

UNIVERSITY OF DERBY

THE EFFECTS OF WEAPONRY AND MATING EXPERIENCE
ON THE LEVEL AND OUTCOME OF AGONISTIC
INTERACTIONS IN MALE FIELD CRICKETS, *GRYLLUS*
BIMACULATUS (ORTHOPTERA: GRYLLIDAE)

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Abstract

A wide variety of factors are predicted to influence the intensity and outcome of agonistic interactions in animals, including the resource holding potential of the opponents and the nature and value of the resource over which the individuals are competing. Field crickets (Orthoptera: Gryllidae) have been used extensively as model organisms with which to study animal contests, but relatively few studies have examined the effect of mandible size or structure, or the level of contact with females on the intensity and outcome of agonistic interactions. To do so was the aim of the present thesis, using *Gryllus bimaculatus* as the study species. The first finding of this study was that there is a significant degree of sexual dimorphism for anterior components of the anatomy in *G. bimaculatus*. The mandibles, head and pronotum of male crickets were all relatively larger than those of females. This indicates that these traits may be acted upon by intrasexual selection. In many animal species that show sexual dimorphism, a trade-off in development sees enhanced weapon growth at the expense of testes size, but no such relationship was seen in this species. A comparison of the mandible structure of males that either won or lost at flaring and or wrestling showed that a relatively wider mandible span was a significant predictor of success during mandible displays. It was also found that specific components of tooth structure, namely the length of the incisor and length to distal tip, were significantly associated with victory at the jaw flaring stage. This is the first time that mandible shape has been shown to affect fight outcome in the Gryllidae, and also the first confirmed identification of a visual cue component of fighting behaviour. Despite the effectiveness of their weapons in fighting, body mass is a primary predictor of victory in combat between *G. bimaculatus* males, with the greater the degree of asymmetry in weight the more likely the heavier fighter will win. However, a study of fighting behaviour between asymmetrically matched opponents found that even males who were out-weighted by 40% were still likely to escalate the fight to grappling. Furthermore, males who were able to fend off their larger opponent in their first clash were significantly more likely to win their overall encounter. This hyper-aggressive response may therefore represent an adaptive mechanism to extreme odds and is worthy of further study. Female contact is known to be a significant promoter of male aggression and fighting enthusiasm, and mate guarding aggression is well documented

in *G. bimaculatus*. A recreation of two contradictory studies, including one which concluded that mating makes males lose fights, highlighted that female contact after spermatophore transfer can overcome the loser effect and cause a male to re-engage with a previously dominant opponent. Fighting behaviour in this species is therefore highly flexible and factors affecting the outcome of contests are complex. There is much scope for further studies on this topic.

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CHAPTER 1. INTRODUCTION

1.1 Animal contests

Whenever access to an essential resource is finite in its availability there will be competition for it (Briffa and Sneddon, 2010). In the animal kingdom this competition is most common between adult males that engage in contests most often over food, territory or access to females. This conflict of interest between individuals is often played out in aggressive interactions that can vary in form and intensity by species (Arnott and Elwood, 2009). However, the term 'aggression' might not always be appropriate to describe these interactions (discussed in Hardy and Briffa, 2013), as it would be a single term describing many separate behaviours and interacting factors that can affect competitive behaviour between males (Hardy and Briffa, 2013). The terms 'animal contests', 'agonistic encounters' or 'male-male competition' will be used throughout to refer to fights between conspecifics because they cover the range of behaviours seen within this area of study, but do not make assumptions about the structure of the conflict itself. 'Aggression' will be discussed, but will be applied within the context of specific behavioural effects that can be incorporated into contest performance (see below).

1.2 Contests and models

1.2.1 Costs and benefits of contests

Conflict revolves around engaging and resolving of contests between individuals over access to a limited resource. As mentioned above, these contests do not necessarily have to involve physical contact or injury, but they are settled by differences in resource holding potential (RHP) between contenders. The study of animal contests and decision-making is based on Game Theory, a mathematical modelling system originally developed for use in human economics (Nash, 1951), that was repurposed by Parker (1974) into Evolutionary Game Theory in order to understand how animals make choices in contests. Parker (1974) applied the method to actions observed during fights between opponents in which behaviours including fighting and other physical signals are used but the fighting itself was not damaging to the contestants, which Parker called 'conventional fighting'. This application sought to explain why fights do not have to incur costs (C), and that the payoff

(E) can depend on strategy and choice of opponent (Maynard Smith and Parker, 1976). These contests often develop in their associated cost the longer the confrontation lasts, from initial signalling (low cost), to high intensity display or fighting (high cost), and is known as contest escalation. How far each contender is willing to go is dependent on the individual's performance in a contest in which costs must be paid in order to secure access to the resource based on that resource's value (V). Any given case of contest escalation is therefore decided by the ability of each contender to absorb the costs (C) associated with competing with a chance of winning (p), and the payoff (E) of winning being enough to make those costs acceptable (Enquist and Leimar, 1987; Hurd, 2006a; Arnott and Elwood, 2008, Briffa and Sneddon, 2010). This can be represented as:

$$E = pV - C$$

(Adapted from Enquist and Leimar, 1987, in Briffa and Sneddon, 2010).

Parker and Maynard Smith's original work in game theory has been instrumental in the development of several key models that aim to understand how animal contests work, especially contests that take place over several stages or involve multiple bouts of repeated interactions, including signals, between males. In conflict that carries an element of risk, such as potential injury from physical contact with an opponent, a male must be able to make his decisions based on the system in which he is competing, taking the resource and his competition into account. When a resource value is relatively low, costs associated with acquiring it must also be low in order for it to be worth the effort, but as resource value increases then decisions have to be made as to whether the cost of playing for ownership are worth it e.g. female *Polistes dominulus* wasps will pass by low quality food resources that are guarded by rivals signalling their ownership, but a high value resource will give them reason to pause and to assess the holder's signals and decide if it is worth competing for (Tibbetts, 2008; Tibbetts et al., 2009). Similarly comparable behavioural changes were shown in food deprived *Astacus astacus* crayfish; small and hungry males were much more likely to engage with dominant resource-holding males over available food. The study also showed that the larger males would often back down from smaller males that initiated fights, indicating that the fight was not worth the costs (Gruber et al., 2016). These examples highlight the importance of accurate decision-making in contest scenarios:

fighting is not always the best course of action, and to engage in high cost physical clashes in competition regardless of potential outcome (or resource value) would be ultimately maladaptive, which is why pre-fight signals are commonplace in the animal kingdom.

1.2.2 Non-injurious contest behaviour and assessment

Even systems that are known to involve direct physical contact and the use of weapons, such as the rutting of red deer, *Cervus elaphus*, begin with lower cost signalling including throat roaring (Reby et al., 2001) before progressing to visual displays and eventually antler locking (Lincoln, 1972; Bartos and Bahbouh, 2006). Despite the variation seen in the animal kingdom, the structure and function of animal contests often follow a common, intuitive pattern of advertisement and escalation, with increasing degrees of cost and risk associated with each rise in intensity. These systems appear to have evolved in order to allow conflict to be resolved more economically as they give the receiver the chance to make an assessment in order to determine their next move, which may involve a confrontation and, depending on species, ultimately result in high intensity fighting (Enquist and Leimar, 1987; Arnott and Elwood, 2008). How animals actually make decisions in fights, and whether they use reciprocal signalling does seem to vary between taxa. While it has been suggested that the evolution of complex systems of signalling and appreciation of an opponent could be limited by cognitive ability of the subject species, discussion of animal cognitive ability in contest signalling and assessment agrees that repeated signalling is an effective method of communication, assuming that signals are used in assessment of fight choices i.e. war of attrition scenarios (see below) (Taylor and Elwood, 2003). This would mean that animal signalling during contests serves the same fundamental purpose in simple and more complex taxa (Elwood and Arnott, 2012; Fawcett and Mowles, 2013).

The manner in which some species compete gives some indication of the methods they might use to determine how they should react as the contest develops. Some species are thought to base their decisions in combat only on their own capabilities as a form of self-assessment, known as the war of attrition, WOA, it relies on both participants competing until their opponent gives up through stamina depletion (Hammerstein and Parker, 1982; Kemp, 2000; Briffa and Hardy, 2013). If the contest involves direct physical contact, once a combatant has sustained costs high enough to warrant giving up they will forfeit and leave

the contest site (Briffa and Elwood, 2000; Morrell et al., 2005a; Mowles et al., 2010). This form of self-assessment is known as the cumulative assessment model, or CAM (Payne and Pagel, 1997; Payne, 1998). In some species the contest is shown to follow a discrete repeated pattern of signals and physical contests that include shows of strength and stamina, known as the Sequential Assessment Model (Enquist and Leimar, 1983). It is thought that this method of interaction centres around mutual-assessment and allows the contestants to learn more about their opponents fighting ability (RHP) relative to their own as the fight continues, by allowing for regular reassessment and consideration of costs incurred in each stage (Enquist et al., 1990; Leimar et al., 1991; Jensen and Yngvesson, 1998).

1.3 Factors affecting fight outcome

1.3.1 Resource Holding Potential in contests

Animal contests are decided by the ability of one the participants to engage with an opponent over access to a limited resource and to be able to drive them away. An individual's ability to outcompete a conspecific in a physical contest is termed its resource holding potential, or RHP (Parker, 1974; Maynard Smith, 1979). In many taxa, studies of RHP asymmetry are primarily centred on aspects of body size/weight (Beeching, 1992; Pavey and Fielder, 1996; Briffa, 2008; Whitman, 2008) and weaponry (Emlen et al., 2005b; reviewed in Emlen, 2008; Fitzpatrick et al., 2012; Buzatto et al., 2017) and their pivotal role in animal contests as a predictor of fight outcome. It is important to understand, however, that RHP is not a single idea, trait or a specific feature of a competition, and nor can it be summed up as an arbitrary value. Within the framework of behavioural ecology, and based on the results of many studies that have examined what constitutes RHP, it could be described as the sum effect of an individual's size, strength and stamina, quality of secondary sexual characteristics such as horns or enlarged mandibles, and the ability to use these traits in combination to gain and maintain ownership of an essential resource against rivals (Payne, 1998; Briffa and Sneddon, 2008; Arnott and Elwood, 2009; Mowles et al., 2011; Zeng et al., 2016).

When conspecifics do compete, the degree of asymmetry in RHP between the two is often the deciding factor in who wins. The exact characteristics that could be considered as

components of RHP are species dependent. Competition in the animal kingdom can take many forms, ranging from the non-contact demonstrations of strength and stamina between male butterflies (Baker, 1972; Kemp 2000; Takeuchi, 2017) to violent and bloody body clashes of male elephant seals (Hayley, 1994; Casey et al., 2015). Even within a single species, the severity or cost associated with contest behaviour over access to a resource can vary significantly, as can the way in which conspecifics interact due to the natural degrees of variation in physical quality and build between individuals (Emlen, 2008; Arnott and Elwood, 2009, McCullough et al., 2016). See section 1.4.2 for information specifically on field cricket RHP.

1.3.2 Sexual dimorphism and weaponry

1.3.2.1 Intrasexual selection on male traits

In species where males engage in contests with other males, their success and failure is determined by performing effectively in whatever form of competition that species has evolved to use. As Darwin (1871) observed, the males of a species can often be identified by the over-development of secondary sexual characteristics such as horns and tusks that they use in combat specifically against other males. What Darwin was seeing was the effect of intrasexual selection for sexually dimorphic traits that contribute to resource holding potential (RHP), or morphology that is most effective in male-male competition (Barnard, 2004; Kardong, 2006; Emlen, et al., 2012). Over evolutionary time, pressure from intrasexual selection has refined, reshaped and in some cases drastically altered the morphology of the adult organism to maximise success in male-male contests that are decided by RHP asymmetry. In many cases this sexual dimorphism results in the male and female having incredibly different appearances, as the male carries the tools he needs for agonistic encounters (see Shine, 1989).

Intra-sexual selection for sexually dimorphic features is commonly associated with exaggerated expression of male traits such as body size, muscle growth, and weaponry such as tusks or horns (Stamps and Krishnan, 1997; Owens and Hartley, 1998; Lindenfors et al., 2002; Preston et al., 2003). Once high quality weaponry or similar traits that covary with fighting success in contests become a standard feature between conspecifics, the same intrasexual processes that produced sexual dimorphism begin working on enhanced trait

refinement (Kawano, 1997; Emlen, 2001; Barnard, 2004; Parzer and Moczek, 2008; Emlen et al., 2012), that focusses on weapon performance as an aspect of male quality. The ability to grow and wield better weaponry therefore becomes a focal point of intrasexual selection and can be seen in many taxa that have been studied at great length. A good example of this selection for better weaponry are the enlarged, curled horns and reinforced skulls that are used in strength based displays of powerful head-butting behaviour between adult *Ovis aries* males- their modified skull suture pattern is also thought to mitigate impact damage when two males clash heads, by acting as a shock absorber during these contests (Shackleton and Shank, 1984; Jaslow, 1989; Preston et al., 2003). The long, branching antlers seen in many species of deer are thought to function as signals of physical quality to be acknowledged as dominant status symbols (Lincoln, 1972; Malyon and Healy, 1994), as well as their use in fencing against other males (Clutton-Brock et al., 1979; Clutton-Brock, 1982; Scribner et al., 1989; McComb, 1991; Pemberton et al., 1992; Caranza and Valencia, 1999; Bartos and Bahbouh, 2006).

1.3.2.2 Positive allometry and weapon size

In some taxa, however, this process of selecting for male competitive effectiveness, with weaponry as the target of selection, has gone beyond refinement for efficiency. It has led to a degree of trait expression that produces forms that scale beyond neutral growth in line with the rest of the wearer's anatomy, but instead become overly large. This effect is known as positive allometric growth, a good example of which is the enlarged cheliped of *Uca pugilator* fiddler crabs that are used in contests between males over burrows (Berglund et al., 1996; Pratt et al., 2003).

Some of the most extreme forms of sexual dimorphism driven by intrasexual competition for weapon performance in combat can be seen in the insect order Coleoptera. For many families in this order, their entire life is one long preparatory stage before the fighting begins. Coleoptera are most commonly associated with this area of study as many species are sexually dimorphic and the males are heavily armed with extensions of the carapace forming horns such as those of the horned flour beetle, *Gnatocerus cornutus* (Okada et al., 2006), and the Lucanidae which includes the stag beetles with greatly enlarged mandibles in males that can display positive allometry (Kawano, 1997; Tatsuka et al., 2001; Knell et al.,

2004; Romiti et al., 2015). Their anatomy displays some of the most extreme examples of sexual dimorphism in the animal kingdom. Selective pressures over millions of years have shaped, modified and refined the morphology of some species such as the incredibly diverse range of *Onthophagus* dung-beetles that possess horn-like projections of the head and pronotum that they use in species-specific fights over resource ownership (Emlen et al., 2005; McCullough et al., 2015). The jaws of male *Cyclommatus metallifer* stag beetles are some of the best examples in the animal kingdom of weaponry scaling beyond isometry and can be as long as the male himself (Figure 1.1).

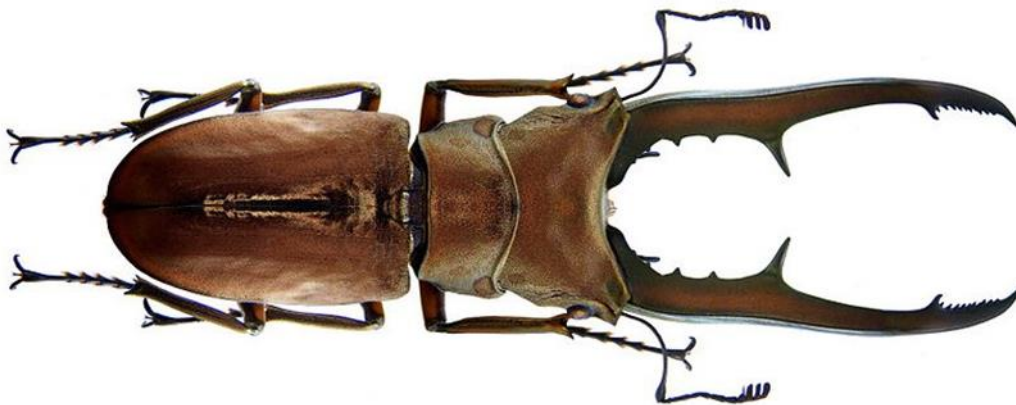


Figure 1.1: A male *Cyclommatus metallifer* has some of the largest mandibles in the animal kingdom and are classic examples of positive allometric growth under sexual selection [adapted from Wikimedia.org]

Enlarged weapons also require that the wearer is capable of paying the associated costs of bearing them- increased weight and reduced manoeuvrability issues need to be overcome for an adult to survive long enough to use them in a fight (Goyens et al., 2015a). For such weaponry to be plausibly grown requires that the beetle in its larval stage has sufficient stored nutrients to successfully metamorphose with the weaponry and associated musculature (as much as 40% more muscle mass than that seen in females) required to operate the mandibles as effective weapons (Moczek and Nijhout, 2004; Goyens et al., 2015a; Goyens et al., 2015b; Mills et al., 2016).

1.3.2.3 Developmental trade-offs

Heavy investment in weapon development has often been linked with a trade-off in resource allocation. Males with high quality mandibles see a reduction in wing growth, but

as the mandibles tend to correlate with greater reproductive success, the dispersal benefit afforded by wings is not so essential (Goyens et al., 2015c). Highly dimorphic forms that demonstrate extreme allometric growth are common among the beetles; a study of 76 species of Lucanid beetles across 9 genera found positive allometry in every species, and reduced wing development in 72 of them (Kawano, 1997). A later comparative study analysed the sexually dimorphic features of 349 Lucanid species and found that positively allometric weapons covaried with negatively allometric wing growth (Kawano, 2006), indicating that the developmental trade-off in favour of weaponry is family wide. While not all examples of male weaponry are quite so dramatic as those of the genus *Cyclommatus*, the relationship between body size and weaponry right across the Coleoptera does not necessarily adhere to a linear scale in many families. Hanley (2001) showed in 5 species of Oxyporin Staphylinids, and Romiti et al., (2015) in a population of European stag beetles *Lucanus cervus*, that a developmental switch point in body size leads to minor and major males, differing in their respective weapon size and therefore approach to mate acquisition. Similar trade-offs were also found in multiple species of *Onthophagus* dung beetles that present two different forms of male (Cook, 1999; Emlen et al., 2005a; Emlen et al., 2005b). Major males possess head and pronotal weaponry used in fights over mates, but a lesser-male form with reduced horn development but greater investment in sperm production was observed (Emlen, 1997; Moczek and Emlen, 1999; Moczek, 2003). Another study found that the lesser males do not succeed in fighting but their smaller head and pronotal ornamentation allowed them to move quickly through the narrow tunnels used by these animals, and that this allowed them to secure matings while their larger opponents were slowly navigating the tunnel network (Madewell and Moczek, 2006). A comparable trade-off mechanism of investment in testes or horn length was also identified in two species of cockroach that have adopted strategies at either end of the trade-off spectrum. Male *Gromphadorhina oblongonota* are large, aggressive and carry horns that they use in male-male competition that grow at the expense of testes mass, while male *Aeluropoda insignis* are less aggressive and show reduced investment in weaponry in favour of relatively greater testes mass (Durrant et al., 2016).

1.3.3 The Anostostomatidae: Large weaponry in the Orthoptera

Males of the Anostostomatidae, known as Wetas in New Zealand and King Crickets in Africa, are quite unique in comparison to the field crickets on which this work is focussed, but they deserve a mention due to the impressive weapons these animals have evolved for use in male-male combat (Toms, 2001). The African King cricket *Libanasidus vittatus* is strongly dimorphic and exhibits elongated tusk-like projections on its mandibles. These weapons are used in preliminary signalling to other males, where size indicates RHP, and in later high-energy stages, the tusks are used to grasp the opponents head to throw him. Research has shown that males with a greater degree of weapon symmetry were more successful in combat than asymmetrical males (Bateman, 2000b). Research on the related Wellington tree weta, *Hemideina crassidens*, showed that the males, that also often possess enlarged and tusk-like mandibles (Figure 1.2), engage in fierce bouts of biting and attempted throwing of their opponents in a similar manner to that seen in Lucanid stag beetles (Field and Deans, 2001). As found in the stag beetles, large male wetas with larger mandibles tend to be more successful in fights than their smaller conspecifics (Kelly, 2007). The location of these fights tends to centre on cavities or holes in tree trunks, referred to as galleries, that act as a valuable resource for competing males. The reason these galleries are competed for is because they provide shelter for adult female wetas, and so the adult male *H. crassidens* will defend his gallery from other males who wish to gain entrance.



Figure 1.2: Male *Hemideina crassidens* with large mandibular weaponry [Adapted from Wikimedia.org]

Securing ownership of a gallery brings mating opportunities as receptive females will mate with the resident male, and a larger gallery often holds more females and larger males with larger weapons tend to control larger galleries (Kelly, 2006a, Kelly, 2007; Kelly, 2008). This leads to a covariance of increased weapon size and reproductive success in a mating system that is essentially harem keeping. Male wetas show a very high degree of sexual dimorphism, with highly developed RHP traits of long tusk-like mandibles, large heads and jaw muscles (Kelly, 2006a; Kelly, 2006b; Kelly, 2007) which exhibit positive allometry (Kelly, 2005). Just as red deer, *Cervus elaphus*, clash to maintain their harem, the weta defends the entrance to his territory against all-comers by blocking the entrance with his body and grappling with his tusks for as long as possible. As in the Coleoptera, RHP asymmetry between aggressor and defender determines the length and often severity of the fight (Kelly, 2008), and as weapon size is a key determinant of dominance it is under positive sexual selection. This has led to positive allometric growth of the mandibles (Kelly, 2005; Kelly, 2006b), although the scaling of this positive allometric development is nothing like that seen in the Coleoptera.

While the resource-defence method of pre-copulatory investment of weaponry and body size is obviously well established in these wetas, not all *H. crassidens* display positive

allometry and find reproductive success by engaging in physical combat over galleries. Just as the *Onthophagus* dung beetle, *O. nigriventris*, males appear as either major morphs that engage in shows of strength with other major males using their horns, or as minor morphs that use their smaller stature to move quickly to evade major males (Madewell and Moczeck, 2006), the males of *H. crassidens* are actually trimorphic (Kelly, 2008). The very largest males, possessing the longest mandibular weaponry mature at the tenth instar and are the males that engage in high risk weapon-centric fighting with other large males over gallery ownership that has led to sexual selection for enlarged weaponry (Kelly, 2007). Smaller males, emerging as adults at the eighth instar show significantly greater developmental investment in spermatogenic tissue, and secure matings by sneaking past fighting males. These males may have relatively fewer mating opportunities, but their investment in a post-copulatory mechanism of sperm competition is effective enough for this to remain as a valid evolutionary strategy (Kelly, 2008). A male that emerges at the ninth instar is a medium morph, possessing intermediate traits of both other forms.

