyses for among-population differences in LS means of each hind leg segment. LS means are mean shape at mean size. Simple allometry is of the form: shape ~ log(size) and common allometry: shape ~ log(size + population) and allows the intercept to vary between populations. Model comparison performed using function *anova.lm.rrpp*() in R package 'RRPP'. See Figs. A1-4 for LS means wireframes.

Leg segment	Model of allometry	Resid. DF	DF	RSS	SS	MS	R^2	F	*Z*	*P*
Femur	Simple	755	1	9.396			0.000			
	Common	745	10	6.844	2.553	0.255	0.265	27.790	8.948	< 0.001
Tibia	Simple	755	1	4.0989			0.000			
	Common	745	10	2.751	1.348	0.135	0.323	36.507	14.428	< 0.001
Tarsus	Simple	755	1	5.271			0.000			
	Common	745	10	4.912	0.359	0.0359	0.0675	5.441	8.590	< 0.001
Claw	Simple	755	1	7.352			0.000			
	Common	745	10	5.582	1.770	0.177	0.229	23.623	8.154	< 0.001

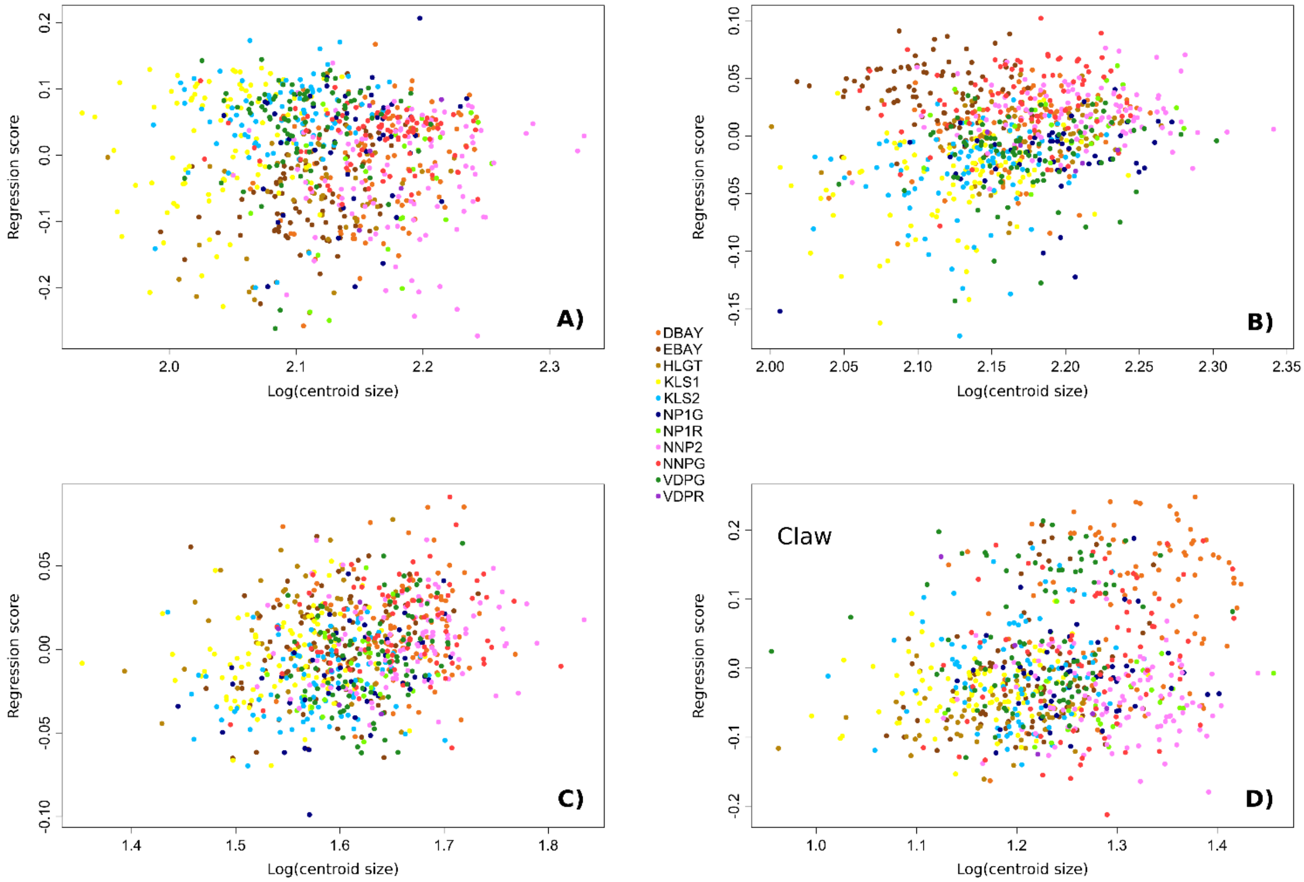

Figure 4.5. Variation in static allometry of each leg segment among populations, as calculated in Procrustes linear regressions: A) femur; B) tibia; C) tarsus; and D) claw. Regression scores are plotted against log(centroid size). Regression scores are standardised shape scores calculated from the regression of shape on centroid size and indicate the amount of shape change associated with change in centroid size and also includes residual shape variation. Results of Procrustes ANOVAs performed on the fit of common allometric models versus simple allometric models to test for differences in LS means (mean shape at mean size) among populations showed a significantly better fit of common allometric models for all leg segments; LS means were significantly different among populations (femur: $F_{745,10} = 27.790$, $R^2 = 0.265$, $Z = 8.948$, $P < 0.001$; tibia: $F_{745,10} = 36.507$, $R^2 = 0.323$, $Z = 14.428$, $P < 0.001$; tarsus: $F_{745,10} = 36.507$, $R^2 = 0.068$, $Z = 8.590$, $P < 0.001$; claw: $F_{745,10} = 23.623$, $R^2 = 0.229$, $Z = 8.154$, $P < 0.001$; see Figs. A1-4 for population LS means). Homogeneity of slopes tests, performed using Procrustes ANOVAs comparing the fit of unique allometric models versus common allometric models, did not support unique allometric slopes for populations (femur: $F_{735,10} = 1.603$, $R^2 = 0.015$, $Z = 1.486$, $P = 0.065$; tibia: $F_{735,10} = 1.297$, $R^2 = 0.011$, $Z = 1.346$, $P = 0.081$; tarsus: $F_{735,10} = 1.410$, $R^2 = 0.017$, $Z = 1.877$, $P = 0.038$; claw: $F_{735,10} = 1.883$, $R^2 = 0.018$, $Z = 1.960$, $P = 0.022$).

Table 4.5. Summaries of Procrustes linear regression models of common allometry for each leg segment. Common allometry is specified as: shape ~ log(CS) + population and allows for variation in LS means (mean shape at mean CS) among populations but constrains the allometric slopes to have a common value. CS = centroid size. Z = multivariate effect size calculated as the difference between the observed F-value and the expected value from an empirically derived sampling distribution of F-values over 1000 permutations, divided by the standard deviation of the sampling distribution. *P* = probability of obtaining an F-value larger than that observed.

Leg segment	Variable	DF	SS	MS	R^2	F	Z	*P*
Femur	Log(size)	1	0.220	0.220	0.0228	23.913	3.272	0.001
	Population	10	2.553	0.255	0.265	27.790	8.948	0.001
	Residuals	745	6.844	0.00919	0.712			
	Total	756	9.616					
Tibia	Log(size)	1	0.0712	0.0712	0.0171	19.285	4.662	0.001
	Population	10	1.348	0.135	0.323	36.507	14.428	0.001
	Residuals	745	2.751	0.00369	0.660			
	Total	756	4.170					
Tarsus	Log(size)	1	0.0459	0.0459	0.00863	6.958	3.797	0.001
	Population	10	0.359	0.0359	0.0675	5.441	8.590	0.001
	Residuals	745	4.912	0.00659	0.924			
	Total	756	5.317					
Claw	Log(size)	1	0.371	0.371	0.0481	49.550	4.320	0.001
	Population	10	1.770	0.177	0.229	23.623	8.154	0.001
	Residuals	745	5.582	0.00749	0.723			
	Total	756	7.723					