Whilst they are related to the Wellington tree weta, males of the mountain stone weta, *Hemideina maori*, are a good example of the effect that natural and sexual selection can have on an organism's evolution. While there is some degree of harem-keeping in this species, and harem size and ownership does correlate with male size, weaponry is not a consistent correlate of mating success for this species (Gwynne and Jamieson, 1998). Whilst the males do possess enlarged sexually dimorphic mandibles that are used as weapons, they do not exhibit the discrete instar-related weaponry associated with their forest-dwelling relatives (Koning and Jamieson, 2001). The rock based habitat in which *H. maori* is found has fewer crevices of a size to shelter multiple females and it is suggested that male weaponry does not follow a similar pattern to *H. crassidens* because body size has been selected for, as opposed to over-sized weapons in this species (Jamieson, 2002). This contrast in mating systems between closely related species demonstrates the different ways that male development can be refined based on degree of female monopolization (Lüpold et al., (2014). The primary difference between the two weta species is their habitat structure – *H. crassidens* defend holes in trees, whilst *H. maori* shelter beneath rocks. Both strategies achieve mating success by defending females, but the traits associated with good reproductive returns are different.

1.3.4 Aggression and contest behaviour

With direct physical contact forming a normal part of intrasexual competition, if the majority of males display the key traits that are required in order to compete, as a result of intrasexual selection, and all males need the finite resource for the same reason, aggression plays a key role in determining how an individual will behave in a confrontation. Carrying superior traits often correlates with success, but aggression is a functional element of animal contests that cannot necessarily be seen by an opponent. Repeatability of behaviour is an important aspect of assessment and decision-making for any male willing to engage in competition (Payne and Pagel, 1997; Hurd, 2006; Tibbetts, 2008), and responses to male agonistic signals would normally be expected to fit within a nominal range, including when choosing to remove themselves from the contest (Matsumura and Hayden, 2006). High aggression in the context of animal contests is often associated with a lack of signal etiquette – the aggressive individual might not act in accordance with signals he receives (Grafen, 1987; Morrell et al., 2005; Elias et al., 2010), or an aggressive individual may not take the time normally required to signal and instead pursue a contest escalation (Rutte et al., 2006; Jang et al., 2008). Additionally, if mutual assessment is a component of the pre-fight sequence (see 1.2.1 for assessment models), highly aggressive individuals might demonstrate a lowered appreciation of an opponent's signals of physical ability, such as weapon size or relative mass (Grafen, 1987; Morrell et al., 2005). Depending on circumstance and species, high aggression could also incorporate a disregard for factors positively associated with opponent victory such as the residency effect (Kemp and Wiklund, 2004). This effectively makes aggression a powerful evolutionary component that may allow for success in circumstances in which a less aggressive individual might fail, circumventing some aspects of sexually dimorphic trait function and stereotyped ritual display and decision-making. See 1.4.4 for how aggression from motivational effects can affect field cricket assessment.

1.3.4.1 The winner effect

Males that have recently been successful in combat against an opponent are more likely to win subsequent bouts, while losers are more likely to lose again (Chase et al., 1994; Rutte et al., 2006). Recent fighting success has been shown to correlate with an increase in

aggression and an improved chance of winning again in a variety of taxa including fish (Chase et al., 1994; Oliveira et al., 2009), mammals (Fuxjager et al., 2009; Jennings et al., 2009), crustaceans (Huber et al., 2003), arachnids (Kasumovic et al., 2010), and insects (Adamo and Hoy, 1995; Khazraïe and Campan, 1999; Rillich and Stevenson, 2011; Goubault and Decuignière, 2012). See 1.5.6.1 for examples of the winner effect in field crickets. The winner effect itself is temporary, although its timeline of dissipation is debated; one study using *G. bimaculatus* stated that the effect begins to dissipate within an hour, although it remains significant for approximately six hours, and disappears within 24 hours (Khazraïe and Campan, 1999), while a more recent study that also used *G. bimaculatus* but utilised endocrinological manipulation to monitor aggression levels, found that the effect is limited to less than twenty minutes (Rillich and Stevenson, 2011).

1.3.5 Motivation

As with innate genetic aggression, a highly motivated individual is capable of affecting fight progression by not respecting signals from conspecifics or by setting acceptable thresholds of energy use or injury risk higher than an opponent might expect on initial assessment (Hofmann and Schildberger, 2001; Hurd, 2006; Buena and Walker, 2008). Whilst all males engaging in competition will be fighting for access to a limited resource and in that sense are already motivated, certain contextual motivational effects on aggressive behaviour in many species have been studied. See section 1.5.6 for information on motivational effects in field crickets

1.3.5.1 Subjective resource value

The value that any one individual will assign to a resource is relative. The value of the resource itself is fixed, but the desire of the individual to take/retain it can vary greatly, hence the term Subjective Resource Value, sometimes abbreviated to SRV or RV (Hurd, 2006; Jang et al., 2008; Rose et al., 2016). In terms of altering behaviour, SRV is shown to have a profound effect on male decision making during asymmetric contests (Enquist and Leimar, 1987; Elwood et al., 1998) and even have fatal consequences if the cost of losing is too great to consider (Grafen, 1987; Enquist and Leimar, 1990; Hayley, 1994). As males normally assess a conspecific's physical traits, such as body and weapon size, during the primary low-cost stages of display (Hack, 1997a; Just and Morris, 2003), a larger male may

not expect a smaller opponent to continue an escalatory attempt at dominance. The effect of motivation via SRV within a RHP comparison scenario is essentially that the value an individual places on the resource at stake can augment an RHP deficit leading to a misread by an opponent (Arnott and Elwood, 2008), especially where mutual assessment plays a role in early escalation decision making. As costs may then accrue at a higher rate than a larger opponent may wish to expend fighting a presumed lesser male, he will disengage as such energy expenditure would normally be associated with decreased asymmetry (Grafen, 1987; Hack, 1997b; Payne, 1998).

1.3.5.2 Residency and territoriality effect

The value of territory is a fundamental component of behavioural ecology and ethology, and in terms of SRV, might drive some males to make riskier decisions, including escalating a fight despite a negative RHP asymmetry with an opponent (Hammerstein, 1981; Parker and Rubenstein, 1981). It is recognized as one of the primary sources of animal competition that has shaped our understanding of motivation in animal conflict scenarios (Maynard Smith, 1974; Maynard Smith and Parker, 1976). The residency effect is perhaps an extension of the subjective resource value effect (see 1.4.3.1) that can affect decision-making in animal contests. Males in possession of a territory are often considered to have an advantage over an intruder, with territory holders being more likely to maintain ownership than lose it (Chapin and Hill-Lindsay, 2016; Rosa et al., 2018). Parker (1974) suggested that an intruder may simply be aware that the male who owns the territory probably earned it, so it would be reasonable to predict him to be a capable fighter. Also territory ownership may be associated with a resource such as food or females, which would benefit the holder and increase his motivational urge to maintain ownership (Nosil, 2002; Kemp and Wiklund, 2004; Kokko, 2013). See 1.4.2.1 for territorial behaviour in field crickets.

1.4 The Gryllidae

1.4.1 Field crickets as model organisms

Field crickets are robust organisms and are very well adapted for survival in a host of conditions (as evidenced by their worldwide distribution (Alexander and Walker, 1962; Masaki, 1967; Wade and Otte, 2000; Rantala and Kortet, 2003)). They are also extremely reliable in their suite of behaviours regarding territoriality (Miyashita et al., 2016), resource defence (Bateman, 2000a), and mate guarding (Parker and Vahed, 2010). In short they are highly aggressive (Dixon and Cade, 1986; Brown et al., 2006; Bertram et al., 2011b) and much of their interaction with conspecifics is agonistic in nature and covaries with lifetime reproductive success in many species (Mallard and Barnard, 2003; Shackelton et al., 2005; Loranger and Bertram, 2016). This has led to them being used widely as model organisms in the study of behaviour, including contest performance (Sakai et al., 2017).

1.4.2 Resource holding potential in the Gryllidae

1.4.2.1 Calling song and territoriality

Mating systems in the Gryllidae are often characterised by being highly social environments where aggressive interaction is common with intense competition for mates and resources (Alexander, 1961; Iba et al., 1995; Tachon et al., 1999; Jang et al., 2008). Unlike the wetas, no species of field cricket has so far been found that includes distinct morphs of adult male that have evolved to express exaggerated mandibular weaponry or sperm production as alternative mating strategies. The populations tend to be mixed sex, in relatively close quarters, and often restricted by habitat structure in terms of securing boundaries. In these aggregated conditions, males restrict their movement, take ownership of a burrow or similar territory from which to call (Hack, 1997b) and via tegminal stridulation attempt to advertise their prowess and availability to both their neighbouring male rivals, and any females who may be out searching for a mate (Brown et al., 2006). Once males have formed territories, spacing between them is generally governed by the use of advertisement calling within the local area (Simmons, 1988; Simmons, 1995; Leonard and Hedrick, 2009). Ritz and Köhler (2007) showed that this spacing is very effective as *G. campestris* maintained a stable 5 metre distance between burrows across a 50 day observation period. Components of this

calling song include information about the resource holding potential (RHP) of the singer i.e. his physical quality including size and stamina, and his ability to use these traits in competition (Simmons, 1988; Simmons, 1995; Brown et al., 2006; Arnott and Elwood, 2009; Leonard and Hedrick, 2009), immune function (as some components of calling song correlate with encapsulation rates; Ryder and Siva-Jothy, 2000; Väänänen et al., 2006), and energetic capacity due to the metabolically expensive costs of calling for prolonged periods of time (Mowles, 2014).

The receiver of that song is then able to compare his own RHP traits with the song content and make a decision on whether or not to change position relative to the sender. The use of dishonest signalling is therefore naturally selected against; males that “lie” about their RHP quality will fare less well if their opponents continually outmatch them (Burke, 1988; Berglund, 1995). This system of space regulation actually works to minimise unnecessary conflict between senders and receivers by preventing multiple physical clashes between opponents of asymmetric RHP, as the song components provide an honest account of the surrounding competition (McGregor, 1993; Stevenson and Rillich, 2013a). Males in the vicinity that do not necessarily have a burrow or shelter can also use the content of this broadcast in order to make a behavioural response i.e. head to the source of the song, or keep looking for an opponent they have a better chance of driving away (Cade and Wyatt, 1984; Simmons, 1986). This makes calling song act as an effective spacing mechanism for males who don't wish to be included in a territory fight (Simmons, 1988). The importance of this innate risk-avoidance strategy to not engage in futile combat was actually demonstrated in *G. bimaculatus* by Simmons (1986) and later by Stevenson and Rillich (2013b). Both studies showed that males engaged in fewer bouts of combat when living in high density conditions i.e. in close proximity to multiple opponents. Simmons (1986) showed that even when males did escalate fights it was only after assessment that their opponent was an acceptable risk in terms of potential reward - normally access to a female or gaining ownership of a burrow. Stevenson and Rillich (2013b) went on to show that the overall aggression level of males in a group setting dropped quickly (less than 10 minutes) after introduction to a group of males. Both of these studies highlight that the decision to minimise injury and capitalise on opportunities for success are an innate component of field cricket behaviour.

When males do meet face to face within such an environment, it may be because RHP components of the calling song were not considered enough to prevent further investigation by a male who might consider himself capable of claiming a new resource (Hack, 1997b; Hofmann and Schildberger, 2001; Fitzsimmons and Bertram, 2013a). It is also important to acknowledge that while cricket advertising song does indicate RHP, no cricket song component has been found to indicate any degree of motivational status (Brown et al., 2006), nor does it indicate the aggressive state of the sender (Fitzsimmons and Bertram, 2013a; Harrison et al., 2017), although some species are by their nature more aggressive than others (Jang et al., 2008). Aggression levels can and do vary by species. Research on aggression levels in multiple Gryllid species has identified a trend in heightened aggressive behaviour in species that utilise burrows (Jang et al., 2008; Bertram et al., 2011a). Between them, the two studies drew a distinction between *G. veletis*, *Teleogryllus oceanicus*, *G. asimilis* and *G. pennsylvanicus*, all species that call from burrows, and the non-burrowing *G. fultoni* and *G. vernalis*. Both papers conclude that burrow use leads to increased escalation rates and more frequent use of grappling behaviour between fighters, whilst Jang et al. (2008) noted that the non-burrowing species were not seen to escalate very highly in any of their observed contests.

1.4.2.2 Body size in Gryllid combat

Prior to actual physical combat, when a pair of males have initially assessed their opponent, mid-level escalatory steps (see levels 3 and 4 in Figure 2.8 of the General Methods) involve direct visual evaluation and comparison of the combatants own mandibles (Hofmann and Schildberger, 2001). When fights between conspecifics escalate beyond visual assessment i.e. Level 5+, field cricket combat becomes a strength and stamina-centric wrestling match in which each fighter attempts to throw back and tip over their opponent. As such, larger males often possess an advantage, not just in terms of their weaponry but in stamina. Physiological costs associated with fighting behaviour increase linearly (Hack, 1997a), and overall costs increase as size asymmetry between opponents decreases (Hack, 1997b; Brown et al., 2006), hence the importance of early stage mutual assessment in decision making and the choice to escalate an encounter between rivals (Hofmann and Schildberger, 2001). The size of mandibles that are used by male field crickets as weapons to physically engage rivals in combat, are correlated with body size or mass in several Gryllid species,

including *G. bimaculatus* (Hoffman and Schildberger, 2001), *A. domesticus* (Bateman, 2008), and *G. pennsylvanicus* (Judge and Bonanno, 2008) and therefore play a key role in mutual assessment and decision making during bouts, as males making decisions to fight or flee from an opponent based on his size are also considering the relative sizes of their jaws (Rillich et al., 2007). Simmons (1986) and Briffa (2008) both demonstrated this decision making based on mutual assessment to minimise risk of injury in *G. bimaculatus* and *A. domesticus* respectively. Male crickets in both studies were shown to preferentially engage in combat against a conspecific only when they perceived their own chances of winning as better than their opponent, with larger body size being a common correlate of success in both of these species.

While fighting style does vary slightly between Gryllid species, the basic stages of the fight follow the same stereotypic escalatory sequence that sees the fighters assessing and escalating the fight based on their own and their opponents' actions. There is a general theme that sees more lightly built species such as *Platygyrillus primiformis* grasping the head of their opponent with their mandibles before tipping them over and chasing them away (pers. obsv), a strategy also seen in *G. pennsylvanicus* (Judge and Bonanno, 2008) and *G. campestris* (Simmons, 1995). Fighting in this manner relies on symmetrical weaponry to allow for effective grasping of the opponent's head, and also makes for dynamic, high energy and fast-paced bouts of combat that see repeated attacks and energetic use of song. These types of confrontations make heavy use of stamina reserves and it was shown in Hack (1997a; 1997b) and Briffa (2008), using *Acheta domesticus*, that body size and energy reserves are highly correlated, with relatively larger males being more successful against smaller opponents, with energy usage increasing as opponent mass asymmetry decreased. Large, thick bodied species of field cricket such as *G. bimaculatus* engage in the same escalatory sequence of combat as their relatives but the highest level of contact (usually designated as Level 6, see Figure 2.7) tends to focus more on shows of strength in which the pair of males strike each other head to head, grappling each other's mandibles, and attempting to push their opponents backwards and off balance (Hoffman and Schildberger, 2001).

1.4.2.3 Gryllid weaponry

When considering smaller orthopterans with substantially smaller mandibles than the wetas and their kin, work on the weaponry of the Gryllidae is very scarce. With such a complex range of mating and calling behaviours in the field crickets, few papers have specifically concentrated on the function of mandibles as weaponry, with many instead focussing on aggressive behaviours associated with mating. The lack of work in this area is possibly because even within the Gryllidae family itself, the use of the mandibles as weapons is inconsistent. Male *G. pennsylvanicus* have been shown to possess sexually dimorphic weaponry that play an important role in energetic and lengthy bouts of ritual combat (Judge and Bonanno, 2008), while the mandibles of *A. domesticus* play less of a role in their fights as body size and stamina levels are more important (Briffa, 2008). Male *G. pennsylvanicus* with proportionately larger jaws than a size-controlled opponent were more successful when fights escalated to high cost signalling e.g. mandible flaring and grappling (Judge and Bonanno, 2008). The authors noted that large mandibles did not affect fight intensity or duration, only that larger mandibles correlated significantly with overall success. Imposing handicaps by disabling the mandibles on fighting males of *G. bimaculatus* also did not affect fight outcome in asymmetric contests in this species (Rillich et al, 2007), supporting the role of mandibles as an accessory to RHP rather than an outright function of it. See section 4.1.2 and 4.1.3 for more detailed discussion of literature associated with Gryllid weaponry during assessment stages of combat and engagement of an opponent.

1.4.3 Assessment and escalation

Once a preliminary antennal confirmation of sex and species by detection of cuticular pheromones has been conducted (Nagamoto et al., 2005), a mutual visual and antennal assessment of their conspecific takes place in order to judge the potential fighting capacity (RHP) of a rival immediately prior to physical contact. In terms of assessment in field crickets, this observation stage at the start of any confrontation is an opportunity to take a measure of the opponent's strength and stamina relative to that of the observer (Hofmann and Schildberger, 2001; Judge and Bonanno, 2008; Reaney et al., 2011, Stevenson and Rillich, 2013a). For example, when pairs of male *A. domesticus* are asymmetrically matched for weight, the larger, heavier males will generally win, though it is noted that the degree of

asymmetry is significant in these contests (Hack, 1997b; Briffa, 2008). Assessment of an opponent in the early stages of a confrontation allows either party to remove themselves from the fight before it can escalate, preventing unnecessary wasting of energy and the risk of significant injury (Hurd, 2006), and it is this assessment of an opponent and consequential retreat or escalation that forms the basis of our understanding of conflict evolution and the importance of ritualised display (Maynard Smith, 1974; Maynard Smith and Parker, 1976). Body size itself can therefore be shown to play a distinct role as a visual signal and component of decision making by mutual assessment prior to engaging in combat with a competitor (Briffa, 2008; Reaney et al., 2011). Beyond this initial stage however, whether a confrontation does indeed escalate beyond posturing and use of visual cues, is often dependent on other size correlated traits, and the body of academic work in this area is vast (Simmons, 1986a; Nosil, 2002; Brown et al., 2006; Whitman, 2008; Reaney et al., 2011).

As the field cricket's basic state can be summed up as aggressive, that is their general day to day behaviour centres around male-male competition (Jang et al., 2008), evolutionary processes have refined their bodies and behaviour such that many traits linked to their body size play a direct role in aggressive signalling and conflict. Therefore body size in multiple Gryllid species has been shown to correlate strongly with aggression, stamina, weapon size, call rate and even mating and fertilization success (Dixon and Cade, 1986; Briffa, 2008; Judge and Bonanno, 2008; Hack, 1997; Hofmann and Schildberger, 2001; Reaney et al., 2011), so it is no surprise that it is the first RHP trait taken into account by an opponent (see 1.4.2). In a competitive system that sees high levels of perceived sperm competition and intrasexual aggression, correlation of physical and physiological traits with body size is a central component of self and mutual assessment between opponents. Preliminary assessment of, and comparison of self to, an opponent is a logical evolutionary step that precedes any potential fight, and is fairly standardised in many species of field cricket (Simmons, 1995; Hofmann and Schildberger, 2000; Brown et al., 2006; Judge and Bonanno, 2008). If neither male backs down then the encounter will escalate, often following a stereotypic sequence of increasingly costly actions, wherein traits linked to body size, and some that aren't, will determine the outcome. As no contest is ever symmetrical, RHP variation between individuals, including that expressed as a function of body size, therefore also drives

selection for aggression and territoriality, both of which also impact on fight outcome (discussed below) (Maynard Smith and Parker, 1976).

Competitive behaviour between males of the same species is often based on taking or maintaining ownership of a vital resource. Behavioural systems of ritualised display and mutual comparison have evolved in many species to minimise futile dominance attempts i.e. where RHP asymmetry is significant, and thereby reduce the risk of injury from entering into a dangerously asymmetric confrontation (Maynard Smith and Price, 1973; Maynard Smith, 1974; Parker, 1974; Enquist et al., 1990; Hill, 1994; Stevenson and Rillich, 2013b). Any male which finds itself in such a match must make a decision to continue beyond simple threat postures if he is to take/maintain ownership of whatever is at stake (Parker, 1974; Enquist and Leimar, 1983). Conversely, if preliminary low level comparison of RHP traits, by visual or at least antennal comparison of relative body or weapon size, does not settle a potential dispute between males then the ability to use those weapons and engage in physical tests of strength and stamina will often follow (Simmons, 1986; Bateman, 2000b; Hoffman and Schildberger, 2001; Judge and Bonanno, 2008). Honest signals of RHP, such as possession of high quality weaponry i.e. that is symmetrical or relatively large (Bateman, 2000b; Hanley, 2001), allow participants to tell whether a significant RHP asymmetry factor is in play and are therefore important in early to mid-level assessment because by allowing either male to leave the fight before it gets going they minimise wasted energy for both parties. Fighting is costly, so incurring physiological costs from display and risking physical injury in fighting for no expected return is a pointless act (Maynard Smith and Parker, 1976; Maynard Smith, 1979; Briffa and Sneddon, 2007), especially when there are other battles to be fought where the chances of winning might be more favourable or the payoff suitably high (Arnott and Elwood, 2008).

1.4.4 Aggression, experience and motivation

1.4.4.1 Recent fight success

When males enter into competition they assess each other and make a judgment of each other's RHP, but during antennal contact they also assess chemical cues from each other that can give more of an insight into what the conspecific has recently experienced, including fight outcome. This has been termed the social-cue hypothesis (Rutte et al., 2006).

A recent win will often result in increased aggression and a tendency to escalate fights despite RHP asymmetries that would normally curtail escalation e.g. when facing a much larger opponent. Reaney et al. (2011) found that small male *Teleogryllus commodus* that had recently won a fight against a weight-matched male would escalate fights against a larger opponent, but would then often lose if the fight escalated to grappling. Prior fighting experience and the change it can evoke in a male cricket in his next encounter with a rival, specifically with regard to his aggressive state and decision-making processes, is known as the 'winner/loser effect' and can have a significant, but not permanent, effect on how a male approaches subsequent confrontations (Adamo and Hoy, 1995; Khazraïe and Campan, 1997; Khazraïe and Campan, 1999; Killian and Allen, 2008). The outcome of an aggressive encounter is either positive or negative in terms of costs accumulated against resources gained (if any). As such, a male that has recently lost a fight, is more likely to lose his next fight if the position he finds himself in, such as his local environment, has not changed. This 'loser effect' was demonstrated in Khazraïe and Campan (1997) and Killian and Allen (2008) when they showed that a male who has lost a fight will not wish to reengage the same opponent unless something in his local environment has changed that forces a re-evaluation of the situation, such as access to a valuable resource. Any given contest between two rival males will be decided by a combination of the fundamental components of resource holding potential, RHP, that are positively correlated with success in competition such as body size/weight (Dixon and Cade, 1986; Hofmann and Schildberger; Brown et al., 2006; Briffa, 2008) and superior weaponry (Rillich et al., 2007; Emlen, 2008; Judge and Bonanno, 2008) and the sum of each males experience and current motivational state.