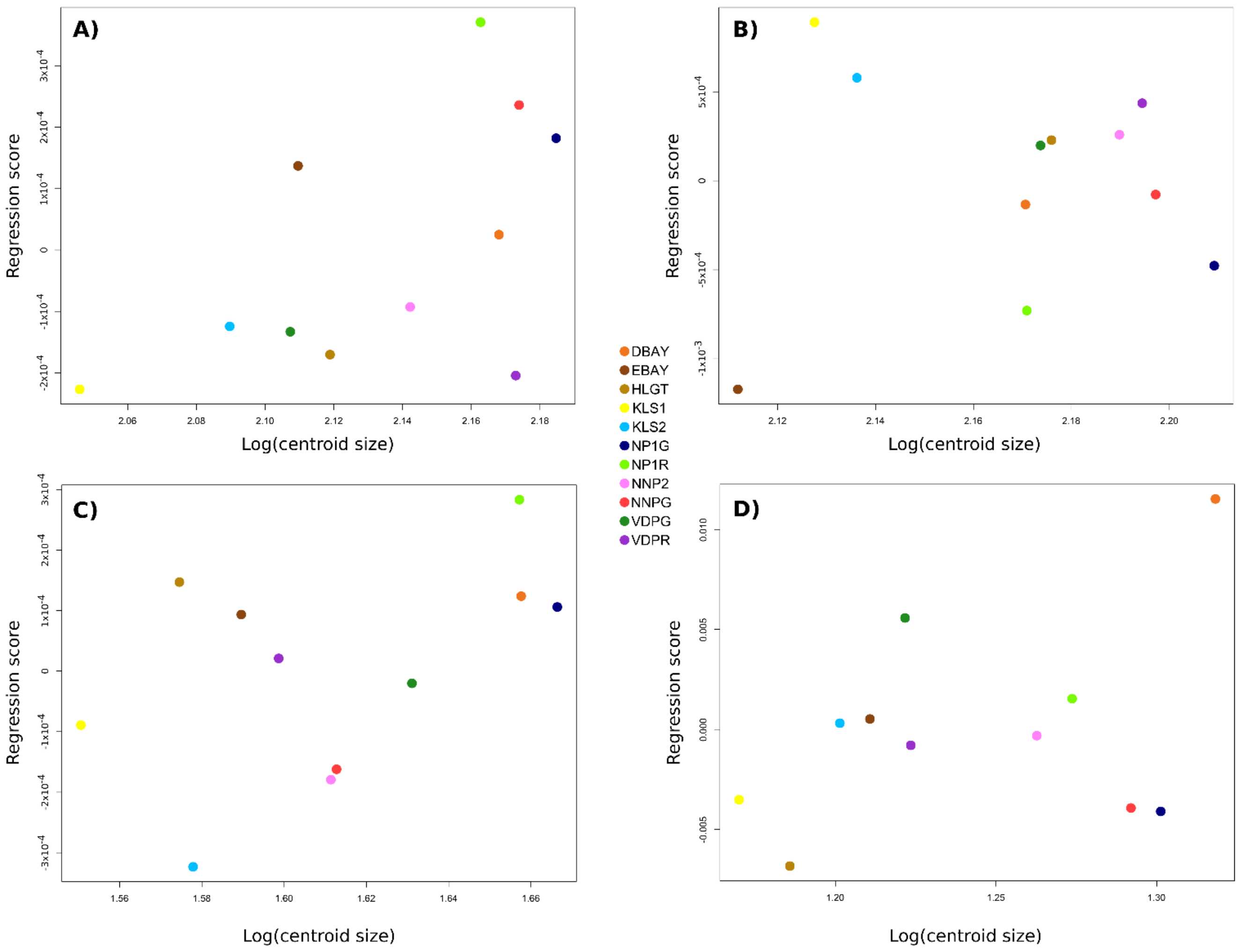

Figure 4.6. Visualisations of the evolutionary shape-size allometric relationships of: A) femur; B) tibia; C) tarsus; and D) claw. Regression scores are plotted against log(centroid size). Regression scores are standardised shape scores calculated from the regression of shape on centroid size and indicate the amount of shape change associated with change in centroid size. See Table 4.6 for summaries of evolutionary allometry models.

Table 4.6. ANOVA tables of Procrustes phylogenetic least-squares (PGLS) linear regression models of evolutionary shape-size allometry for each leg segment. Response variable is the mean leg segment shape of each population. CS = mean centroid size of each population. Z-score = multivariate effect size calculated as the difference between the observed F-value and the expected value from an empirically derived sampling distribution of F-values over 1000 permutations, divided by the standard deviation of the sampling distribution. *P* = probability of obtaining an F-value larger than that observed.

Leg segment	Predictor	DF	SS	MS	R^2	F	Z-score	*P*
Femur	Log(CS)	1	0.00582	0.00582	0.0142	0.130	-1.441	0.928
	Residuals	9	0.404	0.0449	0.986			
	Total	10	0.410					
Tibia	Log(CS)	1	0.00518	0.00518	0.0279	0.258	-1.0888	0.860
	Residuals	9	0.180	0.0200	0.972			
	Total	10	0.185					
Tarsus	Log(CS)	1	0.00563	0.00563	0.0490	0.464	-0.805	0.783
	Residuals	9	0.109	0.0121	0.951			
	Total	10	0.115					
Claw	Log(CS)	1	0.127	0.127	0.264	3.220	1.382	0.079
	Residuals	9	0.356	0.0395	0.736			
	Total	10	0.483					

Table 4.7. Phylogenetic signal of shape and size variation of leg segments among populations. *K* refers to Blomberg's *K* for univariate (size) data and to the multivariate generalisation of Blomberg's *K* for multivariate (shape) data (Adams 2014). Z-score = multivariate effect size calculated as the difference between the observed *K* and the expected value from an empirically derived sampling distribution of *K* values over 1000 permutations, divided by the standard deviation of the sampling distribution.

Leg segment	Variable	*K*	*P*	Z-score
Femur	Shape	1.472	0.003	4.831
	Size	0.867	0.308	0.315
Tibia	Shape	1.697	0.001	5.916
	Size	0.799	0.532	-0.277
Tarsus	Shape	0.971	0.049	1.936
	Size	0.728	0.858	-0.930
Claw	Shape	0.927	0.162	1.00540
	Size	0.796	0.528	-0.293

4.5 DISCUSSION

In this chapter I aimed to assess the extent to which evolutionary constraints may have limited the diversification of animal weaponry in the context of the ‘divergent fighting substrates’ hypothesis, as proposed by McCullough et al. (2016). To achieve this aim, I analysed static and evolutionary patterns of male weapon trait variation and covariation in the sexually dimorphic species complex *S. trimeni*. Males engage in intrasexual contests for mating access to females, using their hind legs as weapons, on disc-shaped flowers. My findings in Chapter 3 show that there is considerable variation in the flower contest substrate and the developmental environment among populations, and that male hind legs have diversified in shape and size across these diverse environments. Here, my results indicate that monkey beetle weapons are relatively unconstrained in their evolutionary diversification. Notably, they exhibit largely independent shape and size variation, while in several other systems, multivariate allometric associations between shape and size account for considerable amounts of trait form variation, for example in the eye stalks of stalk-eyed flies (Worthington et al. 2012), and vertebrate skulls (Cardini et al. 2015). My findings suggest that insect weaponry may exhibit an evolutionarily labile response to variation in the contest substrate and/or developmental environment. Work on *Onthophagus taurus* shows that variation in the developmental environment can underpin variation in competitive traits, even in the absence of variation in the contest environment (Rohner and Moczek 2020). These findings have important implications for hypotheses concerning the role of sexual selection in driving taxonomic diversification and speciation in heterogenous developmental and contest environments (Ingleby et al. 2010; Miller and Svensson 2014).