Rillich and Stevenson (2011) demonstrated that in *G. bimaculatus*, winning fights induces hyperaggression via the action of the biogenic amine octopamine. The 'winner effect' does exhibit a cumulative effect and a cycle of reinforced aggression through winning may be adaptive, at least in the short term, because field cricket distribution is often limited by habitat structure. Dominant males tend to occupy burrows or other features of the terrain that provide shelter, and shelter owning males mate more often (Simmons, 1986a; Hack, 1997b; Jang et al., 2008). The pheromonal cues that indicate a male has been successful in male-male competition are thought to be used by females to select for dominant males (Kortet and Hendrick, 2005), mating preferentially with successful males (Hack, 1997b;

Shackleton et al., 2005; Bretman et al., 2006). In addition, males living in close proximity to each other exhibit a reduction in aggressive behaviour to save energy (Simmons, 1986a), so a male on a hyper-aggressive winning streak may benefit from his neighbours subdued aggression, at least for a time.

1.4.4.2 Motivation and assessment

There are times when a male is confronted with a potential opponent who is influenced by a current motivational state to behave differently than his absolute RHP might indicate is appropriate (Dixon and Cade, 1986; Brown et al., 2007; Bubak et al., 2015; Kar et al., 2016). For a male to escalate a fight beyond what a superior opponent might consider a reasonable point means that he either considers some hitherto hidden aspect of his own RHP (hidden from his opponent) to be of a sufficiently high level as to warrant the risk, or that there is a behavioural effect, often motivational and sometimes even seemingly maladaptive, that is directing his decision making processes to instigate or escalate beyond what any initial or ongoing assessment by an opponent might predict (Hurd, 2006; Morrell et al., 2005; Bubak et al., 2015), and in doing so change the natural point of conflict resolution (Mohamad et al., 2010).

Bringing a hidden, often erratic or at least unexpected, behaviour to a RHP comparison can impact on the normal proceedings of a fight dynamic in that it may not only act to augment an RHP deficit in a male who might not be assessed as a significant threat to a larger opponent, but in doing so can cause an opponent to underestimate their energy budget. This could then see them lose a fight to an opponent that they would consider inferior if rated solely by their apparent RHP (Hack, 1997; Tachon et al., 1999; Just and Morris, 2003). The effect of a male's motivational state on both assessment and escalation phases in agonistic interaction with a conspecific is very difficult to explain in traditional game theory models that only deal with the use of RHP traits and inherent fitness costs associated with them (Maynard Smith, 1974; Parker and Rubinstein, 1981; Hack, 1997), though high resource value is shown to increase the costs a male is willing to incur (Enquist and Leimar, 1987) i.e. males with more to lose can be seen to take greater risks when assessing an opponent (Rutte et al, 2006; Brown et al. 2007; Reaney et al. 2011), at times overcoming divisive asymmetries in RHP that would normally prohibit any kind of escalation.

As might be expected, competition for mating opportunities promotes high levels of aggression between competing males, either over territory that may be associated with female access (Siva-Jothy, 1987; Kelly, 2006; Jang et al., 2008; Bergman et al., 2010; Santos and Peixoto, 2017), or fighting over access to females already present (Kelly, 2006; Casey et al., 2015; Loranger and Bertram, 2016; Montroy et al., 2016). In terms of behaviour-altering effects from a change in motivation, female presence can have profound effects on male status leading to repeated clashes and risk of injury (Tachon et al., 1999; Kelly, 2006; Brown et al., 2007). This change in aggression can be linked to acquiring a high value, defendable resource i.e. a mate, but the effect that mating has on male aggression itself is unclear. Killian and Allen (2008) showed that subordinate males that were mated to a female showed increased aggression toward a dominant male, but Judge et al (2010) stated that mating made males lose fights.

1.5 Aims and scope of this thesis

This thesis aims to examine agonistic behaviour in adult male *Gryllus bimaculatus* in order to determine factors that affect the level and outcome of agonistic interactions, with an emphasis on the roles of mandible size and morphology and of female contact after mating.

Chapter 3. The first study focused on aspects of sexual dimorphism in *G. bimaculatus*. Females do not tend to engage in fighting behaviour with conspecifics, and are never seen to use their jaws as weapons in fights with conspecifics. Males fight often and use their mandibles as weapons to force a retreat from their opponents. This difference in behaviour suggests there could be a degree of sexual dimorphism in the jaws of *G. bimaculatus* arising from different functional requirements between the sexes. As weapon growth has been associated with developmental trade-offs in wetas, this study will also look for any evidence of a potential trade-off between testes and weapon size in male *G. bimaculatus*.

Chapter 4. This study aimed to determine whether there was a difference in mandible size or morphology between male *G. bimaculatus* that either won or lost fights (at either the mandible flaring or the grappling stage).

Chapter 5. This study examined fights between asymmetrically weighted pairs of male *G. bimaculatus*. As weight asymmetry is a key predictor of fighting success, favouring the

heavier male, smaller males that win fights may differ from small losers by possessing significantly larger weaponry that might help them win against a larger opponent. This study looked to see if smaller males that won jaw flaring and grappling fights against larger opponents differed in weapon size from the small males that lost. Fight escalation was also analysed to see if small males could raise their likelihood of winning by escalating fights to higher levels than their large opponent might have expected.

Chapter 6. This study recreated the methodologies of two studies that made apparently contradictory conclusions about the effect of mating on male aggression: 'Mating resets male cricket aggression' (Killian and Allen, 2008) and 'A lover, not a fighter: mating causes male crickets to lose fights' (Judge et al., 2010). Motivational effects on male aggression from female contact, female mounting, and female mating treatments were compared using *G. bimaculatus* as the focal species. Aspects of both original studies were critically compared and contrasted to determine why the studies differed in their conclusions about female contact changing male fighting success.

Chapter 7. As the previous study had looked at the effect that female contact can have on promoting male aggression, this study investigated further the causes of increased male aggression after mating in *G. bimaculatus*. Using a 2 x 2 factorial design, the experiment aimed to determine if increased male aggression after spermatophore transfer was dependent on the presence of the female and/or whether she was carrying his spermatophore.

CHAPTER 2 A. GENERAL METHODS

2.1 Cricket husbandry

All specimens of *Gryllus bimaculatus* used in this set of experiments were selected from healthy, cultured stock maintained at the University of Derby. The insect breeding facilities environmental controls ensured a temperature of 27°C, relative humidity of 33-35% and a light:dark cycle of 12:12 was maintained throughout their development and growth. Crickets were initially reared in mixed sex groups in plastic aquaria measuring approximately 280mm x 180mm x 180mm. All cultured specimens had access to dry rat food, washed raw vegetables and fresh water via water tubes. Shelters were provided by multiple layers of crumpled newspaper.

The male crickets were separated from the females into single sex communities approximately 4 weeks after hatching. It is possible to discern the sexes at this point as the females begin to exhibit an ovipositor. This separation ensured that all individuals used were sexually naïve. Having undergone their ultimate moult in to maturity at approximately 11 weeks old, adult crickets were maintained for one week before use in any experimental work in order to ensure complete developmental maturation of gametogenic tissue.

2.2 Preparation for behaviour trials

2.2.1 Treatment of male crickets used in behavioural trials

Males that were to be used in experiments were caught by hand and visually assessed for injury that might impact behavioural trials, specifically damage to the antennae, legs and wings. See Figure 2.1 as an example of a specimen with damage to the right wing that was not accepted for use in any behavioural trial.



Figure 2.1: An adult, male *G. bimaculatus* with damage to the right wing (photo credit: DG).

The specimens were weighed in grams to 2 decimal places on a Kern EW electronic pan balance and housed in numbered individual plastic food tubs measuring 95mm x 150mm x 50mm; each tub contained a 5ml water tube plugged with cotton wool, a piece of crumpled paper towel for shelter, and dry pellets of rat food (Figure 2.2). Temperature, humidity and light: dark cycles remained unchanged as the individually housed males were kept in the insect rearing room in which they had been bred.



Figure 2.2: An individually housed adult male *G. bimaculatus* with food, shelter and water (photo credit: DG).

Body weight and container number were recorded to keep track of the individuals to allow them to be matched in pairs for use in trials. Where two males had been selected as a pair for behavioural observation, to enable accurate identification, each male in the pairs was

assigned a colour which was applied to the pronotum as a single spot of nail varnish (Figure 2.3). Weight-matched males never outweighed their opponent by more than 0.01g or 2%.



Figure 2.3: A small spot of nail varnish approx. 2-3mm was applied to the pronotum taking care to not touch the wings or antennae during application (photo credit: DG).

Males were then maintained in the solitary enclosures for no less than 24 hours, within the insect breeding room where they were bred. This ensured that temperature and humidity requirements were maintained prior to experimental use. The 24 hour isolation ensured that all experimentally observed behaviours would be those of a naïve cricket where necessary, and that any changes to a cricket's behavioural state were those that were controlled by experimental design. Khazraie and Campan (1999) demonstrated that prior experience of conflict and any confounding influence of winning/losing completely dissipated after a period of 24 hours had passed. Therefore, this physical isolation aimed to remove any previous behavioural cues that might have affected trial fight outcomes and to account for temporary stress caused by handling.

2.2.2 Treatment of female crickets used in behavioural trials

Female crickets used in this experiment were individually taken from a population of mature, adult, virgin females. In each case, the female cricket was chosen from a single sex colony of mature females and in each case was chosen specifically to be a suitable size for the Experimental Male to make a valid mating attempt with, including transference of a spermatophore where it was required by experimental design. The female insects were not

handled at any point prior to use in male treatments, but were caught using a clean plastic sample tub and lid.

The females were captured prior to starting any experiments that required them, and allowed to sit quietly while any initial male-male trials were conducted, for a minimum of 5 minutes. During trial runs of behavioural work, this was found to be a far better method than catching them and putting them straight in with an experimental male.

The female was carefully introduced to the treatment male in each case by carefully removing the lid of the sample tub and allowing the female to climb from the tub and into the treatment area from a height of no more than 2-3 cm. After the treatment phase and any subsequent trial -specific use, the female was carefully recaptured and released into a separate holding tank to ensure that no previously mated/experienced female was reused in any subsequent trial.

2.2.3 Cricket transfer prior to use

Both male and female crickets were moved without direct physical handling, thereby minimising potential stress that would have impacted on their natural behaviours. Males were removed from their individual containers and placed directly into the experimental area by gently lifting a corner of the lid of their enclosure and tilting the tub to allow the male to move himself out of the container, dropping no more than 2-3 cm. Females in the sample tub were allowed to exit the tub by gently removing the lid when the tub was held at 90° to the floor of the treatment area, from a height of no more than 2-3 cm.

2.3 Combat treatment and arena reset

Methodology specific to each study can be found in each results chapter and a full breakdown of fight observation and what constitutes combat resolution can be found in the Full Fight Protocol document, beginning in section 2.7. All combat trials in all studies took place between 11am and 3pm as this is a key time for male calling and fighting behaviour and males are most likely to have a spermatophore in place. Temperature was maintained at 28°C using heat lamps. In all cases, consecutive fights between new crickets did not take place before the arena and the dividing wall were wiped out using cotton wool swabs and 70% ethanol to remove any faecal pellets or other fluids, and to ensure no pheromone

traces persisted that would possibly affect male behaviour during their bouts, as per Brown et al (2006).

2.4 Euthanasia procedure and storage of specimens (when required)

Post-experiment, any males that needed to be euthanized for dissection data were moved to individual 5 ml glass tubes with lids. The 5 ml tube lids were written on with permanent fine-line pen to show experiment number, cricket number and any other relevant identifying/study specific information. The males were then transferred to a lab freezer to be euthanized at -18°C for approximately 15 minutes. Once the males were dead, the tubes were removed from the freezer and filled with a solution of 70% ethanol and stored in a refrigerator.

2.5 General post-mortem measurements and dissection method

2.5.1 Method for taking measurements of cricket physical dimensions

The mass of the whole cricket was the first measurement taken. Using an electronic pan balance, the animal's mass was measured in grams to 2 decimal places, and recorded as Total Mass (g) on the spreadsheet. To gather other post-mortem data, a dissecting microscope with an eyepiece graticule calibrated to a stage micrometer was used. Measurements of male head width, pronotum width and pronotum length, as shown in Figure 2.4, were recorded in mm.

Measurements of the head width, pronotum length, and pronotum width were all made from a dorsal aspect whilst the animal was whole. Tweezers and a mounted needle were used to move the animal into position for measurements to be taken. Measurements were all taken as the longest/widest point on each individual and recorded in millimetres to 1 decimal point.

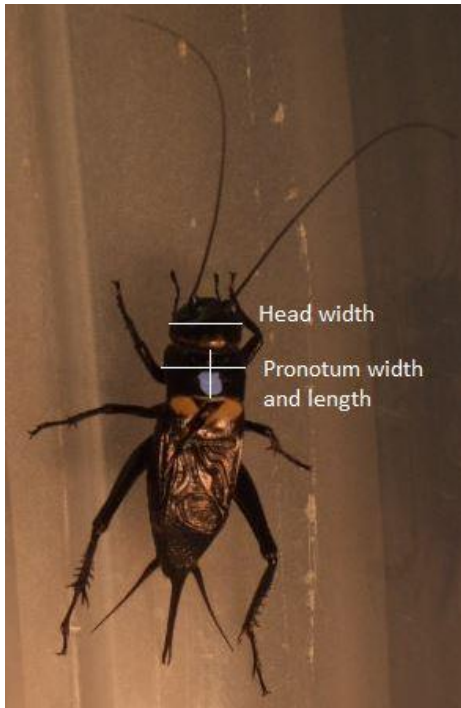


Figure 2.4: Body measure points on an adult male *G. bimaculatus* (photo credit DG).

2.5.2 Method for removing the mandibles

Where experimental design required specifically that mandible measurements were needed, the mandibles were excised from the head by first removing the labial and axillary palps with a scalpel and fine tip tweezers, and by then cutting the muscles at each lateral articulation point.

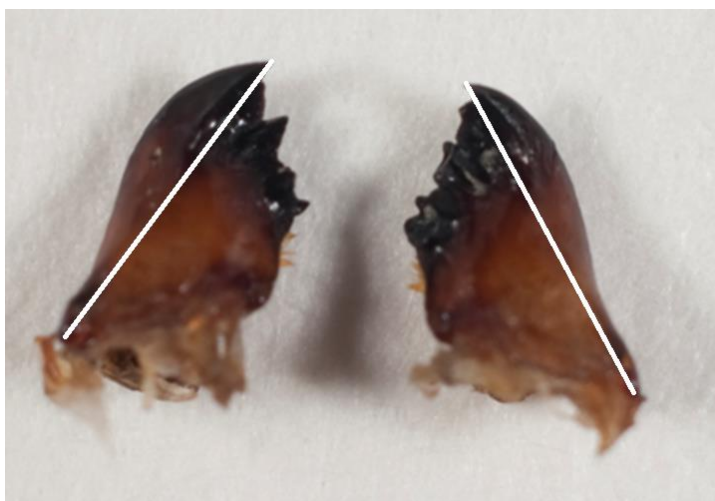


Figure 2.5: Mandibles from a single male *G. bimaculatus* with measure lines from distal tip to lateral articulation marked in white (photo credit DG).

Standard measurements of tooth length were made by measuring the longest line between the distal tip and lateral articulation point (Figure 2.5).

Individual glass jars containing 70% IMS were used to preserve the specimens, including any excised tissues from each the dissected crickets, once any measurements had been made. All storage jars containing cricket bodies were labelled with a fine permanent marker pen with male ID number and study number for potential use in any future work.

2.6 Arena construction and use

The test environment in which behavioural trials took place for all studies making up this thesis was made to fulfil basic requirements for the observation and measurement of normal behaviours between test subjects.

Requirements considered prior to building it were:

- Appropriate dimensions: space for test species to demonstrate normal behaviours.
- Practicality of use: the insects could get traction needed for normal behaviours.
- Reusability in terms of construction: did not become damaged or stained with use
- Maintenance: chemically inert and non-porous material could be easily cleaned

The main body of the arena was a sturdy, stackable storage box with vertical walls, produced by Arca Systems.

A transparent, colourless Perspex plastic wall was installed across the centre line of the tub, halving the floor space, forming an arena measuring 13 x 13 x 18cm. Yellow plastic runners were then installed perpendicular to the floor (as shown in Figure 2.6, below), halving again the floor space i.e. quarters of the original box floor. A black, opaque piece of plastic was then cut to size and slid down between the runners in order to separate the two halves of the arena floor. This black wall was flush to the floor and could be removed.



Figure 2.6: The arena allows the animals room to move and allows observation from above and wider angles (photo credit DG).

All structural fittings were made by myself and held in place with invertebrate-safe aquarium sealant. All surfaces were safe to use under heat lamps, non-porous, and were chemically inert meaning they could be cleaned safely and quickly with 70% ethyl alcohol and cotton wool for the next set of animals. The reasoning behind using a single arena across all behavioural studies was to provide another stable element to data capture; that this itself would provide a consistent and standardised environment that helped to reinforce any data that was recorded.

CHAPTER 2B. OBSERVATION AND UNDERSTANDING OF MALE-MALE AGONISTIC BEHAVIOUR IN FIELD CRICKETS, *GRYLLUS BIMACULATUS* (GRYLLIDAE; ORTHOPTERA): A FULL FIGHT PROTOCOL

2.7 The need for a new standard methodology

Scientific information on fighting behaviour among adult male field crickets is extensive, but a review of the literature indicates that there is considerable variability between methodologies as to what constitutes victory in terms of determining a winner and loser in any given pair of conspecifics. The prevailing methodological view, and that which is typically used in behavioural papers related to male combat in Gryllid crickets, is that when two competing males engage in stereotyped escalatory combat as per Alexander, (1961) and utilising the escalatory chart in Figure 2.7 (below), the winner is determined once one male breaks away from the initial confrontation and his opponent engages in victory song (Khazraïe and Campan, 1999; Hofmann and Schildberger, 2001; Briffa, 2008; Judge and Bonanno, 2008; Judge et al., 2010; Reaney et al., 2011). Variants on this system include deciding on a winner by counting the number of aggressive interactions concluding in victory song he makes per unit of time (Khazraïe and Campan, 1997; Brown et al., 2006); the winner is the male who chases away his opponent twice in succession (Bertram et al., 2011b); or that the loser is the male who spends 10 seconds evading his opponent (Guerra and Pollack, 2010). However, some sources are not so clear in their methods, stating simply that one male is the winner (Zeng et al., 2016); that a male wins by driving his opponent two body lengths away from the fight (Hack, 1997); or that the winner is easily spotted (Adamo and Hoy, 1995).

Stereotyped escalatory scale of male-male fighting in field crickets as used in:

Hofmann, H.A. Schildberger, K. 2001 Assessment of strength and willingness to fight during aggressive encounters in crickets. *Animal Behaviour* 62(2) pp.337-348

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Figure 2.7: A standard aggression escalation scale, from Hofmann and Schildberger (2001).

Whilst conducting pilot studies in preparation for this thesis, I realised that there is a fundamental lack of consistency, not just in standardising observational concepts of fight outcome for data collection, but also with regard to what constitutes a fight as experienced by the subject organism. An inability to reproduce the work of other scientists using their

methodologies has meant that there is a need for a new, thorough, and up to date methodology that addresses common issues and challenges the notion that a fight can be considered won or lost on the basis of an arbitrarily appointed stopping point, when in practice the fight may be far from over.

2.8 Observing different types of encounters between competing males

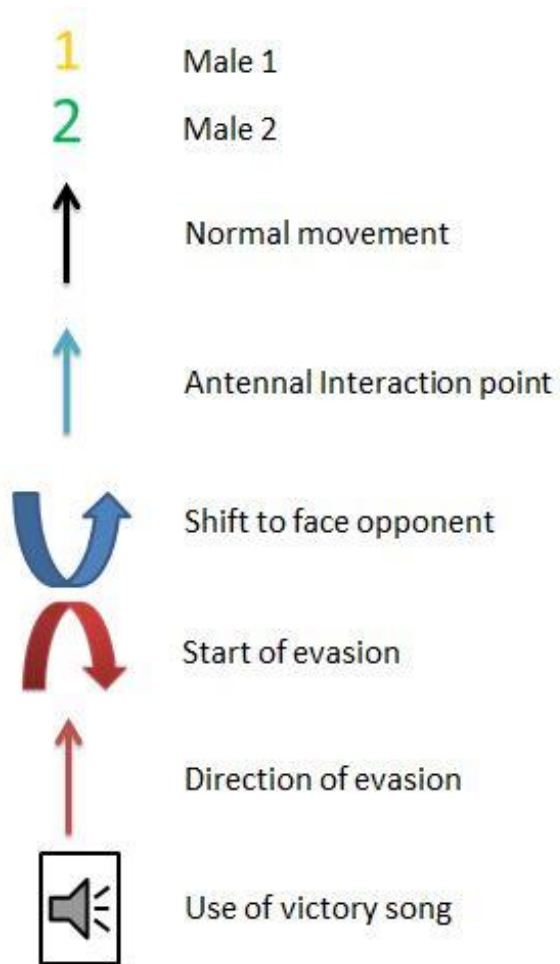
Specific stages of aggression referred to in this work are based on the standard aggression escalation scale (referred to from now as the ES scale) in Figure 2.7, with the additional amendment that fights that escalate to level 5 or 6 are rated simply as 5+. This practice was proposed in Judge and Bonanno (2008), stating that differentiating between the two forms in high level fights is not realistic as elements of both levels often occur in rapid succession.

An encounter between a pair of male crickets may include multiple bouts of fighting and does not always follow a head to head meeting – males will often approach their opponent from lateral or posterior positions – and so the ability to interpret how the animals react to each other on a contextual basis is key to understanding the behaviours exhibited during cricket fights. The following sequences of Fight images (F1-F28) were taken from a digital recording that lasted 150 seconds, and shows multiple types of interaction events between a pair of weight matched, combat naïve, virgin male *G. bimaculatus*, and explains how to read the interactions exhibited by the focal animals. Additional information related to fight interpretation and outcome, including deviation from common practices in scientific literature discussed above, is also covered.

Note:

Any interaction between a pair of males may terminate at ANY point i.e. level 5+ behaviour is not essential for the formation of a behavioural dyad of dominance and subordination.

2.8.1 Key used in F1-F28 to indicate behavioural components



2.9 First contact: Meeting and identifying an opponent

2.9.1 First interaction

Any interaction between males requires that one male must move within antennal range of the other (F1). Any act of aggression, wherein two males are intent on engaging in ritualized combat, will always be preceded by antennal contact (F2) as this is required for species recognition via cuticular pheromones. Antennal fencing (F3), via swift circling of the antennae over the opponent's body is considered the first level of escalation towards establishing a dominant-subordinate relationship. If the pair were to separate at this point the observer, using the ES scale (Figure 2.7), would record an ES Level 2 win for Male 1 (Figure 2.8).

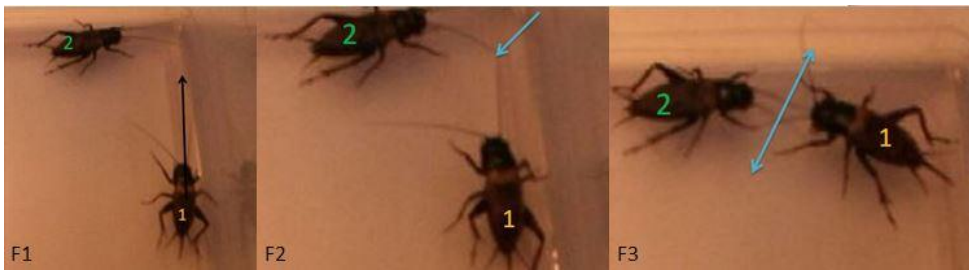


Figure 2.8: An initial encounter between two male *G. bimaculatus* sees Male 1 approach Male 2 (F1), antennal contact is made (F2), resulting in antennal fencing between the pair (F3) (photo credit: DG).

2.9.2 Escalation

Should neither male back away from antennal fencing, the next behaviour on the ES scale is level 3, unilateral mandible flaring. In image F4 (below), Male 1 has flared his jaws in order to demonstrate a measure of his RHP to his opponent. Male 2 does not flare his mandibles in response but begins to break away from the fight. Image F5 shows Male 2 breaking alignment from his opponent (Figure 2.9).

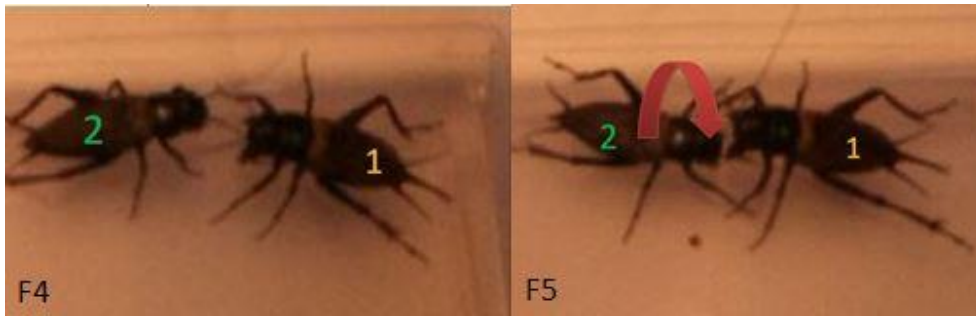


Figure 2.9: An encounter between two male *G. bimaculatus* results in unilateral mandible flaring from Male 1 (F4), and Male 2 choosing to retreat by beginning to break contact (F5) (photo credit: DG).

As Male 2 has chosen to break from combat he moves backwards while still facing Male 1, as shown in F6, before turning directly away from the fight in order to make his escape in F7 (Figure 2.10).

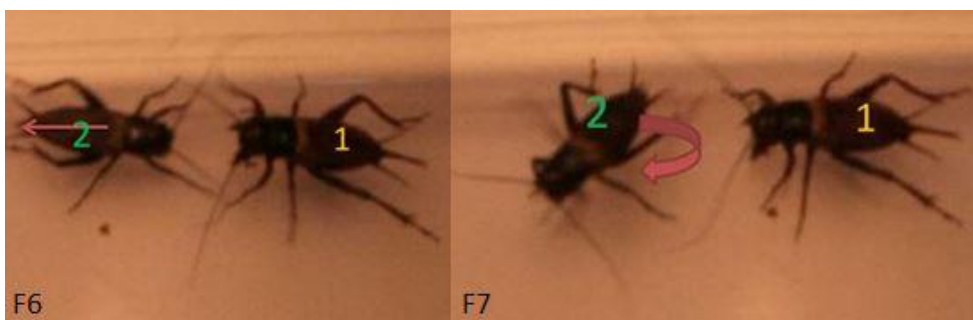


Figure 2.10: An escalating encounter between two male *G. bimaculatus* results in one male moving out of combat (F6) and beginning to retreat from his opponent (F7) (photo credit: DG).

2.9.3 Declaring victory

Male 2 retreats from the fight in F8, taking a path as straight away from his opponent as he can. As the winner of the round of combat, Male 1 engages in victory singing in F9. Some males would chase off their opponent, others stand where they are, singing loudly. This fight would be recorded as a win for Male 1 at ES level 3* (Figure 2.11).



Figure 2.11: An escalating encounter between two male *G. bimaculatus* results in one male escaping the combat area away from his opponent (F8), Male 1 begins victory singing (F9) (photo credit: DG).

*Some observers would now consider this fight to be over, and that male 1 has superior weaponry or some other form of greater RHP than Male 2. Within the confines of their experimental design that may be all that is required, but is not accurate to state that a true dyadic hierarchy has been established based on the observation of a single meeting and middle level escalation. Continued observation of the pair will often result in more interactions, as is discussed below.

2.10 Second contact: Evading a previously dominant opponent when approaching from behind

2.10.1 Making contact

Male 2, having just retreated from a fight, has moved around the experimental arena and in F10 is approaching his opponent, Male 1, from behind (Figure 2.12).

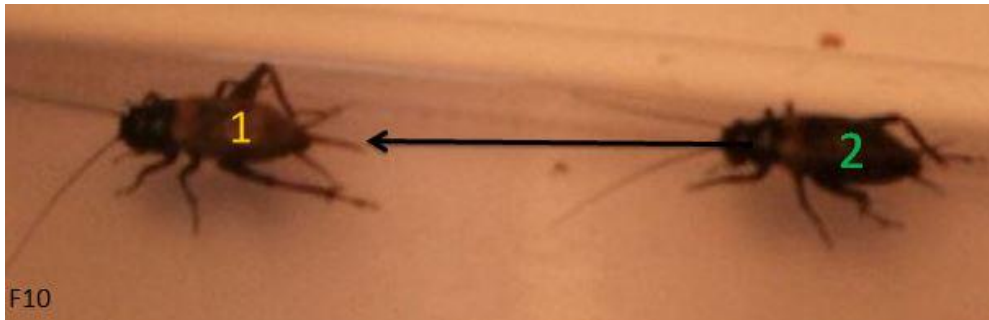


Figure 2.12: In F10, Male 2 approaches his previously dominant opponent from behind (photo credit: DG).

In F11, Male 2 makes contact with the cerci of Male 1 using his antenna and immediately recognises him as a previous opponent. Rather than stopping and turning around, he changes his course in F12 in order to avoid his opponent. Male 1 responds to contact from behind by turning on the spot, in F12, and reacting aggressively to Male 2, despite not making initial antennal contact. Crickets that have recently won a round of combat are more aggressive and less likely to respond to an unknown disturbance such as contact from behind with escape behaviour (Figure 2.13).

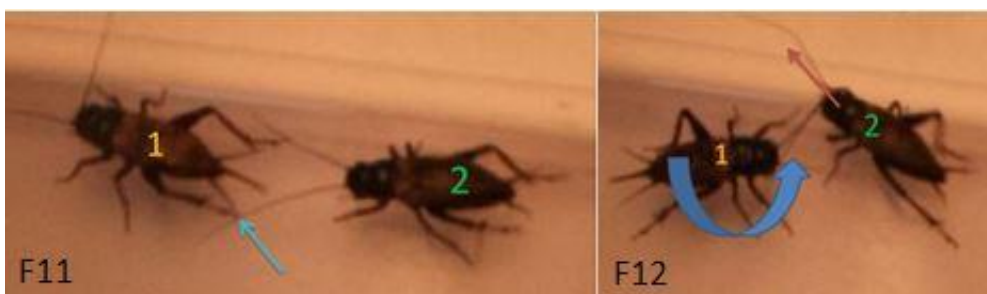


Figure 2.13: Male 2 recognises his previous opponent (F11), and moves quickly around him as Male 1 turns in response to contact with his cerci (F12) (photo credit: DG).

2.10.2 Declaring Victory

In F13, Male 2 quickly runs past Male 1 who tremulates towards his opponent before he emits his victory song in F14. This encounter would be recorded as a win for Male 1 at ES Level 1 (Figure 2.14).

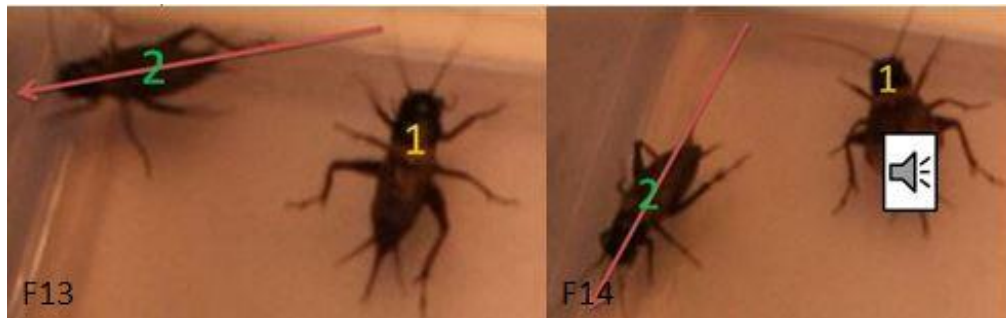


Figure 2.14: Male 2 evades the opponent he has recognised from a previous fight (F13), and Male 1 emits victory song (F14) (photo credit: DG).

2.11 Third contact: Reengaging a previously dominant opponent

2.11.1 Escalation

Male 2 has moved around the arena and in F15 directly approaches Male 1. Antennal identification leads quickly on to bilateral mandible flaring (ES Level 4) in F16 with Male 2 not wishing to back away from his opponent as he has done previously. In F16, Male 1 is stridulating rapidly and loudly, broadcasting victory song whilst still actively engaging in a round of combat that he has not yet won. According to some scientific literature this behaviour is only used by a victor when a fight has been won (Figure 2.15).

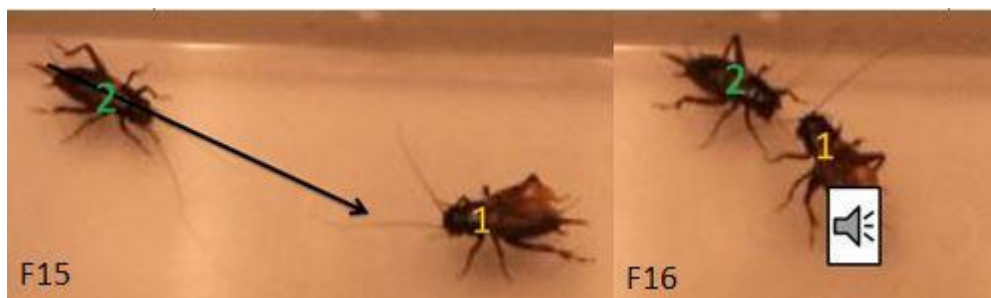


Figure 2.15: Male 1 and 2 meet again head to head (F15) and escalate to bilateral mandibular flaring, with Male 1 also singing victory song as he displays (F16) (photo credit: DG).

2.11.2 Mandibular engagement

As the confrontation escalates in F17, the crickets engage in ES Level 5+, locking their jaws and attempting to push each other back in a show of strength and stamina with a higher risk of injury. Victory song by Male 1 has stopped at this point. Male 2 is ejected from the fight in F18, either through losing traction or by mistiming a jump. Some papers would class this as ES Level 6, but as stated above, it is very difficult to say for sure whether he was thrown by his opponent. The fight does not finish here, though, as Male 2 does not make any attempt to flee (Figure 2.16).

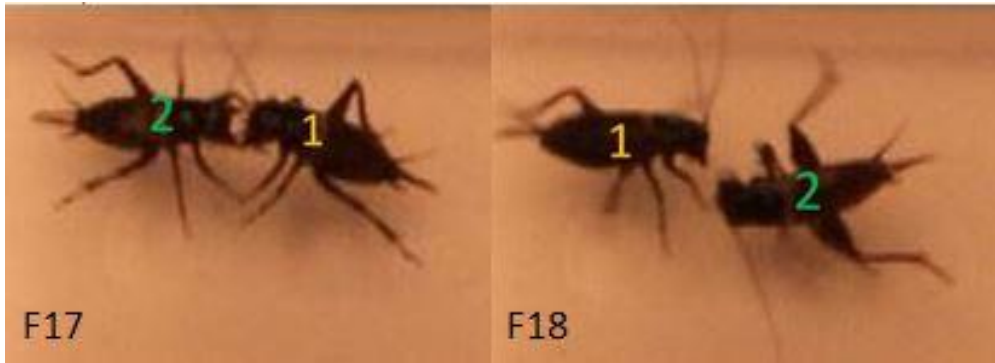


Figure 2.16: The crickets engage in high level displays of aggression at ES 5+ (F17) and Male 2 is ejected from the fight (F18) (photo credit: DG).

2.11.3 Breaking contact and resuming aggressive interaction

Despite being thrown during the fight, F19 shows that Male 2 has immediately reengaged Male 1 in ES 5+. With continued high levels of aggression, the ES 5+ fighting sees the ejection of Male 1 from the combat in F20. This is the first time in the three encounters that he been unable to maintain a dominant position (Figure 2.17).

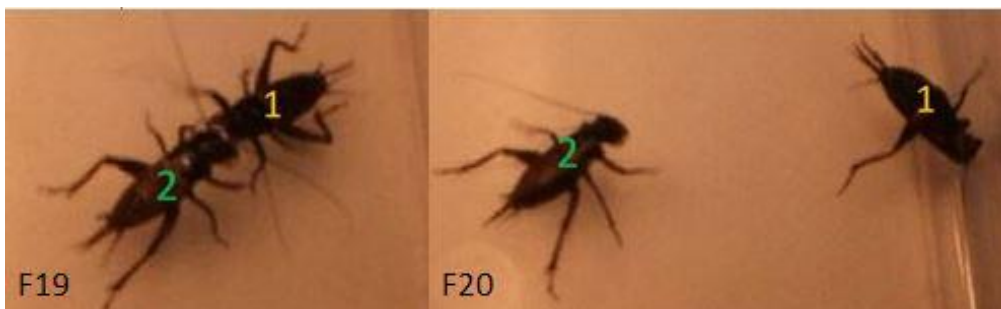


Figure 2.17: The pair return to grappling ES 5+ (F19) and Male 1 is then thrown from the fight (F20) (photo credit: DG).

2.11.4 Breaking contact and deciding to flee

As Male 1 rights himself in F21, Male 2 approaches him stridulating loud victory song. Male 1 makes the decision to break away from the fight, becoming subordinate to Male 2. F22 shows Male 1 breaking alignment with his now dominant opponent, Male 2, as he begins to make his escape. Male 2 does not step back, maintaining his aggressive stance as he continues to broadcast victory song (Figure 2.18).



Figure 2.18: High level fighting proves too costly for Male 1. Male 2 begins victory singing (F21) and Male 1 breaks away from the fight in order to retreat (F22) (photo credit: DG).

2.11.5 Declaring victory

Male 1 continues to make his escape from the fight in F23 by attempting to run directly away from Male 2 but is stopped by the arena wall. As seen in F12 (in Encounter 2, above), a cricket attempting to evade an enemy, rather than stop and reassessing his options, will use a burst of speed to overcome unwanted proximity to a dominant opponent. As Male 1 makes his escape in F24, Male 2 continues to broadcast his victory song in various directions. This encounter would be recorded as a win for Male 2 at ES Level 5+ (Figure 2.19).



Figure 2.19: Male 2 continues his victory song (F23) as Male 1 retreats away from his opponent (F24) (photo credit: DG).

NOTE:

As some studies would have stopped a trial by this point, the importance of plasticity in the formation of a behavioural dyad being exhibited in the continued interactions in Encounter 3 would be lost. This in turn implies that what some studies would call a dominant position is just a state of play in an ongoing power struggle that has not yet been finalised.

2.12 Fourth contact: Escape behaviour in response to contact from behind

2.12.1 The approach and escape response

After Male 1 fled from his opponent both crickets moved freely around the arena. In a scenario similar to Encounter 2 but with reversed dominance and sub-ordinance statuses, F25 shows Male 2 as he approaches Male 1 from behind. As Male 1 touches the rear right leg of Male 2 with an antenna, the now subordinate Male 1 makes brief antennal contact and responds in F26 with the start of an evasion manoeuvre. Lowered aggression associated with losing his recent fight prevents him from turning to face his opponent, as he did in F12 (Figure 2.20).

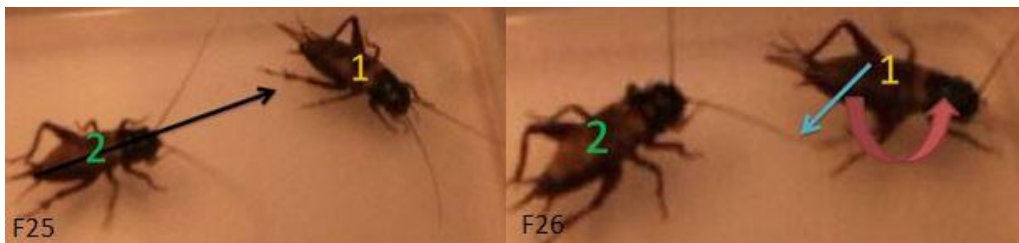


Figure 2.20: Male 2 approaches Male 1 from behind (F25). As Male 1 feels contact he immediately begins to turn away from the source of the contact (F26) (photo credit: DG).

2.12.2 Declaring victory

After turning fully away from his opponent in F27, Male 1 makes his escape. F28 shows Male 2 engaging in victory song having chased Male 1 away, having only recently occupied a subordinate status to the same opponent. This fight result would be recorded as a win for Male 2 at ES Level 1 (Figure 2.21).



Figure 2.21: Male 2 approaches Male 1 who flees from slight contact from behind (F27) and Male 2 begins victory song production (F28) (photo credit: DG).

NOTE:

Had Male 1 not made antennal contact with male 2, it would not be classed as a level 1 fight outcome. Nervous fleeing behaviour due to contact from behind is often seen in males that have lost a fight, regardless of the source of the stimuli.

Individual fights between males can be very energetic, often shifting between levels of escalation and moving around the arena environment as the males grip and wrestle each other, and the number of fights during one encounter can vary tremendously. The ability to read the fights and to understand what constitutes a victory is imperative for accurate data collection. The behavioural examples laid out above may occur in different orders, may occur multiple times, and the male that starts off as dominant may become subordinate over the course of an encounter, and vice versa.

2.13 An explanation of experiment-specific procedures used in this thesis

Four of the five chapters that make up the research components of this work revolve around the observation of fights between adult male *Gryllus bimaculatus*. In all cases the methods described above for observing and accurately deducing the outcome of the fights that occurred during a timed encounter were followed. Methods relating to encounter time window and study specific variations on what constituted a victory did vary by study, however, and so what follows is a chapter-by-chapter breakdown of what differed between studies in how data was collected and why.

2.13.1 Chapter 4

This study focussed on the use of the mandibles in combat, specifically whether mandible shape affected fight outcome. This study used a standard five minute encounter window to observe fight behaviour between naïve males. As time progresses during a fight, males use up their energy reserves and fight frequency can become less regular. As this study only required the more high energy fighting behaviours of mandible flaring and grappling (levels 3, 4 and 5 in Figure 2.7), a five minute encounter window was considered the best option to ensure that the males in use were fighting at a high level of intensity for accurate data collection.

2.13.2 Chapter 5

This study aimed to see how small males fared in a fight against a larger opponent. Five minutes was again used as an appropriate encounter period in order to have the best opportunity to observe high level fighting behaviour between the two males- larger males have higher stamina levels and so would likely beat a smaller opponent over time by default. My reasoning was also that for a small male to achieve dominant status, it would likely need to happen relatively soon into the encounter.

2.13.3 Chapter 6

2.13.3.1: Lover, not a fighter

Fights in the recreation of Judge et al. (2010) followed the methods laid out in the original paper. Judge et al. (2010) did not give a time limit for the encounter that was used to

determine winner or loser status. It was decided that consistently subordinate (level 1) behaviour for a two minute period was enough to say for sure that dominance had been attained.

2.13.3.2: Mating resets aggression

Fights in the recreation of Killian and Allen (2008) followed the methods laid out in the original paper. Their methods required that the males should fight until a level 1 response was observed, and that the males should then just be left for five minutes to maintain that dyadic hierarchy. However, male *G. bimaculatus* are very robust animals and frequently engage in multiple bouts of combat, often reengaging their opponent even after being beaten (as seen in 2.11 above). It was necessary to keep an eye on pairs of *G. bimaculatus* as the dominant-subordinate relationship could change repeatedly before a true dyad was formed.

After treatment and rest periods prescribed by their methodology, Killian Allen (2008) did not specify a time period for the second encounter to see what effect the treatment had had on their experimental male. As the outcome of the study was to see the effect of treatment on male behaviour, I set a three minute limit on the second fight as it was more than enough to register changes in agonistic behaviours from experimental males.

2.13.4 Chapter 7

Fight one: This study required a dominant-subordinate relationship be in effect between the weight-matched males, so unlike in Chapter 4 (above) I gave the pairs of males ten minutes to form a behavioural dyad. Five minutes was not always long enough for one of the males to gain the upper hand.

Fight two: A follow up fight period of ten minutes was utilised in this study because males had been given a motivational treatment to see if it changed their fighting behaviour. As certain aspects of the treatment phase revolved around time fixed biological processes, including the onset of the refractory period (see 7.12), a ten minute encounter window was the most logical choice.

CHAPTER 3. IS THERE A TRADE-OFF BETWEEN TESTES AND MANDIBLES IN ADULT MALE *GRYLLUS BIMACULATUS*?

3.1 INTRODUCTION

3.1.1 Competitive traits

Where males are forced to physically compete for mating rights, tests of strength and stamina have directed the evolutionary paths of male sexual dimorphism, often producing exaggerated or augmented structures that develop as secondary sexual traits and are principally associated with competition against rivals (Parker, 1974; Maynard Smith and Parker, 1976; Hill, 1994; Berglund et al., 1996; Emlen, 2008). The sum effect of these secondary traits and the ability of the bearer to use them in competitive displays against rival males is known as Resource Holding Potential (RHP), in other words the male's fighting ability. Where competition between males of the same species has led to the evolution of secondary traits that are used in direct physical contact as weapons (Emlen et al., 2005; Emlen, 2008) rather than ornaments (McCullough et al., 2016), highly aggressive and costly behaviour involving the use of these weapons against the opponent becomes an integral part of escalated display. Fights that incur greater physical costs on the opponents, and include elevated risk of injury, both from the opponent and themselves, over time will occur more often between individuals who are closely matched in terms of RHP (Alexander, 1961; Parker, 1974; Hofmann and Schildberger, 2001; Rillich et al., 2007; Lane and Briffa, 2017; see also section 1.2).