Patterns of modularity and integration

In a morphological context, integration refers to covariation among traits. Clustered groups of highly integrated traits are called modules. Morphological modularity is the presence of several modules, each exhibiting strong internal integration but weak integration with other such modules (von Dassow and Munro 1999; Wagner et al. 2007; Klingenberg 2014). Patterns of integration and modularity are often studied at the same levels for which allometric relationships are quantified: i.e., ontogenetic, static, and evolutionary (Schlosser and Wagner 2004; Klingenberg and Marugán-Lobón 2013). My findings suggest that the four hind leg segments studied here did not vary in shape independently of one another. Rather, femur covaried with tibia, and tarsus covaried with claw. This two-module pattern of modularity was supported at both static and evolutionary levels. This conserved pattern of modularity may indicate a common functional purpose of the leg segments within each module and/or may arise from the proximodistal patterning of the leg during development (Klingenberg 2005). Observations of intrasexual male contests in monkey beetles suggest that clamping and levering actions, both of which require the simultaneous manoeuvring of multiple hind leg segments, may be common in male-male contests in other monkey beetles (Colville et al. 2018; Rink et al. 2019). Similar behaviour is seen in intrasexual contests in male coreid bugs such as *Acanthocephala declivis*, where a contestant may clamp an opponent's body between his tibia and femora and then lift his opponent off the substrate before dropping him (Miyatake 1997; Eberhard 1998; O'Brien et al. 2018). Levering is also commonly used in horned beetles (Eberhard 1980; Cummings et al. 2018; Buchalski et al. 2019). Developmentally, these modules may be a consequence of the proximodistal gradient of gene expression during leg development in metamorphosis. Research on proximodistal leg patterning in *Drosophila* shows that the tarsus, pretarsus, and distal region of the tibia arise from cells in which the highest expression of the gene *Distal-less* (*Dll*) occurs, while proximal leg segments (the coxa, trochanter, femur, and proximal portion of the tibia) are characterised by low expression of *Dll* but high expression of *homothorax* (*hth*) and *dachshund* (*dac*) (Kojima 2004; Estella et al. 2012). This scheme of leg segment differentiation during development appears to be highly conserved across insects and arthropods in general (Kojima 2004; Moczek and Rose 2009), even underlying horn development in *Onthophagus* beetles (Moczek 2005; Moczek et al. 2006; Moczek and Rose 2009). The low levels of static integration found here between hind leg modules in male *S. trimeni* indicate that they vary in shape relatively independently of one another amongst individuals, possibly due to a lack of developmental, functional, or genetic integration (Klingenberg 2014). Being unconstrained by these factors may, for example, facilitate phenotypic plasticity in hind leg shape through independent responses of each module to the developmental environment (Pigliucci 2003). Additionally, the low level of evolutionary integration between these modules suggests that they can follow independent trajectories of evolutionary diversification (Klingenberg et al. 2001; Emlen et al. 2005; Klingenberg 2005). Most of the current research on morphological integration has focused on integration within vertebrate crania (e.g., Singh et al. 2012; Urošević et al. 2013; Felice et al. 2019); but some studies have investigated morphological integration in invertebrates in the context of sexually selected traits. For example, Tomkins et al. (2005) found that developmental integration between sexually selected traits and nonsexual traits was associated with morphological trade-offs in the dung beetle *Onthophagus taurus* and with concomitant enlargement of supporting traits with weapon size in the earwig *Forficula auricularia*. The similarity between evolutionary and static modularity in *S. trimeni* suggests that this pattern of modularity is evolutionarily conserved. This may constrain the independent evolution of the leg segments within each module, but strong modularity does not imply that evolutionary diversification necessarily be constrained by limited independent variation within module components. Modularity may facilitate evolution, and even diversification, by providing an evolutionary line of least resistance (Hedrick et al. 2019). In *S. trimeni*, all hind leg segment shapes differ significantly among populations; however, in neither module does shape variation exhibit significant phylogenetic signal. These results suggest that modularity has neither constrained nor facilitated male hind leg segment shape diversification within the *S. trimeni* complex and has likely had little influence on the evolutionary response of male weaponry to variation in the contest substrate and developmental environment.

Allometric constraints

For all hind leg segments, I did not find evidence for significant differences in allometric slopes among populations but did obtain support for significant differences in allometric intercepts among populations. Allometric slopes are shown to be evolvable on macroevolutionary timescales (Voje and Hansen 2013; Voje et al. 2014) but conserved over microevolutionary timescales, suggesting that allometric slope can function as an evolutionary constraint over shorter timescales (Egset et al. 2012; Pélabon et al. 2014). My findings support common allometry, which accounts for differences among population in terms of mean shape at mean size (allometric intercept) but not the rate at which size change is associated with shape change (allometric slope). The support for a shared allometric slope (common allometry) suggests that allometric scaling relationships are conserved within *S. trimeni*, although the low amounts of shape variation explained by size suggest that shape and size vary relatively independently of one another among populations. Analysis of shape-size scaling relationships within a phylogenetic context showed that evolutionary shape variation was poorly explained by size variation in all hind leg segments, excluding the claw, for which > 25% of evolutionary shape variation was explained by size. The large proportion of evolutionary variation in hind claw shape associated with size variation suggests that evolutionary diversification of shape in this leg segment may be constrained by size. The hypothesis of "allometry-as-constraint" was proposed with narrow-sense allometry in mind – the scaling relationships between trait size and body size (Huxley 1932; Simpson 1944; Gould 1971). It is unclear whether it is valid to study this hypothesis in the context of multivariate allometry, which examines the scaling relationships of multivariate traits, such as shape, with size (Pélabon et al. 2014; Voje et al. 2014). Nevertheless, shape-size allometric scaling relationships are commonly examined in geometric morphometric studies of trait evolution and undoubtedly are informative of evolutionary constraints in that, like traditional allometry, they quantify constraints on morphological variation due to size variation (Klingenberg 2016; Sansalone et al. 2018; Delgado et al. 2019). My findings suggest that the evolutionary response of male hind leg segment shape to variation in selection pressure is generally unconstrained by allometry.

Phylogenetic constraints

I found that male *S. trimeni* hind leg segment shapes and sizes were generally as diverse as expected under an assumption of trait evolution by Brownian motion. Femur and tibia shape were exceptions, as both exhibited higher-than-expected phylogenetic signal, indicating that closely related populations have more similar femur and tibia shapes than expected under a Brownian motion model of trait evolution. This result suggests that the rate of evolution of femur and tibia shape *may* be constrained, although there is a complex relationship between phylogenetic signal and evolutionary rate and higher-than-expected phylogenetic signal should not be automatically interpreted as reflecting low evolutionary rate (Revell et al. 2008). Given that neither of these two hind leg segments showed strong covariation of shape with size, allometry can be excluded as a source of evolutionary constraint. These hind leg segments may be evolutionarily constrained by some other factor, such as genetic constraints (Hansen 2015). Genetic constraints arising from intralocus sexual conflict may impede the response of exaggerated male traits to sexual selection pressure (Arnqvist and Rowe 2005; Morris et al. 2013). Alternatively, these hind leg segments may already be optimally adapted to the contest substrate and are under stabilising selection (Lande 1980), which has been demonstrated in several insect taxa (Brooks et al. 2005; Robson and Gwynne 2010; O’Brien et al. 2017). Additionally, integration between femur and tibia may have acted as an evolutionary constraint on their shape diversification. In the forelimbs of musteloid carnivorans, bones that comprise functional units exhibit stronger integration, facilitating their coordinated evolution (Fabre et al. 2014). Similarly, male *S. trimeni* hind femurs and tibias may be evolutionarily constrained by their shared functionality as weapon components. In contrast to the strong phylogenetic signal exhibited by femur and tibia shape, the hind leg segments comprising the second module, i.e., the tarsus and tarsal claw, varied in shape as much as expected given the phylogeny and assuming Brownian motion trait evolution. This finding suggests that this second module has not been evolutionarily constrained by the strong integration between its constituent leg segments, and therefore that tarsus and tarsal claw should be able to respond to selection independently of one another. The two morphological modules supported by the best-fitting hypothesis of modularity thus exhibit divergent patterns of diversification along the phylogeny, likely facilitated by the low integration between them. However, the reasons for this disparity in phylogenetic signal are unclear. To tease apart the sources of constraints on shape evolution within each module to understand why they exhibit differing amounts of phylogenetic signal, future work should include quantification of the genetic architecture underlying hind leg segment covariation. It is important to identify the genetic effects that influence sexually selected trait variation because genotype architecture mediates the phenotypic expression of the genotype and thus influences the amount of standing variation on which selection can act (Hansen 2006; Chenoweth and McGuigan 2010). Specifically, pleiotropic effects are important drivers of patterns of modularity and integration and understanding the extent of pleiotropy in monkey beetle hind limbs will provide insights into the extent to which independent evolution of different aspects of monkey beetle weapons are impacted by genetic linkages (Hansen 2003; Chenoweth and McGuigan 2010).

Conclusion

Evolutionary variation in male *S. trimeni* hind legs appears to be characterised by the presence of two well-supported modular components and a marked lack of allometric constraint. Overall hind leg shape and size are as diverse among populations as expected under a simple model of trait evolution. These findings suggest that male *S. trimeni* should be able to exhibit relatively independent evolution of weapon shape from weapon size. Evolutionary variation in *S. trimeni* weapon shape may be influenced by the patterns of integration and modularity supported for the hind leg segments studied here. The relative independence between shape and size, and between the distal and proximal hind leg segments, may enable them to respond flexibly to variation in the contest substrate and developmental environment, but further work is needed to understand the shared genetic, developmental, and functional pathways underpinning the patterns of modularity found here. Overall, the present findings suggest that there is scope for male insect weapons to diversify adaptively in response to variation in the contest substrate and developmental environment, even at the microevolutionary level, but that their evolutionary lability may be subject to several sources of constraints influencing the direction and tempo of their diversification.

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4.7 APPENDIX

Comparisons of modular hypotheses

Table A1. Comparison of modular signal observed under seven alternative hypotheses of modularity (null hypothesis = no modularity present). CR = covariance ratio, Z_{CR} = multivariate effect size, *P* = probability of obtaining a CR coefficient greater than that observed.

Alternative hypothesis	Modularity		
	CR	Z_{CR}	*P*
H1	0.255	-19.366	0.001
H2	0.170	-24.780	0.001
H3	0.140	-21.157	0.001
H4	0.180	-22.785	0.001
H5	0.195	-21.537	0.001
H6	0.240	-21.237	0.001
H7	0.127	-23.043	0.001

Table A2. Z_{CR} associated with pairwise differences among all hypotheses, including the null hypothesis. Hypothesis H2 is the best-performing hypothesis. The difference in modular signal between H2 and the null hypothesis is significant and is associated with the largest effect size of all alternative hypotheses (Z_{CR} of 40.042, *P* < 0.001; Table A3).

	H_{NULL}	H1	H2	H3	H4	H5	H6	H7
H_{NULL}	0.000							
H1	24.555	0.000						
H2	40.042	2.250	0.000					
H3	28.544	2.669	0.862	0.000				
H4	32.960	2.111	0.017	0.789	0.000			
H5	29.596	1.480	0.648	1.321	0.613	0.000		
H6	28.741	0.364	2.025	2.482	1.878	1.203	0.000	
H7	33.756	3.220	1.384	0.361	1.255	1.808	3.075	0.000

Table A3. P-values associated with pairwise differences among all candidate hypotheses, including H_{NULL}. While all alternative hypotheses fit the observed pattern of modularity significantly better than the null hypothesis of no modularity, H2 produced the smallest effect size (i.e., strongest signal of modularity; Table A1).

	H_{NULL}	H1	H2	H3	H4	H5	H6	H7
H_{NULL}	1.000							
H1	0.001	1.000						
H2	0.001	0.024	1.000					
H3	0.001	0.008	0.388	1.000				
H4	0.001	0.035	0.986	0.430	1.000			
H5	0.001	0.139	0.517	0.186	0.540	1.000		
H6	0.001	0.716	0.043	0.013	0.060	0.229	1.000	
H7	0.001	0.001	0.166	0.718	0.209	0.071	0.002	1.000

Comparisons of modularity among populations

Table A4. Z_{CR} associated with pairwise differences in modularity among all populations.

	DBAY	EBAY	HLGT	KLS1	KLS2	NNP2	NNPG	NP1G	NP1R	VDPG	VDPR
DBAY	0.000										
EBAY	4.565	0.000									
HLGT	1.613	4.482	0.000								
KLS1	0.961	5.490	1.237	0.000							
KLS2	6.027	1.806	6.457	7.471	0.000						
NNP2	0.848	7.041	3.773	2.900	8.802	0.000					
NNPG	0.795	6.310	3.171	2.405	7.944	0.018	0.000				
NP1G	2.116	3.559	0.906	2.058	5.482	4.292	3.687	0.000			
NP1R	7.071	2.372	9.092	10.788	0.169	11.667	9.901	7.433	0.000		
VDPG	0.212	5.000	1.985	1.305	6.505	0.624	0.586	2.497	7.731	0.000	
VDPR	13.883	10.747	19.124	21.037	8.368	20.634	17.920	16.967	10.524	14.833	0.000

Table A5. P-values associated with pairwise differences in modularity among populations.

	DBAY	EBAY	HLGT	KLS1	KLS2	NNP2	NNPG	NP1G	NP1R	VDPG	VDPR
DBAY	1.000										
EBAY	0.001	1.000									
HLGT	0.107	0.001	1.000								
KLS1	0.336	0.001	0.216	1.000							
KLS2	0.001	0.071	0.001	0.001	1.000						
NNP2	0.398	0.001	0.001	0.004	0.001	1.000					
NNPG	0.427	0.001	0.002	0.016	0.001	0.987	1.000				
NP1G	0.034	0.001	0.365	0.040	0.001	0.001	0.001	1.000			
NP1R	0.001	0.017	0.001	0.001	0.866	0.001	0.001	0.001	1.000		
VDPG	0.832	0.001	0.047	0.192	0.001	0.532	0.576	0.0125	0.001	1.000	
VDPR	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.000

Comparison of integration among populations

Table A6. Z-scores associated with pairwise differences in integration between modules, as defined in H2, among populations.

	DBAY	EBAY	HLGT	KLS1	KLS2	NNP2	NNPG	NP1G	NP1R	VDPG	VDPR
DBAY	0.000										
EBAY	1.364	0.000									
HLGT	0.120	1.141	0.000								
KLS1	0.965	0.505	0.750	0.000							
KLS2	1.858	0.404	1.578	0.974	0.000						
NNP2	0.418	1.766	0.500	1.408	2.284	0.000					
NNPG	1.068	2.291	1.096	1.997	2.787	0.688	0.000				
NP1G	0.558	1.707	0.624	1.381	2.132	0.209	0.391	0.000			
NP1R	0.053	1.115	0.144	0.777	1.476	0.263	0.784	0.404	0.000		
VDPG	0.075	1.266	0.049	0.864	1.743	0.484	1.117	0.612	0.110	0.000	
VDPR	<0.001	0.782	0.070	0.516	1.032	0.228	0.615	0.342	0.035	0.042	0.000

Table A7. P-values associated with pairwise differences in integration between modules, as defined in H2, among populations.

	DBAY	EBAY	HLGT	KLS1	KLS2	NNP2	NNPG	NP1G	NP1R	VDPG	VDPR
DBAY	1.000										
EBAY	0.172	1.000									
HLGT	0.905	0.254	1.000								
KLS1	0.334	0.614	0.453	1.000							
KLS2	0.063	0.686	0.115	0.330	1.000						
NNP2	0.676	0.077	0.617	0.159	0.022	1.000					
NNPG	0.285	0.022	0.273	0.046	0.005	0.492	1.000				
NP1G	0.577	0.088	0.532	0.167	0.033	0.834	0.695	1.000			
NP1R	0.958	0.265	0.885	0.437	0.140	0.792	0.433	0.686	1.000		
VDPG	0.940	0.205	0.961	0.387	0.081	0.629	0.264	0.540	0.912	1.000	
VDPR	1.000	0.434	0.944	0.605	0.302	0.820	0.539	0.732	0.972	0.967	1.000