The winner is traditionally considered to be the male with a greater RHP (Tachon et al. 1999; Bateman, 2001b; Bateman and Toms, 1998). As male-male conflict often revolves around mating opportunities, either directly if a female is present, or indirectly if fighting over a valuable territory resource that may attract females (such as a burrow), males who defeat their opponents often have better access to receptive females, essentially acting as indirect sexual selection for weapon size (Rantala and Kortet, 2004; Savage et al., 2004). The resulting covariance of dominant status and favourable mating choices by females has made for positive sexual selection on male weapon development that over evolutionary time has led to increased pressure on males to produce larger and more refined traits. The

covariance of dominance and increased reproductive success has resulted in the extremes of sexual dimorphism, specifically with regard to the development of beneficial traits associated with success in contests, seen in the animal kingdom today (Hill, 1994; Houslay and Bussiere, 2012; Emlen, 2008; McCullough et al., 2016).

3.1.2 Fighting or mating

There is however a distinction between acquiring mating rights and achieving successful fertilization (Lemaître and Gaillard, 2013; Ferrandiz-Rovira et al., 2014). The covariance of dominance-linked traits in male animals, such as large body size and weaponry, with increased mating success has been demonstrated in many animal species (Emlen, 2008). In most cases the extremes of sexual dimorphism associated with mating behaviour are the result of a developmental system involving a trade-off between investment in primary traits such as testes against secondary sexual characteristics, including weaponry, though the direction and degree of trade-off is dependent on the behavioural ecology of mating in that species (Lüpold et al., 2014). Some examples of a trade-off mechanism between testes size and weaponry can be seen in male Artiodactyls (Preston et al., 2001), multiple Cetacean species (Dines et al., 2015), multiple Pinnipeds (Fitzpatrick et al., 2012), the Anuran *Crinia georgiana* (Buzatto et al., 2017), many Coleoptera species (Moczek and Nijhout, 2004; Pomfrett and Knell, 2006; Simmons and Emlen, 2006), the Hemipteran *Narnia femorata* (Sasson et al., 2016), and the Blattodean *Gromphadorhina oblongata* (Durrant et al., 2016).

Among the insects, the beetles have been studied in great detail and show high degrees of plasticity in terms of final adult size, the scaling of weaponry within allometric limits, and developmental trade-offs during larval growth (Cook, 1993; Kawano, 1997; Moczek and Emlen, 1999; Moczek, 2003; Knell et al., 2004; Okado et al., 2006, and see the General Introduction). Within the Orthoptera, developmental investment and use of weaponry is most accentuated in the Anostomatidae: the King Crickets of Africa and the Wetas of New Zealand show very high levels of sexual dimorphism; the males of this Family often possess greatly enlarged, tusked mouthparts and this mandibular weaponry plays a central role in securing and maintaining mating rights with groups of receptive females that gather in tree bark hollows, known as galleries, that the males claim and defend (Moller, 1985; Bateman, 2000; Kelly, 2006; Kelly, 2007; Kelly, 2008). This system is analogous to the harem-

keeping behaviour seen in deer and resource-defence observed in many beetle species and in the Wellington tree weta *Hemideina crassidens* has produced multiple male morphs with differing emphases on weapon development or ejaculate production (Kelly, 2008). Smaller males, specifically those that mature at the 8th instar, are less well armed than their 10th instar maturing conspecifics, but produce much larger ejaculates than 10th instar males, despite having no significant difference in testes mass relative to body size (Kelly, 2008). The smaller morph relies on post-copulatory mechanisms of sperm competition in terms of successful fertilization (Kelly, 2008; Lüpold et al., 2014). See section 1.3.4 of the Introduction for more information on weta evolution and mating strategies.

3.1.3 Gryllid systems

3.1.3.1 Spacing between males

Whilst the well-armed wetas establish and maintain ownership of territories and harems, with the result that the paternity of resulting offspring is a reflection of their RHP, for the Gryllid field crickets territoriality and paternal returns are not so clear cut. Population densities can be high for these animals, with many individuals living in close proximity to each other, especially when dispersal is limited by habitat structure. Therefore, stridulatory singing by mature males acts not only as a means of making an individual known to his neighbours, but components of this song act as primary indicators of his RHP (Dixon and Cade, 1986; Hack, 1997a; Simmons, 1986, 1988a; Brown et al., 2006), and therefore acts as a spacing mechanism to minimise fights starting by males running into each other. See section 1.4.2.1 of the Introduction for more information on the function and content of advertisement calling in the Gryllidae.

3.1.3.2 Fighting behaviour in field crickets

Any physical confrontation between male field crickets conforms to a stereotyped escalatory sequence beginning with comparative, mutual visual assessment with the potential to lead on to costlier levels of interaction, during which cricket mandibles are first displayed and then used as weapons for grasping the enemy in physical bouts of aggression and dominance (Alexander, 1961; Hofmann and Schildberger, 2001; Brown et al., 2006; Judge and Bonanno, 2008). The need for a contest system made up of discrete steps on an

escalatory scale allows for damage mitigation by giving males the option to not engage in dangerous or costly contests in an often high density, mixed sex population (Alexander, 1961; Tachon et al., 1999; and see section 1.4.1). Such a high sperm competition environment, with both pre- and post-copulatory pressures from other males and polyandrous females occupying the same habitat, is common among the Gryllidae and while few studies have specifically examined weaponry development within this group, there is some evidence to suggest that weaponry does play a role in achieving paternal success, with females preferentially mating with dominant males (Kortet and Hendrick, 2005; Bretman et al., 2006; Loranger and Bertram, 2016), thus making it likely to be positively selected for. It is worth noting though that among the Gryllidae, body size and weight are considered principle indicators of RHP and a high degree of weight asymmetry between rivals is used as a primary deciding factor during early mutual assessment between males as to whether or not to escalate as larger males can aggressively outcompete smaller rivals (Dixon and Cade, 1986; Hack, 1997a, 1997b; Brown et al., 2006; Briffa, 2008), with only closely matched opponents escalating to a point where comparative mandible size becomes a factor (Hofmann and Schildberger, 2000). While the effect of mandible size on fight outcome in *Acheta domesticus* is marginal (Briffa, 2008) with levels of aggression and resource value having a greater influence on fight outcome (Buena and Walker, 2008), work on *Gryllus bimaculatus* shows that weapon size is an indicator of physical prowess (Hofmann and Schildberger, 2000), mandible flaring by a well-armed male can be enough to cause a more lightly built opponent to flee from combat (Rillich et al., 2007), and male *G. pennsylvanicus* with larger mandibles find greater success in grappling combat against more lightly armed opponents (Judge and Bonanno, 2008; Judge et al., 2010).

3.1.3.3 *Gryllus bimaculatus*

The black field cricket, *Gryllus bimaculatus*, is very commonly used in experimental studies focussed on competition and mate guarding. The males show consistently high levels of aggression towards each other at almost all times once antennal contact is made (Adamo and Hoy, 1995; Hofmann and Schildberger, 2001; Nagamoto et al., 2005; Sakura and Aonuma, 2013), and dominance may require repeated bouts of high energy grappling using mandibles as weapons, especially between evenly matched opponents or between males fighting over a female (Khazraïe and Campan, 1997; Tachon et al., 1999; Aonuma, 2017).

Once a dominant male has transferred a spermatophore to his mate he will engage in post-copulatory mate-guarding, attacking opponents with the same range of behaviours as those exhibited pre-mating. Initially high, the intensity of this post copulatory aggression behaviour diminishes as the male enters a refractory period (Parker and Vahed, 2009). Females are known to preferentially mate with dominant males, and females mated to large aggressive males will leave the spermatophore attached for longer than females mated to small/submissive/loser males (Simmons, 1986; Bateman et al., 2001a; Bretman et al., 2006). This reinforces the notion that weaponry in *G. bimaculatus* may be under positive sexual selection, as it is in other insect taxa, as it plays a key role in terms of dominance and reproductive success. Developmental trade-offs that have already been identified in Gryllid cricket species include males and females in the wing dimorphic *Gryllus firmus*: micropterous (short-winged) morphs whilst unable to disperse as freely as the macropterous (long-winged) forms make higher investment in gamete production and earlier reproductive development leading to improved reproductive success by both males and females (Saglam et al., 2008). In addition male short-winged morphs of *G. firmus* and *Gryllus texensis* spend more energy engaging in calling song than long-winged males (Crnokrak and Roff, 1995; Guerra and Pollack, 2007). Use of weaponry in male-male contests has only been shown to be integral to fight outcome in *Gryllus pennsylvanicus*, with males that have larger teeth than their opponent winning more fights (Judge and Bonanno, 2008). Gryllid agonistic conflict is very commonly mentioned in studies of aggression, especially with regard to competition over females (Simmons, 1986; Tachon et al., 1999; Nagamoto et al., 2005), and whilst studies using male *G. bimaculatus* have shown that males display their mandibles as indicators of fighting ability (Hofmann and Schildberger, 2001) and that males with disabled mandibles lose more fights than unhandicapped opponents (Rillich et al., 2007), no other study has focussed on the weaponry itself.

Given that males of this species engage in prolonged bouts of fierce fighting and that dominant males have greater reproductive success (Simmons, 1986; Bateman et al., 2001a; Bateman et al., 201b; Bretman et al., 2006), it is reasonable to assume that this weaponry is under some form of sexual selection, and that mandibular sexual dimorphism would be apparent. *G. bimaculatus* is known to be sexually dimorphic in terms of mass, with female body weight being on average greater than males, a pattern commonly observed

throughout the Orthoptera (Whitman, 2008). Although the extent of sexual dimorphism in mandibles in this species has yet to be investigated, in the related species *Gryllus pennsylvanicus* sexual dimorphism in mandible length was demonstrated by Judge and Bonanno (2008). This had been predicted in Alexander (1957), and similar descriptions of sexual dimorphism in African Gryllids are mentioned in Otte and Cade (1984). While Judge and Bonanno (2008) studied the sexually dimorphic North American *G. pennsylvanicus* and found males do possess larger weapons than females, the current study is the first to quantify the degree of sexual dimorphism in weapons and other anterior structures in *G. bimaculatus*. There has also been no previous study to examine whether a trade-off between weapon size and testes size occurs in this species, as it does in other insect taxa (Moczek and Nijhout, 2004; Pomfrett and Knell, 2006; Simmons and Emlen, 2006; Sasson et al., 2016; Durrant et al., 2016).

3.1.4 Aims

This study aims to confirm that adult *G. bimaculatus* show sexual dimorphism of the head and mouthparts owing to the fact that males use them in combat against rivals, whilst females mainly use them for processing food. The study also aims to test the prediction that adult male *G. bimaculatus* that have larger and more symmetrical mouthparts will have comparably smaller testes relatively to body size/mass due to developmental trade-offs.

3.2 METHODS

Individual adult male (n=56) and individual adult female (n=43) *Gryllus bimaculatus* were randomly selected from the cultured populations maintained in the breeding room fully described in 2.1.1 of the General Methods. The crickets were then euthanized by freezing and individually stored as per section 2.4 of the General Methods.

3.2.1 Method for measuring cricket body and mandibular teeth size

The basic dissection and measurement of all 99 euthanized specimens was carried out as per standard protocol as detailed in section 2.5 of the General Methods.

3.2.2 Mandible excision and measurement

The mandibles were excised from the head following section 2.5.2 of the General Methods. Measurements for both mandible lengths were taken as the longest point from the distal tip to the lateral articulation (Figure 2.5) as per Judge and Bonanno (2008), and recorded in mm to 1 decimal place using a calibrated eyepiece graticule at 10x magnification. These two measurements were combined for a measure of mandible span. The procedure was identical when excising and measuring male and female mandibles.

3.2.3 Removing and weighing the testes

A mounted needle was pushed through the femur of each hind leg and used to apply light pressure away from the body. The leg itself comes away quite easily at this point.

The thorax was then held firmly with tweezers while a no.11 scalpel blade was used to make a single incision along the lateral surface of the abdomen, starting at the point where the femur met the body. This cut ended at or near the cerci. This was repeated on both sides of the abdomen. A small cut on the ventral surface was then made between the saltatorial appendage points under the body to allow access to the body cavity. The three cuts allow for the abdomen to be neatly opened. The gut and bowel were removed from the abdominal cavity and discarded. Once the individual testes were located, they were removed from the surrounding tissue using a mounted needle (Figure 3.1).



Figure 3.1: Excised *G. bimaculatus* testes.

Any remnants of the nearby accessory organ were removed using tweezers and a mounted needle. The accessory organ was not considered necessary for the line of investigation carried out here. Whilst the organ does play a role in spermatophore formation in the Gryllidae, it is not used to adapt the spermatophore to include anti-aphrodisiac components, including allomones, that have evolved to impact female receptivity as is seen in many Tettigonid bush crickets; female field crickets appear to lack a mating refractory period (Fleischman and Sakaluk, 2004). Any excess body fluid was removed by capillary action using a paper towel and the testes were weighed on a set of AND HR-120 electrical scales accurate to 0.0001g, and recorded on the spreadsheet as actual mass and as a percentage of the animal's total mass.

Individual glass jars containing 70% IMS were used to preserve the specimens, including any excised tissues from each the dissected crickets, once all measurements had been made. All storage jars containing cricket bodies were labelled with a fine permanent marker pen with ID number and study number for potential use in any future work.

3.2.4 Statistical Methods

3.2.4.1 Sexual dimorphism in G. bimaculatus

To look at whether there was sexual dimorphism in the mouthparts and other anterior structures of *G. bimaculatus*, morphological measurements were checked for normality and a general linear model was built with mandible span as a response variable with sex, head

width and their interaction as predictor variables. Individual linear models of mandible span against head width and pronotum width in both males and females were then made to see how the slopes varied between the sexes.

3.2.4.2 Trade off in testes and weapon size in male G. bimaculatus

Individual linear models looking at trait size relationships in adult male *G. bimaculatus* were built to see which traits scaled positively and whether any traits exhibited signs of a trade-off in development. All statistical work was carried out using R Studio, R version 3.4.1. GGplot2 and visreg packages were used to produce the figures used in the results section.

3.3 RESULTS

3.3.1 Section A: Is sexual dimorphism of the mandibles and associated anatomy evident in *G. bimaculatus*?

Preliminary visual checks (histograms and box plots) indicated a normal distribution.

Unpaired t-tests were used to compare the measurements from both sexes. Males and females differed significantly in mean body mass, with females being significantly heavier than males ($t=3.203$, $d.f.=74$, $p=0.002$, Fig 3.2).

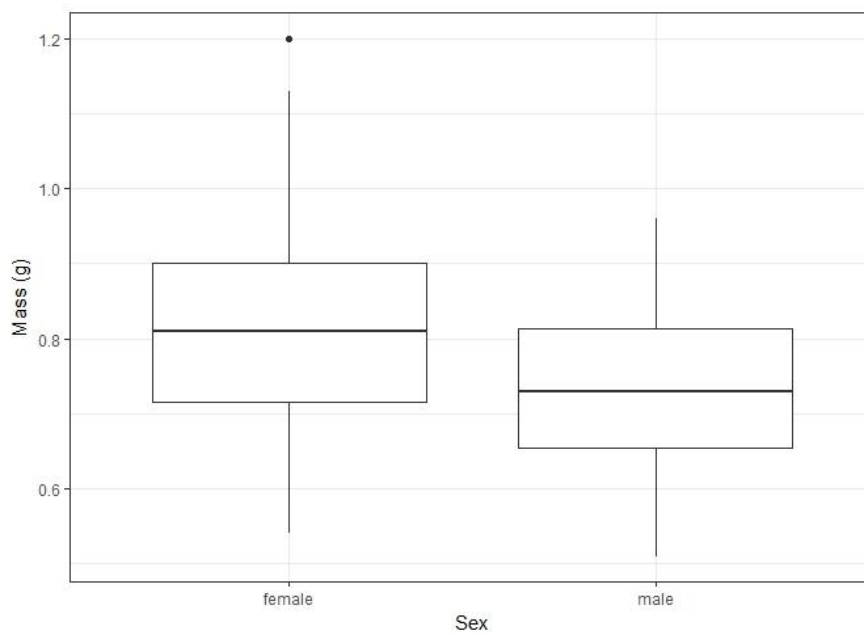


Figure 3.2: Boxplot showing median and interquartile range comparing female and male mass (g).

As predicted, pronotum width was significantly different between males and females ($t=-9.066$, $d.f.=95.34$, $p=1.594e-14$, Fig 3.3).

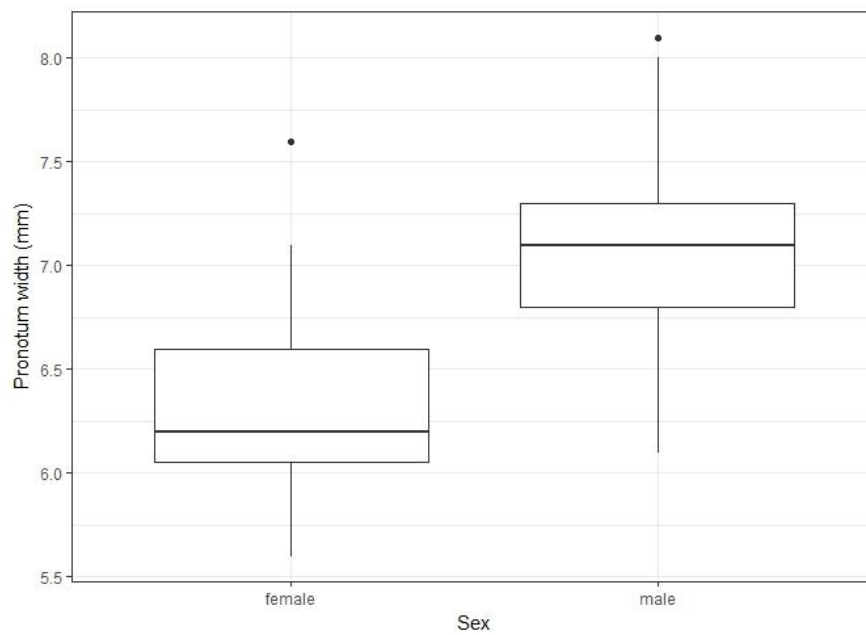


Figure 3.3: Boxplot showing median and interquartile range comparing female and male pronotum width (mm).

Head width was also significantly different between males and females ($t = -12.437$, $d.f. = 95.994$, $p < 2.2e-16$, Fig 3.4).

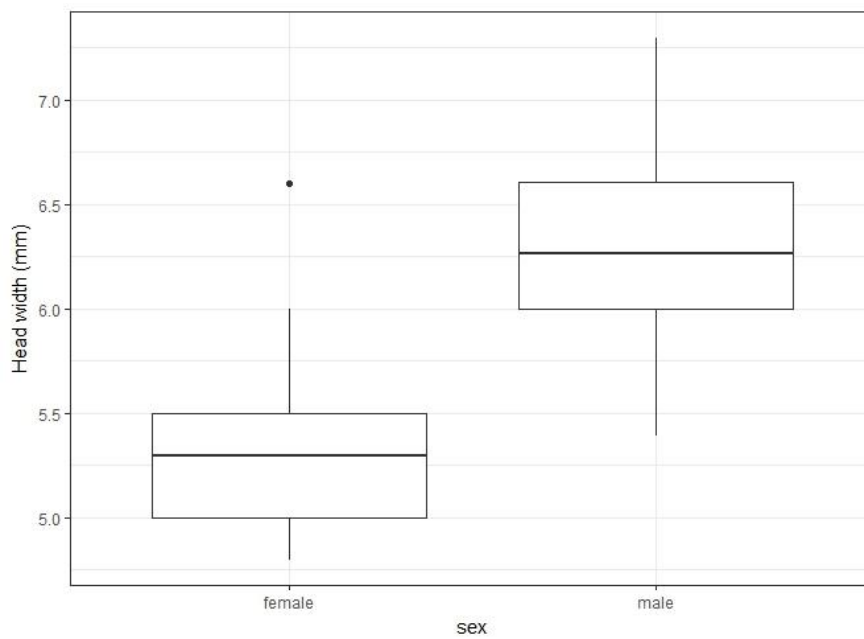


Figure 3.4: Boxplot showing median and interquartile range comparing female and male head width (mm).

Finally, mandible span was also significantly different between males and females ($t = -16.04$, $d.f.=86.557$, $p < 2.2e-16$, Fig 3.5).

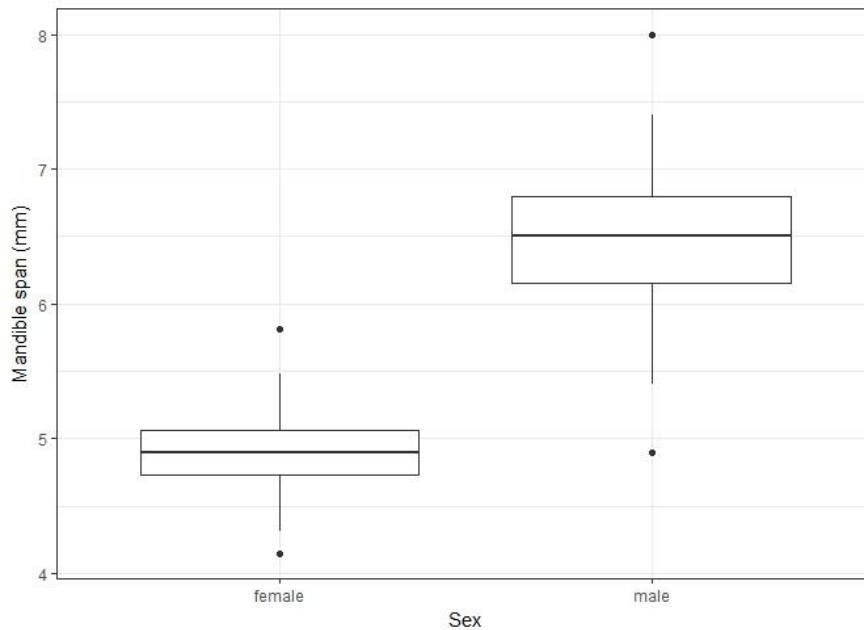


Figure 3.5: Boxplot showing median and interquartile range comparing female and male mandible span (mm).

Males were shown to be significantly larger in all traits except adult body weight.