Static allometry

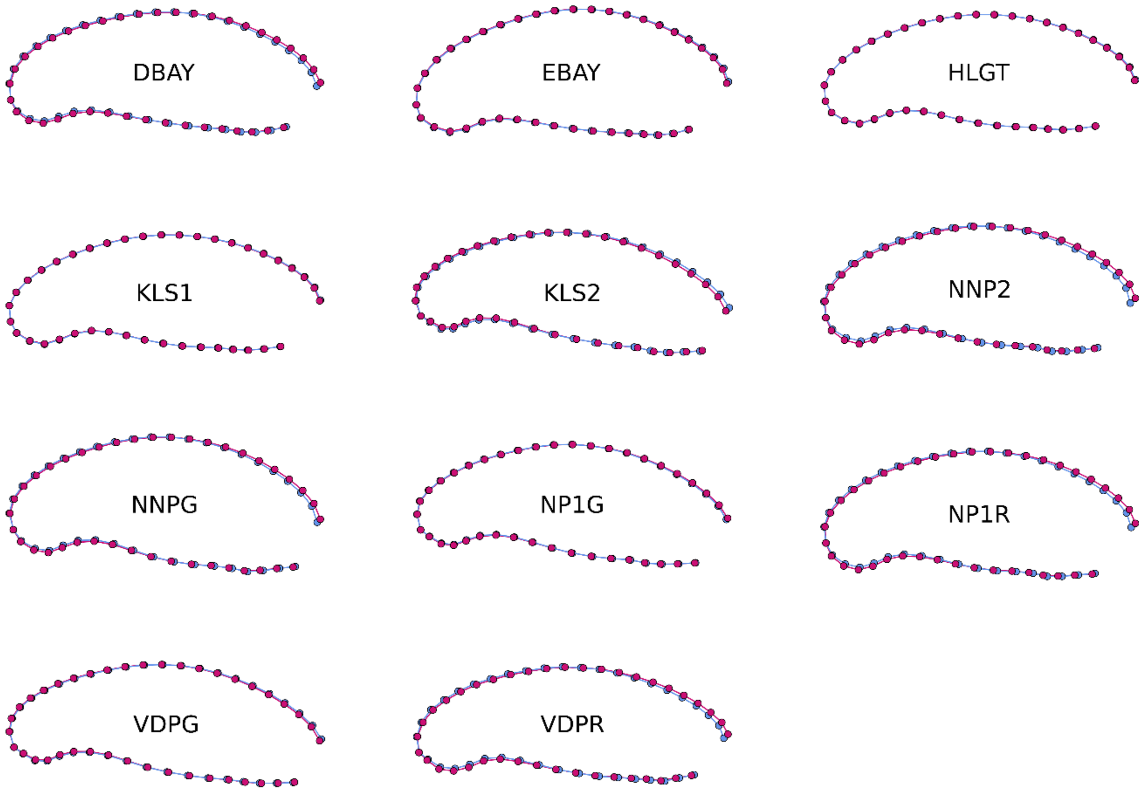

Figure A1. Least-squares mean shapes of femurs in each population. Dots represent landmarks and are connected by lines of the same colour to indicate landmark relationships. Pink landmarks and lines represent LS means, while blue landmarks and lines represent the mean shape before correction for allometry. Greater difference between the two configurations indicates a greater amount of shape covariation with size.

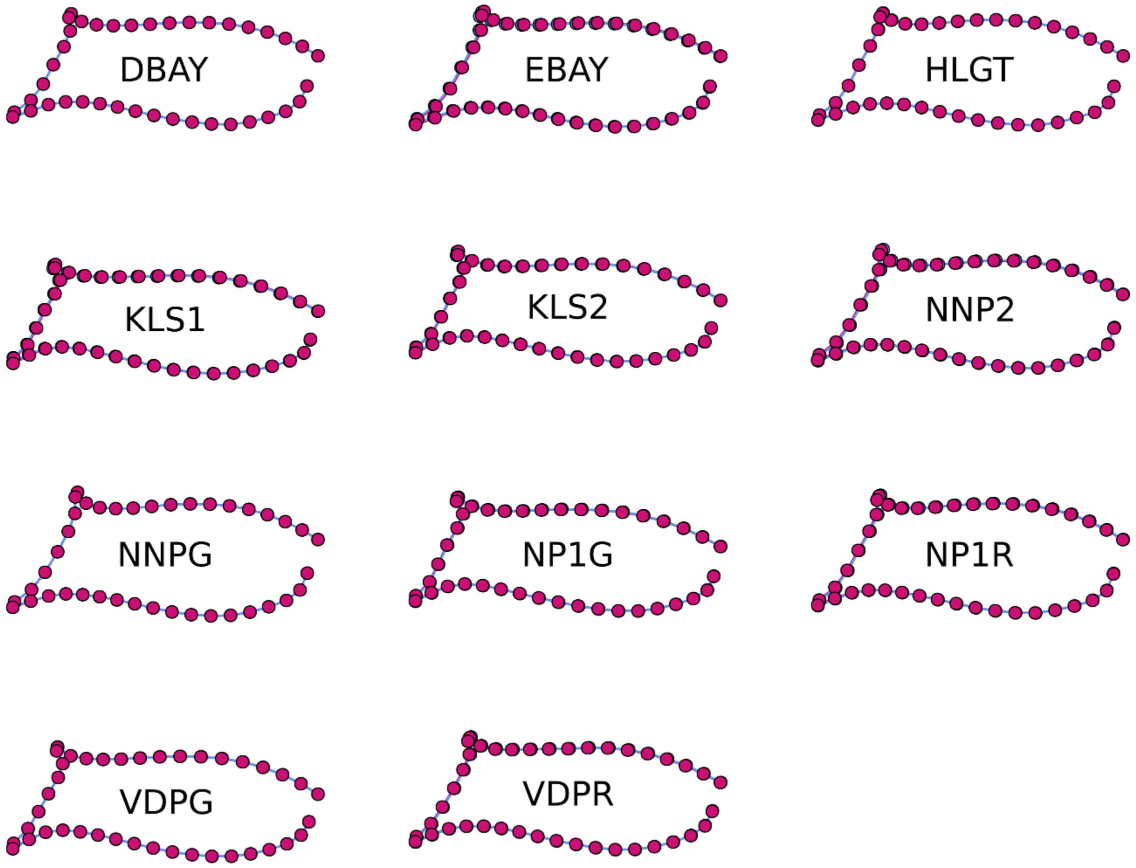

Figure A2. Least-squares mean shapes of tibias in each population. Dots represent landmarks and are connected by lines of the same colour to indicate landmark relationships. Pink landmarks and lines represent LS means, while blue landmarks and lines represent the mean shape before correction for allometry. Greater difference between the two configurations indicates a greater amount of shape covariation with size.

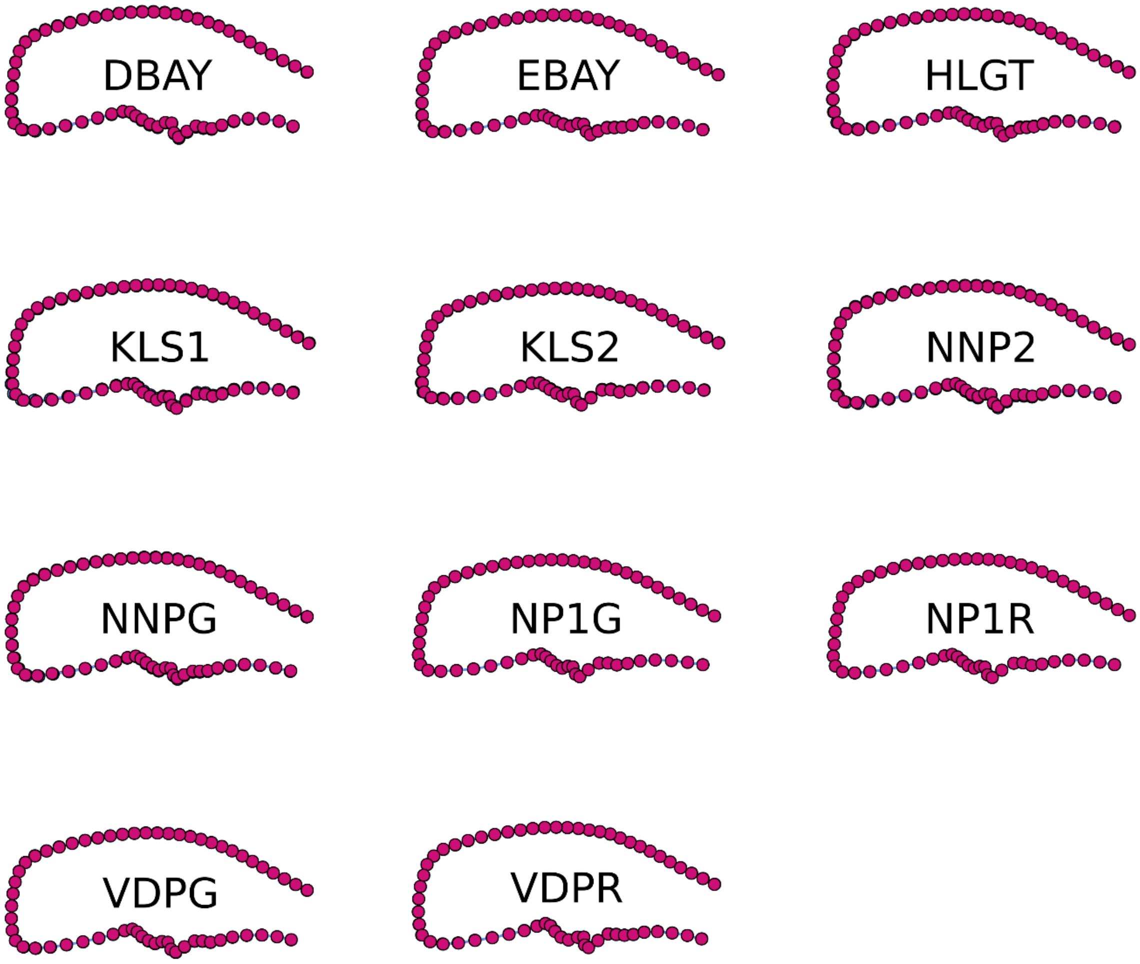

Figure A3. Least-squares mean shapes of tarsi in each population. Dots represent landmarks and are connected by lines of the same colour to indicate landmark relationships. Pink landmarks and lines represent LS means, while blue landmarks and lines represent the mean shape before correction for allometry. Greater difference between the two configurations indicates a greater amount of shape covariation with size.