A combined linear model with mandible span as the dependent variable with sex, head width and their interaction as predictor variables, showed that while mandible span increased with head width for both males and females ($\text{slope} \pm \text{S.E.} = 0.459 \pm 0.128$, $t = 3.597$, $d.f. = 95$, $p = 0.0005$), sex was a significant predictor of mandible span, with males having greater mandible span than females ($\text{slope} = -3.593 \pm 0.907$, $t = -3.96$, $d.f. = 95$, $p = 0.001$).

Furthermore, a significant interaction between sex and head-width indicates that mandible span increased with head-width at a greater rate for males than for females ($\text{slope} = 0.739 \pm 0.159$, $t = 4.461$, $d.f. = 95$, $p = 1.11e-05$) (Figure 3.6).

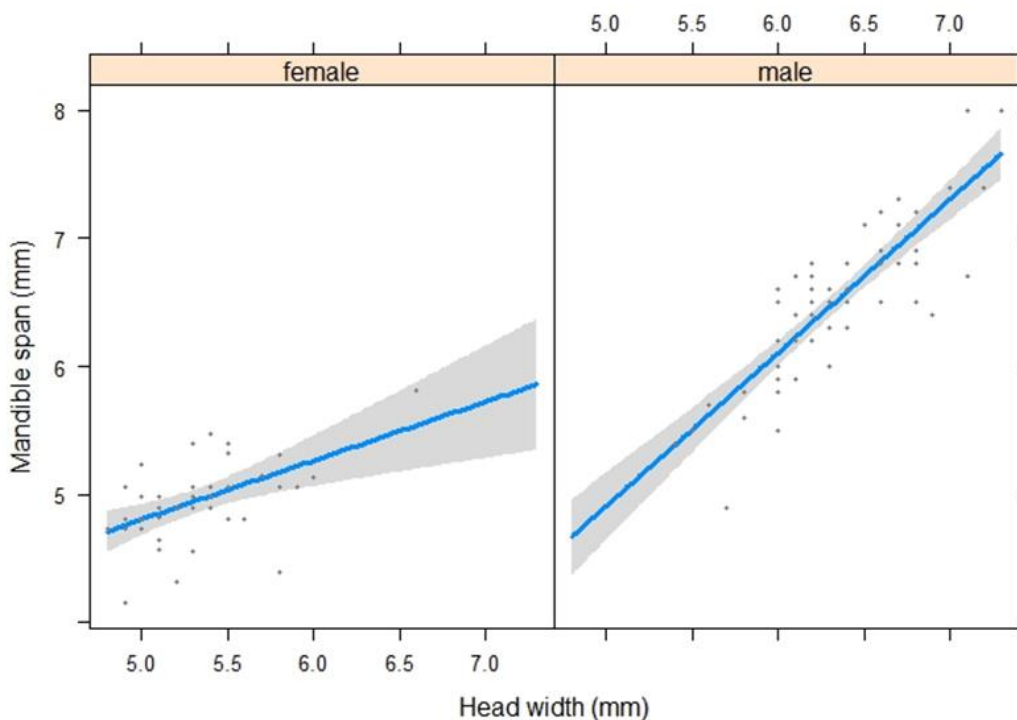


Figure 3.6: Scatterplot of mandible span modelled on head width, in female and male *G. bimaculatus*, with linear regression lines fitted.

Linear regressions revealed significant positive relationships between male mandible span and both head width (slope= 1.2 ± 0.1 , $t=11.96$, d.f.=54, $p < 2e-16$) and pronotum width (slope= 1.06 ± 0.11 , $t=9.87$, d.f.=54, $p=1.06e-13$). Significant positive relationships were also found between female mandible span and both head width (slope= 0.45 ± 0.11 , $t=4.02$, d.f.=41, $p=0.0002$) and pronotum width (slope= 0.49 ± 0.09 , $t=5.05$, d.f.=41, $p=9.5e-06$).

3.3.2 Section B: Is there a trade-off between weapon size and testes investment in *G. bimaculatus*?

In addition to the positive relationship between mandible span and head width in males, above, linear regressions revealed positive relationships between head width and both pronotum width (slope= 0.87 ± 0.05 , $t=17.55$, d.f.=54, $p < 2e-16$) and length (slope= 0.91 ± 0.09 , $t=9.45$, d.f.=54, $p=4.78e-13$) (Figure 3.7). There was no significant relationship between body mass and testes mass (slope= 0.01 ± 0.01 , $t=1.39$, d.f.=54, $p=0.17$) or between mandible span and testes mass (slope= 29.2 ± 17.7 , $t=1.62$, d.f.=54, $p=0.11$), (Figure 3.8).

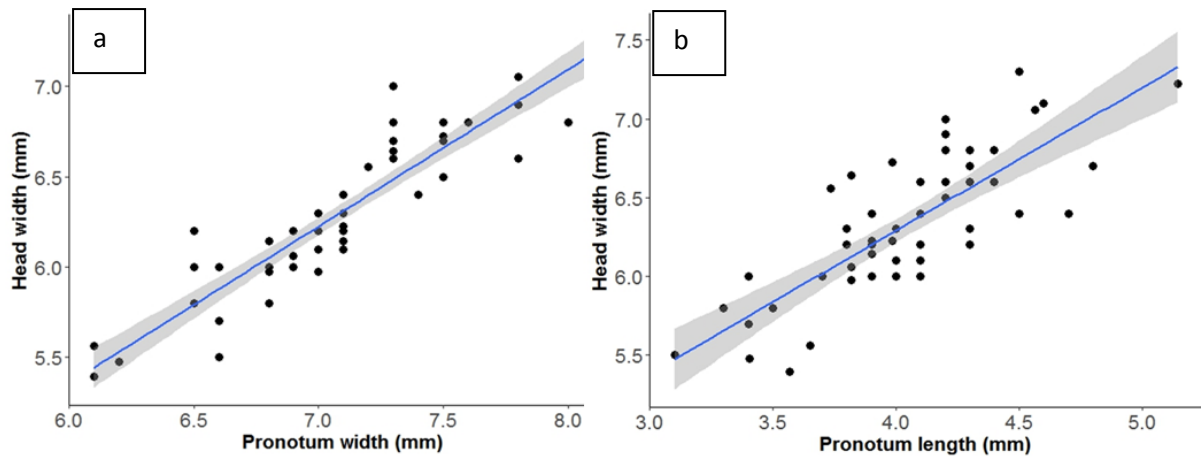


Figure 3.7: Linear regression of male *G. bimaculatus* head width modelled against pronotum width (a), and linear regression of male *G. bimaculatus* head width modelled against pronotum length (b).

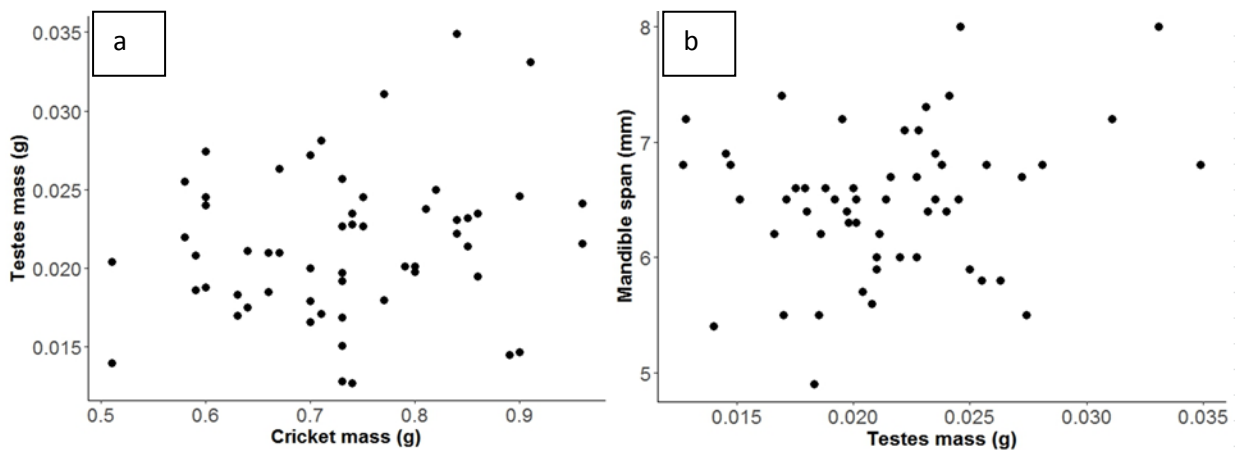


Figure 3.8: The relationship between adult *G. bimaculatus* testes mass and cricket mass (a), and the relationship between adult *G. bimaculatus* mandible span and testes mass (b).

A linear model with testes mass as the dependent variable and both body mass and mandible span, and their interaction, as predictor variables, showed that neither male mass (slope= -0.05 ± 0.06 , $t = -0.94$, d.f.=52, $p = 0.35$), mandible span (slope= -0.01 ± 0.01 , $t = -0.78$, d.f.=52, $p = 0.44$), nor their interaction (slope= 0.01 ± 0.01 , $t = 1.01$, d.f.=52, $p = 0.32$) were significant predictors of testes mass. Removing the non-significant interaction term did not improve the fit of the model ($\Delta AIC < 2$).

3.4 DISCUSSION

3.4.1 Is sexual dimorphism of the mandibles and associated anatomy evident in *G. bimaculatus*?

Analysis of morphological characters of male and female *G. bimaculatus* has shown that there is significant sexual dimorphism in mandible size, with males possessing larger jaws than females. The way in which mandible size scales with body size also differed between the sexes, with the slope of the relationship between mandible length and head width being greater in males than in females. For both sexes the mandibles are a means of processing food – mouthparts from both sexes feature a sharpened proximal edge that acts as a blade and a molar structure close to the oral opening which is used for grinding food prior to ingestion. In terms of functionality the mandibles of both sexes are very capable of managing dietary requirements, effectively working on most available foodstuffs and allowing the species to accept a wide range of substrates on which to feed. Dietary fulfilment aside, the main difference between male and female field crickets is that males will readily and repeatedly engage in bouts of violent combat against their rivals (Simmons, 1986; Adamo and Hoy, 1995; Tachon et al., 1999), and a central component of this combat involves using the mandibles as weapons for signalling threat and physical engagement of a rival.

Whilst the Orthoptera are highly regarded for their aggression and enthusiasm to engage in escalatory behaviour that involves the display and use of their mandibles in combat (Hofmann and Schildberger, 2001; Rillich et al., 2007; Judge and Bonanno, 2008), the majority of weapon research that has been produced on the Orthoptera has used the Anostostomatid wetas (Jamieson, 2002; Kelly, 2006; Kelly, 2007) and king crickets (Bateman, 2000). However, even in this family, adult mandible size has not evolved to the point of hyper-allometric scaling that is so often the case with beetles, despite their similar use in male-male combat. This may be due to natural selection acting in a different way to restrict final adult size in crickets and beetles. Whilst larval foraging success mediates the scale and effectiveness of beetle weapons and associated morphology (Hanley, 2001; Emlen et al., 2005; Emlen, 2008), crickets need their mouthparts to maintain their effectiveness into adulthood as they do not undergo a metamorphic shift in form or function of their anatomy.

Crickets are highly territorial and use their mandibles in repeated displays of aggression and stamina for food and mates (Alexander, 1961; Adamo and Hoy, 1995; Briffa, 2008). Their effective use as weapons and tools is likely to be under selective pressure both in terms of natural selection for base functions and survivability but also indirect sexual selection once mature; dominant and successful males are often well armed and get to mate more often (Bretman et al., 2006; Emlen, 2008). Reproductive functionality presents itself very differently in the two orders, and while the wetas and king crickets in one cricket family might demonstrate some aspects of mating behaviour that are reminiscent of those seen in certain beetle species (Bateman, 2000; Kelly, 2006; Kelly, 2007), the fundamental differences in life cycle and development are a possible reason as to why hyper-allometry has not been seen among the Orthoptera. Given that male Gryllids engage in vigorous and violent competition, that the effect of male weaponry on fight outcome has been highlighted in other studies using *G. pennsylvanicus* and *G. bimaculatus*, and that aggression studies focussing on mate-guarding and female mate selection for dominant males in this genus are many (Hack, 1997; Nelson and Nolan, 1997; Hack, 1998; Bateman et al., 2001b; Bretman et al., 2006), the fact that *G. bimaculatus* exhibits sexual dimorphism in physical parameters related to success in combat is not surprising.

The sexually dimorphic traits highlighted in this work, specifically the fact that pronotum width, head width and combined mandible span all display male bias, relate to combat performance. High level escalation during fighting in *G. bimaculatus* (Level 5 grappling) involves taking hold of the opponents head if possible, or at least locking jaws, before pushing him and attempting to tip him over (Alexander, 1961; Adamo and Hoy, 1995; Hofmann and Schildberger, 2001). Goyens et al. (2015c) showed that the pronotum of male stag beetles houses hypertrophied muscle mass to aid lifting and pushing during fights, and it is therefore reasonable to assume that Gryllid pronotal width might function in a similar capacity, supporting the head during a fight between male crickets whilst mandibular grappling is ongoing. The head itself is the location of the adductor muscles used to bring the mandibles together in a bite motion when wrestling an opponent. In other insects that use the jaws as weapons, longer muscle attachment points are needed to compensate for longer mandibles and still provide input leverage power (Goyens et al., 2014a). Therefore a larger head for larger adductor attachment area would increase bite pressure which would

be advantageous when trying to beat a conspecific. This factor has been measured in *Teleogryllus commodus*; males that won fights were shown to exhibit significantly more powerful biting ability (Hall et al., 2010). The actual length of the mandibles in this situation equates to the degree of reach that a male has relative to his opponent – a male in possession of longer mandibles than his opponent not only benefits from being able to close his jaws over a wider portion of his opponents head but longer mandibles might also function to keep the enemy further away from his more delicate facial structures such as the maxillary and labial palps. Unilateral and bilateral stages of escalation (Level 3 and 4 on Figure 2.7) during male-male confrontation specifically involve flaring of the mandibles toward the opponent and would therefore act as a direct visual comparison for males assessing their opponent's weaponry relative to their own mandible span. Identification of any potential asymmetries in weapon size and consideration of the potential risks of escalating to full grappling behaviour may be an important component of assessment strategy that acts to minimise futile and potentially harmful escalation.

It should be noted that while female Gryllids do not often enter into combat they are not passive creatures. Under experimental conditions with limited available resources they have been observed participating in escalatory combat with conspecifics over food in *T. oceanicus* (Fuentes and Shaw, 1986) and *A. domesticus* (Nosil, 2002). Aggression from female *A. domesticus* over food as a resource was even shown to be enough to drive away males (Adamo and Hoy, 1995) and in a kin recognition study female *G. bimaculatus* were seen to aggressively attack courting males if the females sensed they were related as half siblings or more (Simmons, 1989). When multiple female *G. campestris* were exposed to recorded mating song but not allowed to mate they were seen to enter into full escalatory combat with other females, and although females cannot sing to indicate RHP or victory, *T. oceanicus* were observed in a few instances raising their wings in a stridulatory-like manner (Fuentes and Shaw, 1986). Whilst this female-centred aggression was shown following the same escalation as males, including mandible flaring (Rillich et al., 2009) it was noted that females did not use their mandibles as primary weapons, but were seen to use their powerful hind legs to kick their opponents in displays of strength and aggression.

3.4.2 Is there a trade-off between weapon size and testes investment in *G. bimaculatus*?

This study aimed to identify whether adult *G. bimaculatus* exhibited signs of a trade-off system that would result in greater developmental emphasis on weaponry size or testes size. Linear regressions conducted on various combat-oriented aspects of male anatomy (head width, mandible span, pronotum width, pronotum length) showed high degrees of relatedness. However, no significant relationships were found from linear regression of testes mass and body mass or, importantly, between mandible span and testes mass.

Even though no relationship, not even a neutral isometric increase based on final adult size, could be found between the size of testes and mandibles in the sample of adult male *G. bimaculatus* specimens used for this study, this does not mean that all males see a relatively identical return in terms of sperm competition and reproductive fitness. Given the fact that male *G. bimaculatus* do not seem to invest in weaponry preferentially to testes mass, one conclusion that can be drawn is that mating success and reproductive fitness look to be primarily dictated by behavioural mechanisms rather than investment in weaponry. Their highly aggressive nature both before and after mating indicates that male field crickets engage in both pre- and post-copulatory mechanisms of sperm competition (Parker and Vahed, 2009; Sakai et al., 2017), including mate guarding the female into the refractory period to prevent premature spermatophore removal by the female herself or by competing males (Bateman, 1998; Bateman et al., 2001a; Wynn and Vahed, 2004), and that their weaponry is actually just one component of this conflict-focussed strategy.

In some insect species, the “decision” to invest more heavily in either weapons or testes occurs during larval development and is thought to be mediated by nutrient availability during the larval stages of development; that is males that find greater success whilst foraging experience greater growth and store more nutrients for use during metamorphosis, triggering a physiological trigger point leading to greater investment in weaponry (Hanley, 2001; Madewell and Moczek, 2006). This is thought to be the primary factor leading to positive allometric expression in the largest adult males, and explains the degree of intrasexual dimorphism of the jaws and associated structures in many groups, including male Staphylinid dung beetles (Forsyth and Alcock, 1990), Lucanid stag beetles (Knell et al., 2004), flour beetles, *Gnathocerus cornutus* (Okada et al., 2006), and scarabs (Simmons and

Emlen, 2006). Species in which extremes of intrasexual variation occur often also exhibit 'lesser' males that, rather than possessing the enlarged weaponry of the 'major' males, are lightly built, possessing only rudimentary male traits and do not engage in agonistic behaviour with other males, but instead find reproductive success as sneaker or satellite males by evading or out-manoeuvring their larger peers, and invest more heavily in testes size (Emlen, 1997; Madewell and Moczek, 2006). This alternative evolutionary strategy is effective, so much so that sneaker adult male Staphylinid beetles, *Leistotrophus versicolor* have been observed to achieve successful mating with a female even as the lesser male himself is being courted by a major male (Forsyth and Alcock, 1995). The Orthoptera it seems are just not as phenotypically plastic as their beetle relatives. Within this order only the wetas exhibit anything like the degree of developmental trade-off seen in the beetles. The wetas' mating system centres around resource defence polygyny (Kelly, 2006) and the resultant trimorphic forms of male *H. crassidens* (Kelly, 2008) that mirror the resultant forms seen in similar mating systems in the Coleoptera support the notion that developmental trade-offs do work for that system. Such mating arrangements do not really appear within the Gryllidae, or at least not for long, as the extremely changeable nature of resource holding and polyandry/polygyny makes for a far more plastic set of behaviours that cover all scenarios.

Studies examining cricket development and growth have demonstrated differences in adult males based on increased nutrition during nymphal stages, but, unlike coleopteran species in similar circumstances, adult male *G. lineaticeps* fed high calorie and protein rich food were shown to allocate their additional resources to song production and were able to spend considerably longer periods producing metabolically expensive mate-calling with no statistical morphological difference in body shape, size or mass when compared to males fed a control foodstuff (Wagner Jr and Hoback, 1999). The same conclusion that body size has no effect on song content specifically is also noted in *G. bimaculatus* (Miyashita et al., 2016). Components of mating and calling song including chirp rate and amplitude and the ability to sing for longer at greater range are informative of phenotype and RHP in *G. bimaculatus* (Simmons, 1988; Zhemchuzhnikov et al., 2017), *G. veletis* (Zuk, 1987), *G. pennsylvanicus* (Zuk, 1987), *A. domesticus* (Gray, 1997; Brown et al., 2006), and *G.*

campestris (Simmons, 1995), and as phonotactic females do elect to move toward potential mates based on singing ability, this is an understandable form of investment.

In conclusion, although a high degree of sexual dimorphism of the head and mouthparts was identified in *G. bimaculatus*, there was no evidence of developmental trade-offs in testes mass-weapon production. The role of relative mandible size in determining the outcome of aggressive encounters between males deserves further study in this species, and is the subject of the following chapter.

CHAPTER 4. DOES MANDIBLE SIZE AND SHAPE HAVE AN EFFECT ON FIGHT OUTCOME IN *GRYLLUS BIMACULATUS*?

4.1 INTRODUCTION

Males that are successful in terms of securing reproductive success often do so by engaging in displays of aggression and fierce fighting with their conspecifics in order to establish ownership of a high value resource (Parker, 1974; Maynard Smith, 1991; Berglund et al., 1995; Reichert and Quinn, 2017). Fighting often involves the use of enlarged or elaborate extensions of morphological structures, such as the jaws of stag beetles or the horns of ungulates (Barrette and Vandal, 1990; Bartos and Bahbouh, 2006), as weapons that have evolved to be expressed as secondary sexual traits for the purpose of engaging in male-male agonistic conflict (Emlen and Nijhout, 2000; Emlen, 2006; Husak et al., 2009; Okada et al., 2012). As the actions of males in agonistic conflict have been studied for many years, how an individual behaves during a fight is now commonly referred to as his 'resource holding potential'. First coined by (Parker (1974), it is a frequent subject of scientific investigation, and RHP has been studied in the context of asymmetric contests and decision-making (Maynard Smith and Parker, 1976; Parker and Rubenstein, 1981; Arnott and Elwood, 2008); individual fighting ability and the use of weaponry (Barrette et al., 1990; Bartos and Bahbouh, 2006; Madewell and Moczek, 2006); skill in competition (Briffa and Sneddon, 2017), energy costs associated with display and fighting (Andersson, 1982; McComb, 1991; Briffa and Sneddon, 2006), reaction to injury and risk (Ercit and Gwynne, 2015), and even how an individual performs when costs associated with fighting are very high and might include death (Le Boeuf, 1974; Cook et al., 1999; Batchelor and Briffa, 2010). See sections 1.2.3 for a more in-depth look at RHP components in general animal contests, and 1.4.2 for RHP factors in field cricket contests.

4.1.1 Assessment

Contests over mating opportunities often begin with an initial assessment for purposes of species identification and sex confirmation (Adamo and Hoy, 1995; Nagamoto et al., 2005). In addition, rival males are also expected to assess the value of the resource and potentially the RHP of their opponent, relative to their own RHP (Maynard Smith and Parker, 1976;

Hurd, 2006). However, the capacity of fighting animals to engage in such ‘mutual assessment’ of RHP might be subject to constraints centred on the cognitive complexity of this task (Arnott and Elwood, 2009; although see Fawcett & Mowles 2013) or simply on the availability of reliable information during a dynamic interaction. Thus, an alternative hypothesis of ‘self-assessment’ (Payne & Pagel 1997; Arnott & Elwood 2009; Briffa & Elwood 2009) has been proposed, where animals fight up to an individual threshold of costs accrued during the fight. Nevertheless, mid-stage assessment often involves visual components that, at least in principle, could allow for a mutual comparison of RHP indicator traits such as cheliped size in *Diogenes nitidimanus* hermit crabs (Yoshino et al., 2011), eye span in *Taleopsis dalmanni* stalk-eyed flies (Small et al., 2009), body size in *Phidippus clarus* jumping spiders (Elias et al., 2010), tail length in *Xiphophorus helleri* swordtail fish (Benson and Basolo, 2006), and antler size and degree of branching in *Cervus elaphus* red deer (Bartos and Bahouh, 2006). There is good evidence to suggest that the display of such physical attributes is an advertisement of the owner’s physiology and health as it reflects his life history. Adult weapon size is limited by foraging success during the juvenile stage (Hanley, 2001; Knell et al., 2004; Okada et al., 2006; Lavine et al., 2015) and physiological trade-offs necessary for growth (Rantala and Roff, 2005; Okada et al., 2012; Goyens et al., 2015a; Körner et al., 2017), especially in traits that follow positive allometric development. Therefore, weapon size is likely to reflect the male’s potential fighting ability should the encounter escalate to physical combat (Emlen et al., 2012; Lavine et al., 2015), and may be an honest indicator of RHP (Parker, 1974; West-Eberhard, 1979). Once direct physical combat ensues, both parties begin using up energy reserves and risk injury the longer the fight continues (Parker, 1974; Maynard Smith, 1979; Hack, 1998).

4.1.2 Fighting and reward

The outcome of these escalated bouts of fighting is driven by differences in stamina and strength, in other words the ability to bear energetic costs and (in the case of injurious fights) inflict injuries on the opponent. Thus, escalated fighting is often viewed as a ‘war of attrition’ (Maynard Smith, 1979; Parker and Rubenstein, 1981; Payne & Pagel 1997), where the first individual to cross a threshold of accumulated costs will withdraw, relinquishing the contested resource. Taking ownership of a valued resource brings benefits for the winner, including potential mating opportunities if the resource at stake was territory in which

females might be found, as is often seen in harem-keeping species such as red deer (Reby et al., 2001) and elephant seals (Le Bouef, 1974; Hoelzel et al., 1999). A victory may also lead to mating opportunities with females in the local area because they will preferentially mate with victorious and therefore dominant males (Simmons, 1986a; Nelson and Nolan, 1997; Shackleton et al., 2005; Lailvaux and Irschick, 2006), and who may have even formed an audience whilst the males fought (Tachon et al., 1999; Fitzsimmons and Bertram, 2013b; Montroy et al., 2016).

As males who out-perform their peers often get to mate more often, their own physical quality is rewarded in reproductive fitness returns, either through monopolizing female attention in harem keeping species (Le Bouef, 1974; Hoelzel et al., 1999; Simmons et al., 1999; Kelly, 2006), or by obtaining repeated mating with individual females (Weatherhead and Robertson, 1979; Hosken et al., 2008) and the resultant offspring will represent the success of that mating competition (Darwin, 1871; Weatherhead and Robertson, 1979). Therefore, sons fathered by high quality males should have the potential to repeat his success if they make it to adulthood (Hill, 1994). Studies of the evolution of male weaponry and its use in competition tend to focus primarily on absolute size as an indication of strength/health of the bearer and constraints on adult size across the animal kingdom (Gould, 1974; Barrette and Vandal, 1990; Eberhard and Guttierrez, 1991; Hanley, 2001; Knell et al., 2004; McCulloch and Emlen, 2013), with some consideration of alternative successful evolutionary strategies. In species that include both heavily armed and lightly armed individuals (dimorphic males), alternative mating strategies related to satellite or sneaker male behaviour have evolved as effective alternatives to combat (Eberhard, 1982; Eberhard, 1987; Gadgil, 1972; Oliveira and Almada, 1998; Simmons et al., 1999; Zatz et al., 2011).

4.1.3 Fighting and field crickets

Field crickets of the Gryllidae family, including *G. bimaculatus*, engage in highly aggressive male-male fighting over resources, including females and the mating behaviour of both sexes is highly promiscuous (Simmons, 1986b; Simmons, 1988; Tregenza and Wedell, 1998; Wynn and Vahed, 2004). Body size is often cited as the primary aspect of RHP that mostly affects fight outcome. Due to increased energy reserves and the costs associated with fighting at high intensity, body weight and size correlates very strongly with fighting success

in many insects, including the field crickets *Acheta domesticus* (Hack, 1997; Brown et al., 2006; Briffa, 2008), *Gryllus campestris* (Simmons, 1995) and *G. bimaculatus* (Hofmann and Schildberger, 2001; Rilich et al., 2007). However, while it has been shown that male *G. bimaculatus* exhibit significant sexual dimorphism of the head and mouthparts (see previous chapter), and that larger males have greater fighting and reproductive success (Simmons, 1986a; Bateman, 2001b; Hofmann and Schildberger, 2001; Rilich et al., 2007), very few papers have considered weaponry as a specific component of RHP in crickets of the family Gryllidae. Males of the horn-headed field cricket, *Loxoblemmus doenitzi*, actually possess sexually dimorphic weaponry in the form of triangular protrusions of their flattened head that is used in pushing matches against conspecifics in much the same way as mandibular engagement functions in other field crickets, though horn size does not correlate with increased fighting success (Kim et al., 2011). Male *G. pennsylvanicus* have been shown to possess sexually dimorphic mandibles that play an important role in ritual combat as males with proportionately larger jaws than a size-controlled opponent were more successful when fights escalated to grappling, though no effect was found on flaring of the jaws (Judge and Bonanno, 2008), and male *G. bimaculatus* that had their jaws disabled won fewer fights than untreated males (Hofmann and Schildberger, 2001), but mandible size had little effect on contest outcome in *A. domesticus* (Briffa, 2008).

When a pair of adult male *G. bimaculatus* meets, if neither male is inclined to retreat from the confrontation, a stereotyped escalatory sequence of ritualized behaviours ensues that eventually culminates in grappling behaviour. Gryllid combat also often involves mandibular engagement and though the fights do not necessarily end in tipping or throwing, but rather a sustained charge from both males as they clash head to head, and might see one male destabilize and tip his opponent backwards, sometimes forcing his retreat, the importance of the mandibles in high energy display and RHP assessment is clear. Physical engagement of the jaws in combat against a rival in order to attain a position of dominance, and the covariance of dominance with reproductive fitness would logically imply that the jaws are under a degree of selective pressure (Lavine et al., 2015). Therefore it follows that successful males would possess mandibles that bestow an advantage in terms of fitness (Kelly, 2006; Judge and Bonanno, 2008). Combat that escalates to the point at which the mandibles form part of the assessment is usually observed in pairs of conspecifics wherein

physical parameters of body size, weight etc. are considered relatively equal (Bateman, 2000; Hofmann and Schildberger, 2001; Judge and Bonanno, 2008), and such escalations that see the males involved flaring their jaws and physically grappling with each other would logically be the focus of intrasexual selective pressures for weapon effectiveness.

4.1.4 Aims

This study aimed to test the following predictions in *G. bimaculatus*:

Prediction 1: males with wider anterior features relative to their opponent, including head and pronotum width, will be more successful at fights that escalate to grappling against an evenly weighted male.

Prediction 2: males with wider or larger jaws and with more pronounced incisor portions of the tooth edge will win more often when fights escalate to jaw flaring stages against an evenly weighted male.

Prediction 3: males with more symmetrical jaws will be more successful in fights that escalate to grappling than males with asymmetric jaws.

4.2 METHODS

4.2.1 Pre-fight

Twenty eight pairs of weight-matched (in grams to 2 decimal places), adult, undamaged, virgin, naïve, male *Gryllus bimaculatus* were selected from a cultured population. All pre-fight animal protocols were followed as per section 2.2.1 to remove behavioural effects from handling or prior fights (either wins or losses) that might affect experimental performance.

4.2.2 Fight Stage

All combat experiments took place in the custom built arena measuring 180mm high x 130mm x 130mm described in 2.6 and shown below in Figure 4.1.

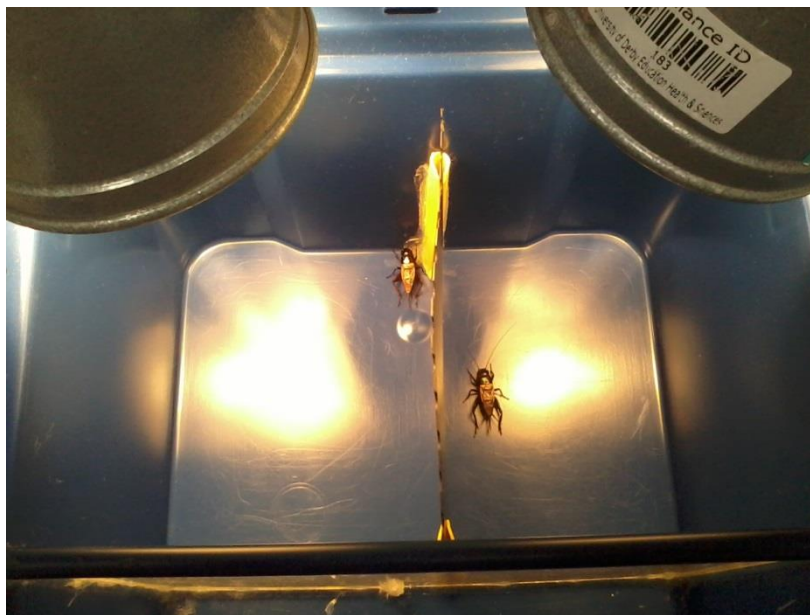


Figure 4.1: A pair of male crickets, individually marked on the pronotum, placed either side of a dividing wall. Heat lamps maintain the arena temperature at 27°C (photo credit: DG).

Pairs of males were introduced to the arena on either side of an opaque dividing wall. At no point during this transfer were the males handled. This was to minimise disturbance and stress to the test subjects. Once in the arena the males were allowed to acclimatize for two minutes (as seen in Figure 4.1) before the divider was removed for the interaction to begin.

Once a male crossed the central line, a 5 minute countdown timer was started. All fights that took place in the five minute encounter time between opponents were watched and accurately scored with the level of escalation and the winner in each case using the scale in Figure 2.7 of the General Methods and following the fight protocol laid out in sections 2.8 and 2.9 of the General Methods. The males were observed during their 5 minute encounter for their performance in combat with regard to fights ending in jaw flaring and grappling. As such there was no final winner declared. After 5 minutes had passed, the males were separated again and placed in 5ml glass tubes for euthanasia following section 2.4.

Consecutive fights between subsequent pairs of males were conducted in an identical manner, but before each new set of males was introduced to the arena, the arena floor, walls and the dividing wall were wiped down using cotton wool soaked with 70% alcohol to ensure no pheromone traces persisted that would possibly affect male behaviour during their bouts, as per Brown et al. (2006).

4.2.3 Post-fight dissection and measurement

Dissection of male specimens was carried out as per section 2.5 and all physical data were recorded on a spreadsheet alongside the fight scores from the behavioural component of this work. Variables measured and recorded were: Head width (mm), Pronotum width (mm), Pronotum length (mm), Right mandible length (mm), Left mandible length (mm).

4.2.4 Imaging and measurement of mandible structure

Measurements of the tooth edge were taken by first laying the tooth on its dorsal surface and manually posing it so that the distal tip, incisor and molar portions of the tooth were aligned when viewed from above. A digital image was then taken via a digital camera mounted on a dissecting microscope. Using the computer program ScpelImage 9.0 calibrated to a stage micrometre, the digital photographs were then used to determine measurements of distance from the lateral articulation to the distal tip, incisor and molar portions of the Right mandible from each male cricket (see Figure 4.2, which shows the measurement points generated using ScpelImage 9.0).

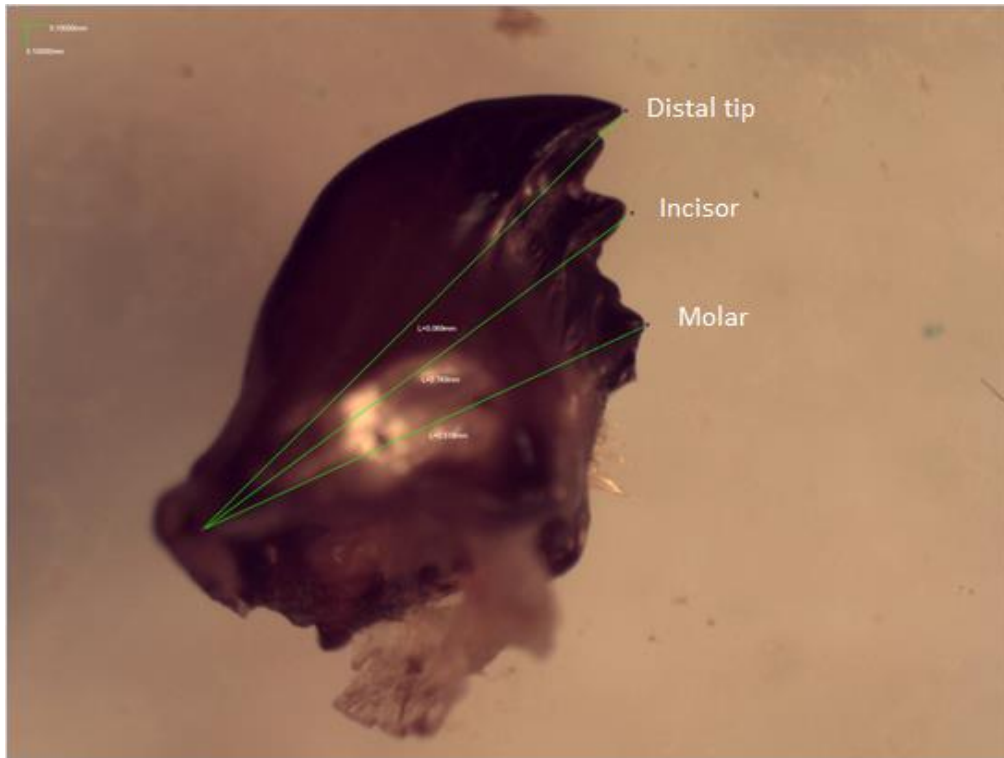


Figure 4.2: The right mandible from an adult male *G. bimaculatus* with measure points labelled. The green lines indicate the measurement lines taken using ScopelImage 9.0 (photo credit: DG).

Tooth dimensions were then added to the spreadsheet containing the rest of the dissection data and the fight results from each 5-minute encounter. The Right mandible on these animals features more pronounced processes than the Left, which acts as a sheath for the Right. See Figure 4.3, for an example of how each mandible fits a knife: sheath relationship, especially when considering the mandible's use as a weapon. This form of directional asymmetry is also important with regard to using the mandibles as effective tools for processing food (Okada et al., 2008).

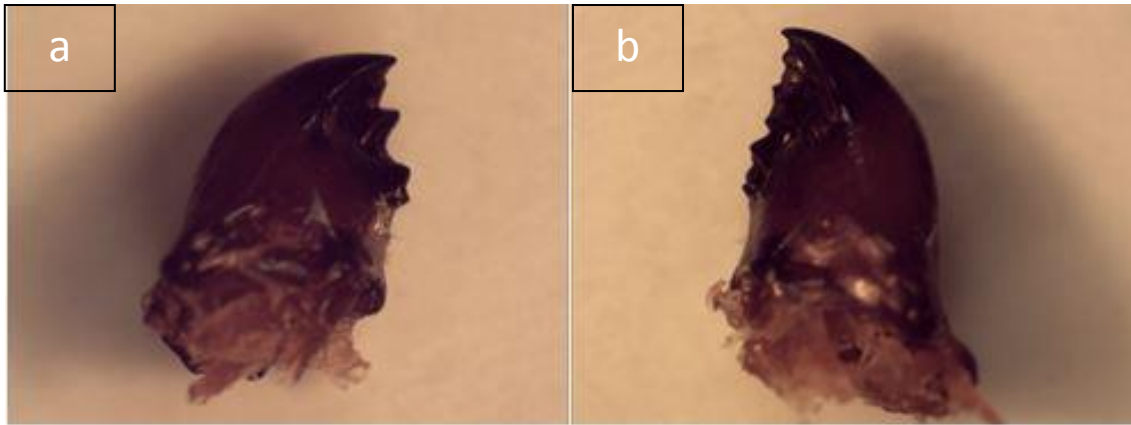


Figure 4.3: Ventral view showing differing shapes of the Right (a) and Left (b) mandible from the same specimen of adult male *G. bimaculatus* (photo credit: DG).

The decision to take measurements of tooth shape from a ventral position was made because the mandibles are overtly dome-shaped when viewed from a dorsal aspect, and as such much of the mandible-edge cannot be properly seen from this position (see Figure 4.4). In life, the cricket mouth parts are held in an alignment that allows for them to close neatly, but this view cannot be seen in dissected specimens.

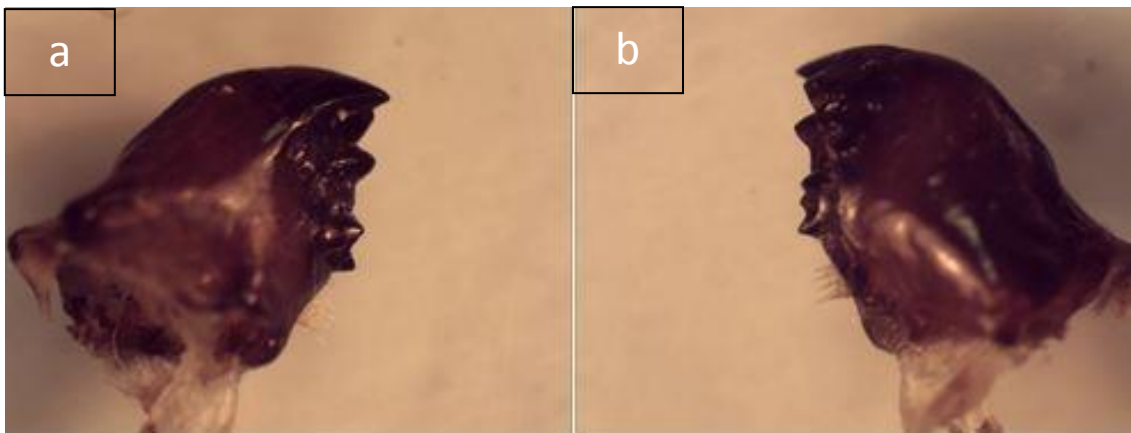


Figure 4.4: Edge structures visible from the ventral (a) as opposed to dorsal (b) aspect on the same right mandible from an adult male *G. bimaculatus* (photo credit: DG).

4.2.5 Statistical Methods

Two data categories were highlighted for analysis: values based on males that won and lost fights that concluded at levels 3 and 4 (jaw flaring) (n=30), and values based on males that won and lost fights that concluded at level 5+ (grappling using the mandibles as weapons) (n=42). As pairs of crickets used in this study represented the normal range of adult body

sizes and males were paired with their opponents based on mass, rather than using absolute weapon size difference to compare individual males, relative figures that compared trait size between winners and losers (calculated as winner trait divided by loser trait) were produced in order to be able to analyse data from all male pairs across the full range of body weight and trait size represented in the specimens used for the study. Correlation tests highlighted a degree of co-linearity between Pronotum Length and Pronotum width. To prevent this impacting data analysis, Pronotum length was removed as a covariate. R Studio was used to carry out binomial logistic regressions with backward stepwise deletion in order to identify the traits most important to fight outcome at different levels of intensity, with fight result treated as a binary outcome, win = 1, lose = 0. The Akaike Information Criterion (AIC) was used to compare the fit and quality of relative models during backward step-wise deletions. Model improvement was considered as significant if the AIC value was reduced (represented in text as Δ AIC) by 2 or more.

4.3 RESULTS

For the analysis of fights that ended at mandible flaring, the initial model included fight outcome (win vs lose) as the dependent variable and relative head width, relative pronotum width, relative mandible asymmetry, relative mandible span, relative length of the distal tip of the mandible and relative incisor length as independent variables. Because we did not have the statistical power to include all possible interactions, we included only the interaction that we considered to be the most biologically meaningful. This was the interaction between relative head width and relative distal tip length, which could show any effect of weapon size relative to body size. Following model reduction, the final model included a highly significant positive effect of relative distal tip length (slope=89.086, S.E.=34.008, $p=0.009$) and a significant negative effect of relative incisor length on successful fight outcome (slope=-80.145, S.E.=32.135, $p=0.013$).

For the analysis of fights that ended at grappling, the initial model included fight outcome (win vs lose) as the dependent variable and relative head width, relative pronotum width, relative mandible asymmetry, relative mandible span, relative length of the distal tip of the mandible and relative incisor length as independent variables. Again, the only interaction included in the analysis was that of relative head width with relative distal tip. Following model reduction, the final model included a highly significant positive effect of the interaction between relative head width and relative distal tip length (slope=95.81, S.E.=36.46, $p=0.009$), significant positive effect of relative mandible asymmetry (slope=26.78, S.E.=13.65, $p=0.05$), a positive, but not significant effect of relative mandible span (slope=23.11, S.E.=13.79, $p=0.09$), a highly significant negative effect of relative incisor length (slope=-101.03, S.E.=36.26, $p=0.005$), and a significant negative effect of relative head width (slope=-75.87, S.E.=24.66, $p=0.03$).

4.4 DISCUSSION

By controlling for cricket mass and using pairs of fighters weight matched in body weight to 2 decimal places, this study was designed to neutralise the effect of relative mass on fight progression and cricket decision making, as studies suggest that relative body mass is an important predictor of fight outcome in *G. bimaculatus* (Alexander, 1961; Simmons, 1986a; Adamo and Hoy, 2005; Hofmann and Schildberger, 2001). The results of this study demonstrate that anterior morphological structures, including head and mandible size and tooth structure all have an effect on fight outcome. Fights ending in jaw flaring and fights that escalated to grappling also showed different emphases on traits most likely to be used as predictors of success.

Whilst relative head width or length of distal tip were not found to be individually positive predictors of grappling victory, a strongly positive interaction effect between the traits was shown to be significant in predicting grappling fight outcome. As relative head width alone was negatively associated with victory in grappling, and pronotum width was not included in the final model, Prediction 1 is not accepted. Prediction 2 cannot be fully accepted as larger incisor length was not significantly linked to flaring display victory, but length to distal tip was. Prediction 3 can be rejected as relative mandible asymmetry did not return significant predictor values in flaring or grappling stages of fighting. This is the first study to examine the role of weapon size in determining the outcome of aggressive interactions in *Gryllus bimaculatus*. The only other similar studies of crickets to examine the effect of jaw size on fight outcome in species of Gryllidae were Briffa (2008), using *A. domesticus*, and Judge and Bonanno (2008), using *G. pennsylvanicus*. While Briffa (2008) found only a marginal effect of mandible size on fight outcome, the results of Judge and Bonanno (2008) were similar to those of the present study: males with proportionately larger jaws than a size-controlled opponent were more successful when fights escalated to grappling. They did not however report any findings on the size of specific mandible structures that might affect decision-making in mandible flaring stages of combat. No other study has identified components of the mandible, specifically the distal tip and incisor length, that are used as RHP indicators in visual assessment during jaw flaring contests between male *G. bimaculatus* or any other species in the Gryllidae.