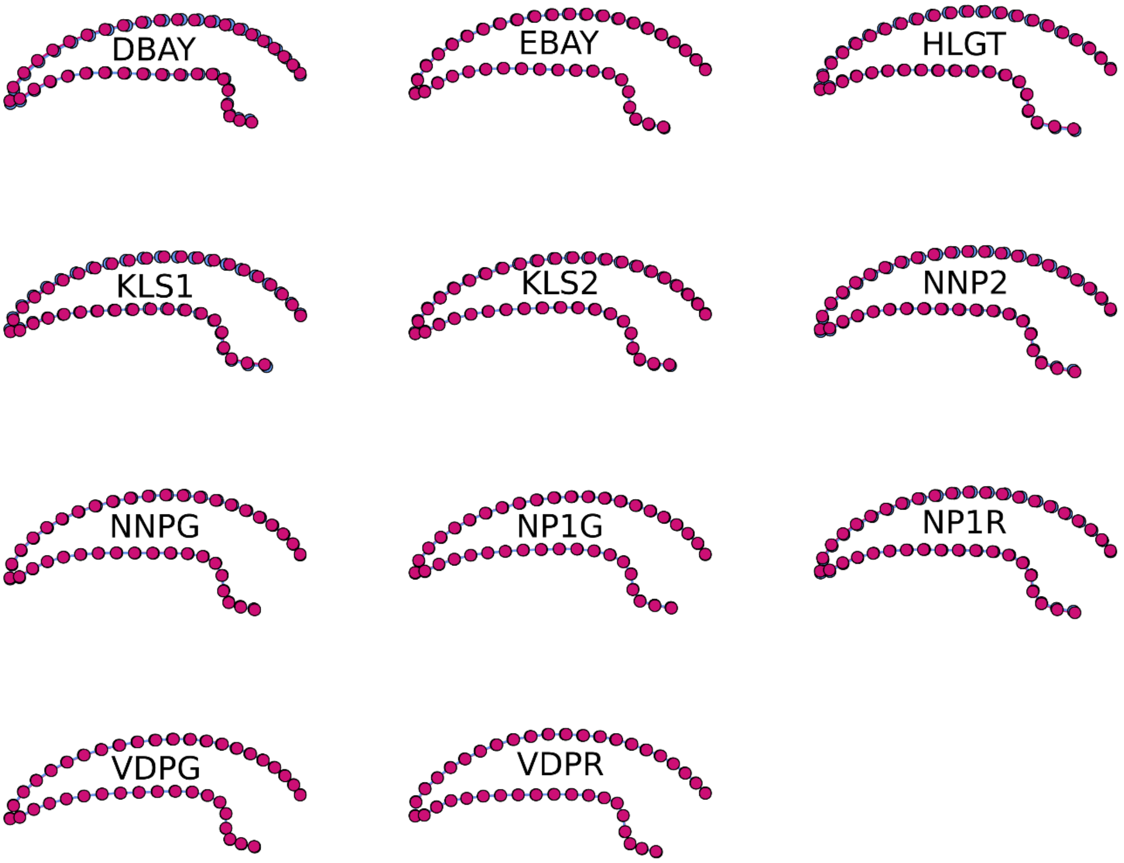

Figure A4. Least-squares mean shapes of claws in each population. Dots represent landmarks and are connected by lines of the same colour to indicate landmark relationships. Pink landmarks and lines represent LS means, while blue landmarks and lines represent the mean shape before correction for allometry. Greater difference between the two configurations indicates a greater amount of shape covariation with size.

5. CONCLUDING CHAPTER

5.1 BACKGROUND

Darwin recognised that biodiversity is driven by two sources of selection: natural selection that favours traits that maximise survival and sexual selection for traits that increase reproductive success through competition for mates (Darwin 1859; Darwin 1871; Lyon and Montgomerie 2012). Habitat heterogeneity can mediate variation in the intensity of competition for mates (Todd and Miller 1997; Panhuis et al. 2001; Svensson and Gosden 2007; Maan and Seehausen 2011; Maia et al. 2013; Servedio and Boughman 2017), influencing the strength and direction of sexual selection and potentially driving taxonomic diversification and speciation (Miller and Svensson 2014). For example, there is strong evidence that the rapid radiations of African cichlids have been driven by variation in female choice for visual male ornaments in heterogeneous lake environments, facilitating assortative mating despite gene flow between sympatric taxa (Wilson et al. 2000; Gonzalez-Voyer and Kolm 2011; Maan and Sefc 2013). Work on several animal groups provides evidence in support of sexual selection as a driver of diversification when acting in conjunction with natural selection (e.g., Scordato et al. 2014; Bay et al. 2017; van Rijssel et al. 2018; Scordato 2018). However, the evolutionary importance of sexual selection has overwhelmingly been studied in the context of mate choice, and only recently has research expanded to explore the mechanisms by which intrasexual contest competition may drive evolutionary diversification and speciation (e.g., Emlen and Nijhout 2000; Rutte et al. 2006; Arnott and Elwood 2008; Emlen 2008; Hunt et al. 2009; Qvarnström et al. 2012; Lackey and Boughman 2013; Miller 2013; Mesterton-Gibbons and Heap 2014; McCullough et al. 2016; Boughman 2018; Dijkstra and Border 2018; Tinghitella et al. 2018; Rico-Guevara and Hurme 2019).

In **Chapter 1** I summarised the hypotheses that have recently been proposed concerning the mechanisms through which intrasexual contest competition may lead to weapon divergence, assortative mating, reproductive isolation, and ultimately, speciation (Qvarnström et al. 2012;

McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018). These hypotheses were developed to provide a sound theoretical framework within which future work could address questions concerning the role of male-male competition in influencing the speciation process (Lackey et al. 2018). Despite the provision of this framework, the few studies that have investigated the role of intrasexual contest competition as an evolutionary mechanism have examined systems in which female choice occurs in addition to male-male competition (Martin and Mendelson 2016a, Martin and Mendelson 2016b; Bierbach et al. 2018; but see Yang et al. 2020). Thus, our understanding of intrasexual contest competition as an evolutionary mechanism in the absence of female choice remains in need of research attention.

5.2 AIMS AND METHODS

In this thesis I aimed to assess the importance of sexual selection as a driver of microevolutionary weapon diversification in monkey beetles. In **Chapter 1** I provided a synthesis of sexual selection research, focussing on intrasexual contest competition, and introduced my study system in this context and put forward several aspects that make monkey beetles a model system in this regard. In **Chapter 2**, using the sexually dimorphic monkey beetle *Heterochelus chiragricus* as a model species, I aimed to verify the occurrence of intrasexual contest competition, to establish the determinants of RHP, and to identify the competitive assessment strategy used by male monkey beetles. In the following two chapters I examined several widely dispersed populations of the cryptic species complex *Scelophysa trimeni*. In **Chapter 3** I examined the evidence for a role of sexual selection in driving microevolutionary weapon divergence across gradients of male contest substrate variability and in the context of abiotic environmental variation, while accounting for gene flow across distance and the relatedness among populations. In **Chapter 4**, I quantified patterns of male hind leg variation and covariation to assess the potential of evolutionary constraints to limit competitive trait divergence along selection gradients. In this **Concluding Chapter** I synthesize the findings from the data reported in **Chapters 2 - 4** and suggest directions for future research.

5.3 KEY FINDINGS

The nature of male-male contest competition in monkey beetles

The South African monkey beetle fauna was first described in detail by Péringuey (1902) at the turn of the last century. The function of the exaggerated hind legs present in several Hopliine taxa was long misattributed to the feeding behaviour of the embedding guild (Péringuey 1902; Scholtz and Holm 1985). It was not until Midgley (1992) suggested a combatant function of males' hind legs that a role of sexual selection in influencing the diversification of the sexually dimorphic taxa was recognised. Further work by Colville (2009) and Colville et al. (2018) quantified patterns of hind leg and colour sexual dimorphism across the Hopliini and reiterated the potential for a role of sexual selection, over and above that of natural selection, in driving phenotypic and taxonomic diversity in this group. The research presented in **Chapter 2** (Rink et al. 2019) of my thesis constitutes the first comprehensive examination of sexual selection in monkey beetles, not only quantifying sexual dimorphism with respect to hind leg size and allometry, but also determining the correlates of contest success, possible means of contest assessment, and the role of residency. This system exhibits the three prerequisites for the occurrence of animal competition, as defined by Emlen (2014): females were a scarce resource (the sex-ratio was significantly male-skewed), they were spatially restricted and easily defended (being restricted to flower heads and sedentary), and males fought in one-on-one contests for mating access to females (a resident male mate-guarding or *in copula* with a female would be approached by a single intruding male). My findings demonstrated significant sexual dimorphism in *H. chiragricus* hind leg size and allometry. Moreover, hind leg traits influenced males' competitive success, with males bearing larger hind femurs significantly more likely to win contests. Residency effects were also important, allowing males in prior possession of a female to hold a competitive advantage over intruders (see supplementary video of males fighting). Enlarged hind legs are used in male-male contest competition by several insect taxa and are especially prevalent in the coreid bugs (Mitchell 1980; Miyatake 1997; Eberhard 1998).

They are unusual, however, in the scarab beetles, which, in the combatant species, are typically characterised by myriad cephalic and thoracic horns (Emlen et al. 2005; Moczek 2005). Monkey beetle weaponry is thus unusual in the Scarabaeidae, but parallels can be drawn with the use of weaponised hind legs in chrysomelid beetles (Eberhard and Marin 1996; Katsuki et al. 2014; O'Brien et al. 2017), and coreid bugs (Miyatake 1997; Eberhard 1998; Somjee et al. 2015; Miller et al. 2016), all of which exhibit sexual dimorphism of hind leg morphology and the use of males' enlarged hind legs as weapons during intrasexual contest competition for reproductive access to females. I found that residency effects biased contest outcome in the favour of the resident male in *H. chiragricus*. Residency effects are widespread where males compete for females and may even outweigh RHP in determining contest success, as for contests between male jumping spiders *Phidipus clarus* (Kasumovic et al. 2011). Males may be resident because they are *in copula* with the female, or because they are mate-guarding, a behaviour that is motivated by sperm competition (Harts and Kokko 2013). Mate guarding duration is proportional to the male bias of the sex ratio (Berger-Tal and Lubin 2011; Weir et al. 2011), and in Japanese beetles *Popillia japonica* (Scarabaeidae), the duration of mate-guarding increases with female size, suggesting post-copulatory sperm competition (Saeki et al. 2005).

Overall, my **Chapter 2** (Rink et al. 2019) findings confirm that sexual selection, in the form of intrasexual male contest competition, does occur in hind leg dimorphic monkey beetles, and is probably driven by the significantly male-skewed sex ratio in monkey beetles (Louw 1987; Midgley 1992; Colville et al. 2018; Rink et al. 2019) and the spatially clumped, sedentary females that are easily defended by males. I proceed, in **Chapter 3**, to study the variation of male competitive traits along environmental gradients. Specifically, I examine correlations between male traits and the developmental environment and contest substrate (*sensu* McCullough et al. 2016).

Support for the 'divergent fighting contexts' hypothesis of diversification by intrasexual contest competition

The potential of intrasexual male competition to drive evolutionary processes has long been a topic of contention, with the prevailing view of the past being that divergence in female choice was a prerequisite for the development of reproductive isolation, which is required under the ecological species definition. The surge of interest in intrasexual contest competition as an evolutionary driver has produced a number of hypotheses outlining scenarios in which intrasexual male competition might lead to divergence, and possibly speciation, in sympatry and allopatry, in combination with input from female choice and natural selection, and alone (Qvarnström et al. 2012; McCullough et al. 2016; Lackey et al. 2018; Tinghitella et al. 2018). The development of these hypotheses was hoped to spur a new wave of empirical research, especially on wild, naturally behaving populations, that would test these ideas and further the field, but to date few studies have sought to test these hypotheses (McCullough et al. 2016, Tinghitella et al. 2018). One promising hypothesis concerning the role of male competition in evolutionary diversification suggests that divergence in weaponry across gradients of contest substrate variation may be capable of enforcing reproductive isolation between locally adapted populations through selection for different fighting styles which in turn select for changes in weapon morphology to maximise style-specific competitive ability (McCullough et al. 2016). To my knowledge, this hypothesis has never been formally tested. In **Chapter 3** I tested this hypothesis in several, spatially distributed populations of the monkey beetle species complex *Scelophysa trimeni*. As in *H. chiragricus*, male *S. trimeni* compete on flower heads, using their hind legs as weapons, to win reproductive access to sedentary females. I found that divergence of male *S. trimeni* weapon size and elytral colour have occurred along gradients of flower size and colour variation, suggesting that variation in the contest substrate and signalling background may have mediated selection for diverging fighting styles and signalling traits across the eleven populations that I studied. In rhinoceros beetles, horns are structurally adapted to the typical forces experienced during contests using the fighting style exhibited by each species but are more likely to fracture when subjected to different forces, as expected if using a fighting style maladapted to the fighting substrate (McCullough et al. 2015). These findings suggest that fighting style selects for horn morphology in this group. If the optimal fighting style varies across fighting substrates, then horn morphology may be under selection to maximise performance in different contest contexts (McCullough et al. 2016). For bovids, Lundrigan (1996) found correlations between fighting style and weapon morphology, suggesting that the direction of selection on bovid horn shape is related to fighting behaviour.

Mean male UV chroma was consistently greater than petal UV chroma in all populations (Fig. 3.20, **Chapter 3**). This is consistent with the patterns expected for traits that function as signals of fitness or quality to conspecifics and thus need to be visible against the signalling background (Miller and Svensson 2014; Wellenreuther et al. 2014), as in female mantids (White et al. 2014), male dewlap colouration in male West Indian *Anolis* lizards (Leal and Fleishman 2004), and male visual signalling in the wolf spider *Schizocosa ocreata* (Clark et al. 2011).

My findings in **Chapter 3** indicate that male colour in *S. trimeni* shows some degree of condition-dependence, as evidenced by its covariation with climatic and nutrient gradients, and therefore may function as an indicator of fitness (Berglund et al. 2013). Male *S. trimeni* colour also exhibits covariation with the flower substrate, suggesting that selection on male colour may be mediated by the signalling background to influence conspicuousness of males to potential competitors (e.g., Lynn and Cole 2019). *H. chiragricus* also exhibits pronounced sexual colour dimorphism, with males having high-contrast structurally based colouration (Fig. 2.1, **Chapter 2**; Rink et al. 2019), a phenomenon often associated with female choice (Keyser and Hill 1999; Endler and Day 2006; Lim and Li 2007). However, female choice is apparently absent in both of my study species (**Chapter 2**; Rink et al. 2019; pers. obs.), as appears to be the case for combatant monkey beetles in general (Louw 1987; Midgley 1992; Colville et al. 2018), suggesting that male monkey beetle colouration is not an ornament driven by female choice. Male colour can also function as a signal of resource-holding potential (RHP; Parker 1974) to competitors (Arnott and Elwood 2009), for example in territorial competition between male eastern bluebirds *Sialis* for nesting boxes (Siefferman and Hill 2005). The role of male colour in contest, and the accuracy and type of information it conveys to competitors, should be explored further in monkey beetles, perhaps in staged contests where male colour is experimentally manipulated to elucidate the role of male colouration in contests, as has been done in lizards (Stuart-Fox and Johnston 2005; Bajer et al. 2011). As of yet, no work has been done to assess whether cryptic female choice occurs in combatant monkey beetles. This topic deserves investigation as cryptic female choice can ignite sexually antagonistic coevolution through sexual conflict, driving evolutionary diversification (Eberhard 1991; Eberhard and Cordero 1995; Firman et al. 2017; Simmons 2017; see also section on sexual conflict below).

Sexually selected traits are known to exhibit heightened condition-dependence and phenotypic plasticity relative to non-sexually selected traits, as in the dung beetles *Onthophagus* (Moczek and Emlen 2000; Emlen et al. 2005), where males exhibit horn dimorphism contingent on their body size. My findings suggest that several aspects of the competitive phenotype in monkey beetles, namely weapon shape and the UV and blue components of their elytral colouration, are highly sensitive to nutrient availability and climate during larval development. This phenotypic variation among populations may reflect underlying genetic variation but may also be a consequence of phenotypic plasticity (Casasa and Moczek 2018; Miller and Svensson 2014). Heightened phenotypic plasticity is common in sexually selected traits and may increase the opportunity for sexual selection to drive the divergence of male competitive behaviour among populations experiencing different developmental environments (Moczek 2010).

In a broader definition of the contest environment that extends past the physical substrate and also encompasses the social environment (West-Eberhard 1983), variation in demographics, such as population density, affect the intensity of selection for alternate male phenotypes associated with alternate reproductive strategies, such as sneaking behaviours in small-horned male *Onthophagus* (Casasa and Moczek 2018).

The patterns of competitive trait variation among populations that I quantified for male *S. trimeni* are therefore reflective both of sexual selection for optimally performing weapons and conspicuous signals for different contest substrates and of phenotypic plasticity resulting from heterogeneity in the developmental environment in terms of nutrient availability, climate, and possibly demography.

These findings support the potential for evolutionary diversification in monkey beetles to have occurred through divergence in weaponry along variable contest substrates, and underscore the importance of natural selection, acting through variation in the developmental environment, in constraining the phenotypic variation of animal weapons within and among taxa. My findings provide a baseline for future studies of weapon variation in response to environmental heterogeneity at multiple levels: the contest substrate, the developmental environment, and the social environment.

Limited constraints on weapon evolution

In addition to external constraints on weapon form, such as the contest substrate (variation in which may be an evolutionary diversifying force), trait responses to selection pressures may be internally constrained, for example by developmental, functional, allometric, and phylogenetic effects (Breuker et al. 2006; Futuyma 2010; Voje et al. 2014; Hansen 2015; Bright et al. 2016). The implications of evolutionary constraints for diversification and speciation are well documented for non-sexually selected traits (e.g., Frankino et al. 2005; Young and Hallgrímsson 2005; Costello et al. 2008; Watanabe 2018), but the roles of evolutionary constraints in limiting or mediating the responses of sexually selected traits, specifically male weaponry, to selection pressures are largely unknown (Emlen and Nijhout 2000; Pélabon et al. 2014; Voje 2016). In **Chapter 4** I assessed the evidence for several evolutionary constraints to influence male weapon diversification within *S. trimeni*. The findings made in this chapter suggest that male hind legs are relatively unconstrained and thus evolutionarily labile in their responses to changes in selection pressure. The low amount of leg shape explained by leg size suggests that these two aspects of weapon form are able to respond independently to selection pressures, as was observed in **Chapter 3** where shape and size exhibited different levels of condition-dependence and different responses to variation in the contest substrate. This phenotypic plasticity, together with the modularity identified in **Chapter 4**, may have facilitated diversification of the sexually dimorphic monkey beetles along gradients of abiotic environmental variation and across spatially and temporally variable flower sizes, as seen for other scarab beetles (Moczek 2010; Sanger and Rajakumar 2019).

5.4 DIRECTIONS FOR FUTURE RESEARCH

Determinants of reproductive success

In **Chapter 2**, I quantified the relationship between weapon size and procurement of mating opportunities in *H. chiragricus*, by examining multiple males over a span of a few days. I did not control for adult age, nor measure variation in fitness over a male's lifetime. Monkey beetles have relatively short adulthoods, living above-ground for approximately one week after completing the soil-dwelling larval and pupal stages (Colville 2009). Fitness is expected to vary over an individual's lifetime, in response to various factors including internal energetic state (Kemp 2006). Male monkey beetle fitness may decline dramatically over their brief adulthood because agonistic encounters with other males may deplete their energy reserves and/or damage weaponry (Saeki et al. 2005; Lane and Briffa 2017). Energy may also be depleted by sperm production (Parker et al. 2012). The extensive periods of time that male monkey beetles spend mate-guarding (**Chapter 2**; Colville 2009; Karolyi et al. 2016; Colville et al. 2018; Rink et al. 2019), perhaps to reduce sperm competition (Elias et al. 2014), entail a trade-off between maximising past reproductive success against the cost of lost future mating opportunities (Harts and Kokko 2013). As adults age, they may adjust their reproductive strategy according to their fitness, and vary the time and energy spent on competing for additional females and sperm and ejaculate production, versus on mate-guarding

(Parker and Pizzari 2010; Parker et al. 2012). In hide beetles *Dermestes maculatus*, Jones et al. (2007) found that middle-aged males were generally more successful at inseminating females and tended to transfer more sperm than did younger and older males, suggesting a role of sexual maturation in enhancing mating success. A game theory-informed study of the relative importance of age, weapon performance, and reproductive success in determining contest outcomes in monkey beetles (*sensu* Korona 1991) may provide insights into temporal variation in sexual selection on weaponry at the scale of the individual's lifespan.

Sexual conflict

Most systems in which intrasexual contest competition occurs are thought to also exhibit female choice. However, no obvious female choice behaviour has been observed in monkey beetles (**Chapter 2**; Colville et al. 2018; Rink et al. 2019). Female monkey beetles may indeed be passive, or they may exert cryptic choice (e.g., over seminal products; Eberhard and Cordero 1995; Birkhead 1998; Eberhard 2009) or have traits that enable them to avoid or resist mating attempts (Parker 2006). Females of *S. trimeni* and other monkey beetle species are colour polymorphic, sometimes matching their host flowers, and sometimes mimicking male colouration (Colville et al. 2018). Such "androchromism" is well-documented in damselflies as an effective means of male-avoidance by females (Svensson and Abbott 2005; Svensson et al. 2009). In addition to mate choice and intrasexual contest competition, sexual conflict is another manifestation of sexual selection that may be able to drive reproductive isolation and thereby speciation (Parker and Partridge 1998; Martin and Hosken 2003; Gavrilets 2014). Understanding sexual conflict dynamics within monkey beetles may therefore further our understanding of the importance of sexual selection in shaping evolutionary diversification within this group.

Reproductive isolation

Species are generally defined as such under the biological species concept, which describes a species as a group of organisms that can breed with one another but are reproductively isolated from other such groups (Mayr 1942). Under this species concept, divergent fighting contexts must be capable of driving reproductive isolation among populations adapted to different contest substrates to be able to drive speciation (McCullough et al. 2016; Tinghitella et al. 2018).

Chapter 3 results show that male weapons do vary along flower size gradients, but does variation in weapon morphology among populations reflect an underlying difference in fighting style? If fighting styles do differ among populations, is this sufficient to create reproductive isolation among the allopatric populations upon secondary contact, due to the competitive inferiority of immigrant males? Staged contests among males from different populations on flowers of varying sizes may reveal incompatible fighting styles among populations. Addressing these questions is essential for understanding whether divergent fighting contexts can drive speciation in the embedding monkey beetle guild and so elucidate the role of the diverse floristic environment of the Greater Cape Floristic Region (GCFR) in contributing to the diversification of monkey beetles.

5.5 CONCLUDING REMARKS

My thesis has revealed intricate selection dynamics in monkey beetles, arising from the interaction between sexual selection and the floristic and abiotic environments that these pollinators inhabit. I found support for sexual selection to be an important driver of monkey beetle diversification at the microevolutionary level, but the extent to which spatial and temporal variation in the hyper-diverse floristic environment of the GCFR has influenced the strength, direction, and tempo of sexual selection in this group on macroevolutionary timescales deserves the attention of future work, as do the topics suggested above. Further work should also examine the evolution of sexual selection in monkey beetles within a phylogenetic context to understand the evolutionary relationships between feeding guild type and contest competition. Intrasexual contest competition also occurs in non-anthophyllic monkey beetles, which fight on plant stems and on the soil surface, and these contest substrates may exert strong selection pressures, leading to fighting styles and weapon morphologies that are distinct from those in the anthophyllic embedding monkey beetles.

This work forms a point of departure for understanding how male-male contest competition, as an agent of sexual selection, can drive diversification along gradients of contest substrate variation in the context of broader environmental selective pressures which influence evolutionary processes in combatant animals.

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