4.4.1 Mandibular flaring

Some male *G. bimaculatus* were observed in experimental trials to win fights despite not being in physical contact with their opponent, meaning that they relied on other visual and tactile cues to settle their confrontations. For males to win at fight stages 3 and 4 (mandibular flaring in Figure 2.7 of the General Methods), the only physical contact either male experiences is via the antennae to establish sex, species and intent. As neither male was inclined to back away from antennal contact, for a male to drive off his opponent without touching him, the losing party must detect a component of his conspecific's aggressive display that indicates the likelihood of beating him is low, or the costs associated with winning would be too high (Maynard Smith and Price, 1973; Parker, 1974; Hurd, 2006). This implies that mandibular flaring therefore acts as an important visual signal of RHP for the purpose of self and mutual assessment between the opponents (Figure 4.5). Significant predictor variables of success in mandibular flaring include relative distal tip length and incisor size. These traits seem to direct the decision of an opponent to flee from combat before grappling begins. Although this is the first work to highlight the significance of variation in mandible structure on male-male assessment in *G. bimaculatus*, the use of visual cues by opponents in fight decision making has been demonstrated in other studies using this species by blinding males before behavioural tests. The results of such trials showed that blinded males did not back down from fights against males with larger jaws (Rillich et al., 2007) and that blinded male pairs escalated significantly more fights to direct grappling than mandible flaring (Hofmann and Schildberger, 2001).



Figure 4.5: A pair of male *G. bimaculatus* engaged in bilateral mandibular flaring (photo credit: DG).

For the mandibles to be used as an effective signal of RHP that can be assessed by an opponent, they must be either morphologically functional in combat i.e. more likely to effectively damage the opponent, or they must be representative of some other aspect of the owner's biology i.e. stamina, physiology, strength, and bite pressure. Mandible size and morphology can be used as reliable signals of male quality (Emlen, 2008) as they represent life history foraging success and physiological health in many insect species that use them as weapons, including the beetles of the Dynastinae (Emlen et al., 2012), the Scarabaeidae (Moczek and Emlen, 1999), the Staphylinidae (Hanley, 2001), and the Lucanidae (Knell et al., 2004). However, this system of advertising life history success and fitness by wielding superior weapons has not been reported in the Gryllidae. Lifetime foraging success and allocation of nutrients during development has been examined in several species of cricket, but trials found that allocation in energy reserves specifically used in singing and mate calling were prioritised (Wagner Jr and Hoback, 1999; Judge et al., 2008). Song components reflective of physiological quality and RHP, that are particularly important in maintaining territories and putting off opponents, have been identified in *Gryllus campestris* (Simmons,

1995), *Acheta domesticus* (Hack, 1998; Ryder and Siva-Jothy, 2000; Brown et al., 2006) and *G. bimaculatus* (Simmons, 1986a; Simmons, 1988).

4.4.2 Grappling

A fight that escalates to grappling becomes a whole body exercise in strength and stamina and does not rest on just one or two traits acting in unison but requires dedication and acceptance of risk by both fighters. *G. bimaculatus* is a stout bodied insect that readily engages in agonistic display but rather than grasp an opponent by its head, lift and throw him aside, the male *G. bimaculatus* instead engages his opponent head to head, and pushes his opponent up and back (Figure 4.6), in a show of strength that does appear to require the use of the mandibles to grasp the mouthparts of the other male and then push the opponent backwards, although a larger relative mandible span was not shown to be significant predictor of grappling success.



Figure 4.6: A pair of *G. bimaculatus* males engaged in mandibular grappling (Photo credit: DG).

Other insect species that rely on anterior power and weapon size to unseat a rival also possess increased prothoracic muscle mass to drive this biting and pushing action (Goyens et al., 2014a) and also in order to absorb compression and generally increase robustness

(Goyens et al., 2015b), especially where fighting requires one male to displace the entire weight of his opponent (Goyens et al., 2015c). Although *G. bimaculatus* does exhibit sexual dimorphism of the mandibles and pronotum (see previous chapter) pronotum size did not predict fight outcome in either mandibular flaring or grappling stages in this study. Additionally, the mandibles really only act as dual pressure points that don't grasp the opponent fully by the head but are used to lock up against the opponent's mouth prior to shoving (pers.obs), unlike the biting and holding behaviour seen in *G. pennsylvanicus* (Judge and Bonanno, 2008) or *Teleogryllus commodus* (Hall et al., 2010). This form of grappling behaviour is used in a very similar manner to that seen in competition between males of another Gryllid species, the horn-headed cricket *Loxoblemmus doenitzi*. Although horn-headed cricket males use the large, flattened, anterior surface of their head as a focal point when two males engage in shoving matches as opposed to the jaw-locking behaviours seen in *G. bimaculatus* (Kim et al., 2011).

As this study has identified specific components of mandible shape that are used as assessment criteria during mandible flaring stages of agonistic display, the potential for future research includes the possibility of using trait manipulation to deduce how morphological scale and variation is processed during male-male conflict. As distal tip and incisor length were both shown to be significant predictors of success during mandibular flaring displays but less significant when fighting escalated to grappling, the act of modifying the mandible edge would be an interesting avenue of investigation. As it is now known that characteristics of the tooth edge are taken as RHP indicators, a methodology involving minor mechanical ablation of the mandible to accentuate or minimise trait size could be implemented to examine their effect on assessment.

As certain morphological characteristics have now been shown to direct male-male assessment and have an effect on fight outcome between weight-matched opponents, aspects of RHP in asymmetric contests must also be considered. Small male behaviour and fight performance against a larger opponent will be the focus of the following chapter.

CHAPTER 5. AN INVESTIGATION OF SOME OF THE FACTORS THAT MAY RESULT IN A SMALL MALE SECURING VICTORY AGAINST A LARGER OPPONENT

5.1 INTRODUCTION

5.1.1 Decision-making

When a pair of competing individuals comes face to face, they are predicted to engage in initial assessment of their opponent in order to make a decision as to whether conflict is likely to end with success and whether the resource is worth the cost of energy expenditure and any potential risks (Enquist and Leimar, 1987; Hack, 1997a; Buena and Walker, 2008). Body size and weight are key predictors of fight outcome between field crickets, and according to aggression and combat theory, an asymmetric fight will generally end in the larger male's favour, becoming more likely as the degree of asymmetry between opponents increases (Dixon and Cade, 1986; Hofmann and Schildberger, 2001; Nosil, 2002; Buena and Walker, 2008). Initial visual and antennal scans may give each male an idea of their opponent's sex and species by detecting cuticular pheromones (Tregenza and Weddell, 1997; Nagamoto et al., 2005). It was suggested by Kortet and Hendrick (2007) that the pheromone signatures are used to gather social cues relating to local sperm competition. A study using *Teleogryllus oceanicus* found that previous fighting success affects dominant male pheromone expression in a manner that can be detected by subordinate males, which will avoid combat and invest in other mating strategies instead of fighting, namely ejaculate production (Thomas and Simmons, 2009a).

All information that can be deduced about the status of their opponent allows for each male to determine their course of action, and the ability to make accurate observations in this way can be considered adaptive (Arnott and Elwood, 2009). Initial decisions made during preliminary assessment include forecasts (predictions of what the opponent is capable of, and projected acceptable costs of escalation, including damage incurred during the fight, both from the opponent and self-inflicted) essentially setting up thresholds of acceptable costs relative to the benefit of winning (Payne, 1998; Rillich et al., 2007; Briffa and Lane, 2017). Accurate assessment of an opponent is imperative from a natural selection perspective if an individual is to make efficient use of its physical resources while minimising the risk of injury from engaging in bouts of combat that it will not likely win due to

insurmountable asymmetries in RHP (Maynard Smith and Parker, 1976; Parker and Rubenstein, 1981). Multiple forms of assessment used in judging a potential opponent have therefore evolved in order to allow for accurate decision-making before competition can become too costly (Payne, 1998; Arnott and Elwood, 2009; Lane and Briffa, 2017).

5.1.2 Weaponry

The use of the mandibles as weapons in male-male conflict is standard throughout the field crickets and, while relatively understudied, the role of weaponry in combat has been demonstrated in *Gryllus pennsylvanicus*, where males with relatively larger weapons than their opponents tend to win fights more often (Judge and Bonanno, 2008; Judge et al., 2010). In *Acheta domesticus* male weapon size is not as important as relative body size in affecting fight outcome (Briffa, 2008). Male *Gryllus bimaculatus* use their mandibles in aggressive signalling to indicate fighting ability; this was demonstrated experimentally by blinding smaller opponents who would escalate fights against a larger opponent more often than unblinded controls (Hofmann and Schildberger, 2001; Rillich et al., 2007). Sexual dimorphism in the mouthparts of *G. bimaculatus* has been identified, and the size and shape of the teeth has been shown to have an effect on fight outcome between weight-matched males (See chapter 3 and 4).

5.1.3 Asymmetric contests and behavioural effects

What has not been examined in field crickets before, however, is the effect of weapon size on the outcome of aggression in opponents that differ in body mass (males were matched in body mass in chapters 3 and 4 and in Judge & Bonanno, 2008). As has already been established, the Gryllidae do not possess mandibular weaponry that even resembles that seen in members of the Coleoptera, or even the wetas in terms of size and shape (see section 1.5.2.3 of the Introduction for more information). Their weaponry could, however, show differences between individuals, potentially providing slight advantages in combat. No extremes of mandible variation in *G. bimaculatus* has been identified in the literature, but Hofmann and Schildberger (2001) made an observation that during fights between asymmetrically matched males (in terms of them being of different weights), the smaller male won the fight thirty percent of the time on average, and this figure actually reached forty-one percent for smaller males winning at the highest level of escalation: grappling,

using the mandibles as weapons. The authors could not say for sure what was causing the larger males to lose their fights in this way. Manipulation of combatants by blinding males and shortening antenna affected assessment times but not fight intensity, and Hofmann and Schildberger (2001) could only suggest that motivation to fight or aggressiveness could be a factor.

Previous fight outcome and other motivational status effects such as mating experience can all play a major role in male decision making when he is choosing to engage in combat (Brown et al., 2006; Brown et al., 2007; Killian and Allen, 2008; Stevenson and Rillich, 2013a) (see also chapter 4 for the effect of weapon size on fight escalation, and chapters 6 and 7 for the impact of previous fight experience and motivational status effects on aggression). Hofmann and Schildberger (2001) made use of a controlled environment; males were selected from an isolated population with no previous fight experience effects or mating contacts that would affect aggression levels, such as female contact. This group of smaller males that beat larger opponents could be naturally more aggressive than normal. Aggression is normally suppressed in smaller individuals that detect asymmetric cues during physical assessment, although stalk-eyed flies with abnormally high serotonin levels have been shown to escalate fights despite such an asymmetry (Bubak et al., 2015). High aggression in a small male that might not be considered a threat could then in theory force a reassessment, or even retreat, by a larger opponent if costs start mounting at a higher rate than he initially predicted upon first meeting his smaller opponent (Hurd, 2006). This cryptic aggression component could account for the unexplained thirty percent loss rates by larger males in Hofmann and Schildberger (2001), but it could also be a function of morphology i.e. that some smaller males had mandibles sufficiently large enough to force a reassessment by their larger opponent.

5.1.4 Aims and predictions

This study sought to identify factors that can influence fight outcome between asymmetrically weighted opponents in favour of the smaller male. As body size and weight are such major predictors of fighting success (Hack, 1997b; Hofmann and Schildberger, 2001; Brown et al., 2006; Briffa, 2008; Reaney et al., 2011), smaller males that win such fights may look or behave differently during combat. Potential areas of variation that may

increase success rate are 1) possession of physical traits that make up for the mass asymmetry, such as larger relative than average weaponry than his opponent to use in combat, or, 2) the use of behavioural strategies that other males of his size might not normally employ.

An experimental design that involved matching pairs of naïve, but asymmetrically weighted, male *G. bimaculatus* against each other in a novel arena environment was used to collect fight data when the males interacted. This experimental design is in contrast to those used in chapters 2, 4, and 5 that matched males by weight to within 5%. The focus of this study was on the smaller males and whether they won or lost their encounter with a larger opponent. Specific components of fight behaviour, including escalation level, may give some insight as to whether small males rely on aggressiveness or superior weaponry to beat a larger opponent.

This study aims to test the following predictions:

Prediction1: The larger males will win more often against their smaller opponents.

Prediction 2: Small males that beat a larger male at jaw flaring stages of escalation will have comparatively larger mandibles than small males that lose fights at this level.

Prediction 3: Small males that are willing to escalate the first encounter to high levels of intensity will be more likely to win the overall encounter (note that repeated bouts of aggression frequently occur between opponents in field crickets, see Chapter 2).

5.2 METHODS

This experiment made use of 54 asymmetrically weighted pairs of adult male *G. bimaculatus*. All selection and handling procedures took place as laid out in sections 2.1 and 2.2 in the General Methodology.

5.2.1 Male cricket selection

To form pairs of opponents for this trial a large number of male crickets (approximately 300 over multiple sessions) were weighed and placed in plastic containers marked with their weight in grams to 2 decimal places. Enough crickets were taken from the captive population to give an accurate representation of the weight range of the adult animals, normally 0.4 – 1.0 grams. Pairs were then formed by taking males from opposite ends of the size scale and calculating their weight asymmetry. The calculation used was:

$$(\text{Small Male Mass}/\text{Large Male Mass}) \times 100 = \text{Mass asymmetry}$$

Pairing of larger and smaller males from the cultured population produced pairs of males with degrees of asymmetry ranging from 12% to 40%. Those males that were paired against an opponent that took their asymmetry value below or above this range were re-paired with another until a suitably weighted opponent was found. Once weighed, the pairs were marked on the pronotum with different colours of nail polish and housed individually as per section 2.2.1.

5.2.2 Aggressive encounter and data collection

5.2.2.1 Pre-fight preparation

For each trial, an asymmetrically weighted, prepared pair of crickets was taken from the insect culture room and carefully introduced to an encounter arena, one male on either side of a removable dividing wall. See 2.6 of the General Methodology for notes on construction, dimensions, and parameters of the arena. At no point were the animals physically handled. After introduction to the arena environment the males were given a 2 minute period to acclimate to the arena after the transfer stage.

5.2.2.2 Beginning the trial

Once 2 minutes had passed to allow the males to settle down, the dividing wall across the middle of the arena was removed. Once the males began to move they were watched and when antennal contact of an opponent was made by either male a digital 5 minute timer was started.

5.2.2.3 Observation of fighting behaviour

Using the stereotyped escalatory scale as used in Hofmann and Schildberger (2001) and described in the General Methodology, interactions between the males were observed and the level of fight intensity and winner of each fight was noted until the 5 minute encounter period ended.

5.2.2.4 Post-trial procedure

The pair of opponents was removed from the arena using a plastic, freezer safe sampling cup with a lid. The sampling cup containing the pair of crickets was then placed in the laboratory freezer for euthanasia as described in 2.4 of the General Methodology. The arena was cleaned and reset for the next encounter as described in 2.3 of the General Methodology. Euthanized specimens were then dissected and morphological measurements of the mouth parts, head and pronotum were taken, as described in 2.5 of the General Methodology.

5.2.3 Statistical Methods

An initial round of tests were carried out to assess the effect of mass and weapon asymmetry on the likelihood of a small male being beaten by a larger opponent. Then, to determine whether small males that were successful in fights against a larger opponent differed in morphology to those that were unsuccessful, binary logistic regression models were built with fight escalation/outcome as a dependent variable, and measures of relative weapon size and relative mass to the opponent were built. Model reduction and selection was based on change in AIC. Welch 2 sample T-tests were also used to compare differences between groups. These statistical analyses were performed using R studio running R v3.4.1 with ggplot2. Bar charts were made using Microsoft Excel.

To determine whether large males that lost fights had significantly smaller mandibles than would be expected, and whether small males that won fights had larger mandibles than would be expected, a linear regression model of head width modelled against mandible span (as discussed in Chapter 3) was conducted. Analysis of the extracted residuals from that model were then tested using a Mann-Whitney U test. This portion of the analysis was conducted in SPSS.

5.3 RESULTS

5.3.1 Large and small male pairings

Preliminary visual checks indicated normally distributed data for both mandible span and mass, and Levene's test showed homogeneity of variance. Mass ranges were also significantly different ($t=8.283$, $d.f.=97.86$, $p=6.361e-13$, Figure 5.1a).

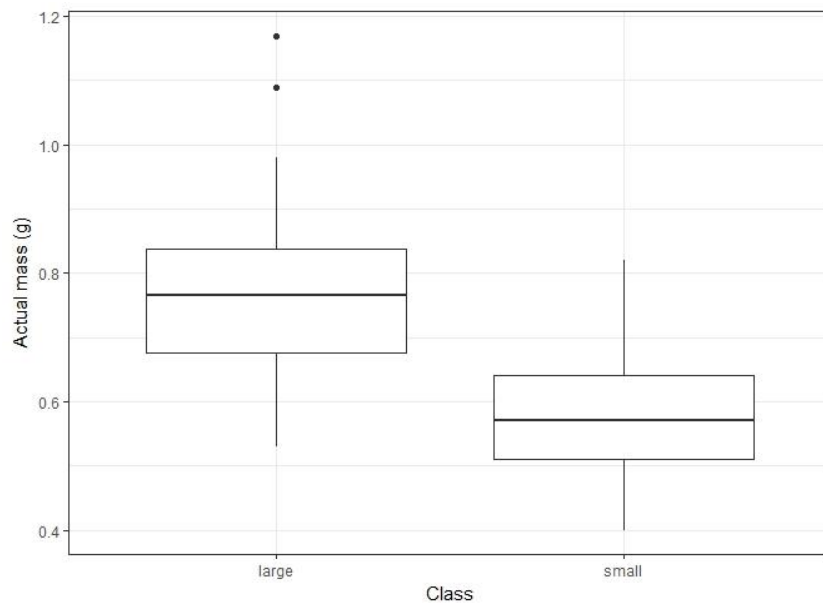


Figure 5.1a: Boxplot showing median and interquartile range of the mass ranges of the large (n=54) and small (n=54) males used in this work.

Mandible spans of large males were also significantly larger than those of the small males ($t=6.981$, $d.f.=105.33$, $p=2.715e-10$, Figure 5.1b).

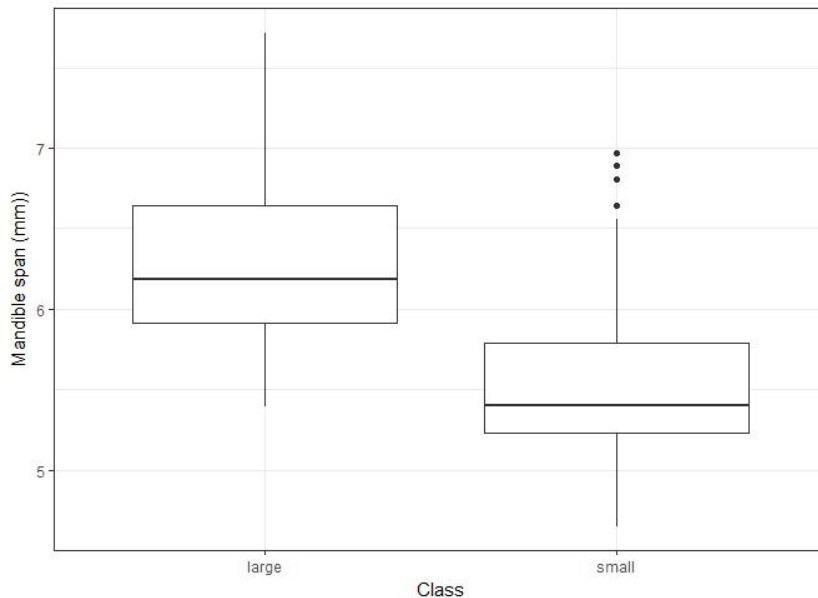


Figure 5.1b: Boxplot showing median and interquartile range of mandible spans of the large ($n=54$) and small ($n=54$) males used in this work.

A logistic model was run with fight outcome as a binomial dependent variable (1=win, 0=loss) and both percentage mass difference and relative mandible span difference between opponents as independent variables, plus their interaction. There was no significant interaction between mass and weapon difference ($\chi^2=0.002$, $p=0.89$) and mandible difference was not significant ($\chi^2=1.76$, $p=0.18$). Mass difference was a significant predictor of fight outcome however ($\chi^2=3.83$, $p=0.05$, Model AIC=61.998, Figure 5.2). Removal of the interaction term improved the fit of the model but did not change the p values of mass and weapon differences in the model (Model AIC=60.017). Removal of weapon difference did not improve model fit ($\Delta AIC < 2$).

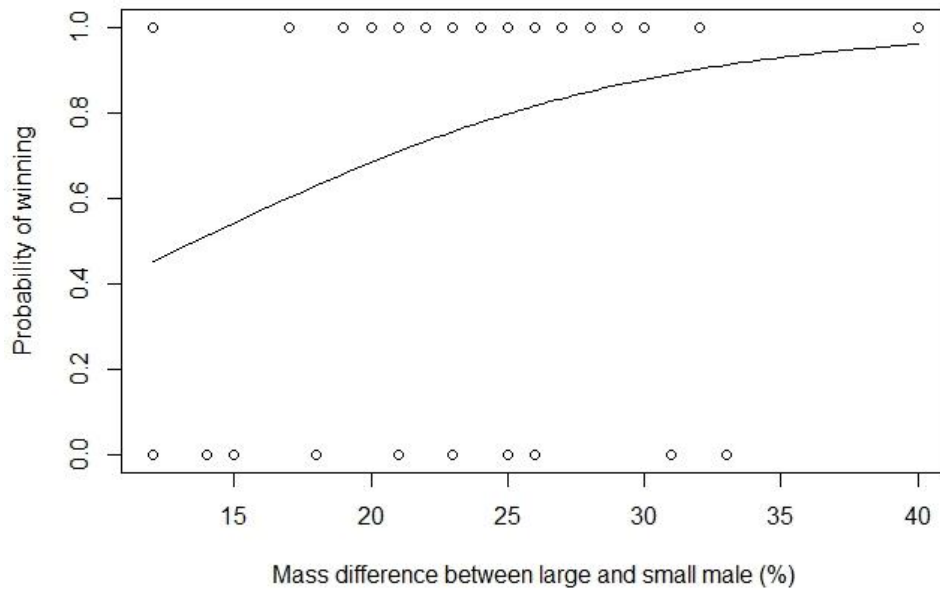


Figure 5.2: Probability of large male winning based on degree of weight asymmetry with the smaller male.

5.3.2 Jaw flare fighters, comparing small winners and small losers

Preliminary visual checks indicated normally distributed data, and Levene’s test showed homogeneity of variance. Winners and losers of fights that ended in mandibular flaring did not significantly differ in mandible span ($t=-0.065$, $d.f.=24$, $p=0.95$, Figure 5.3a).

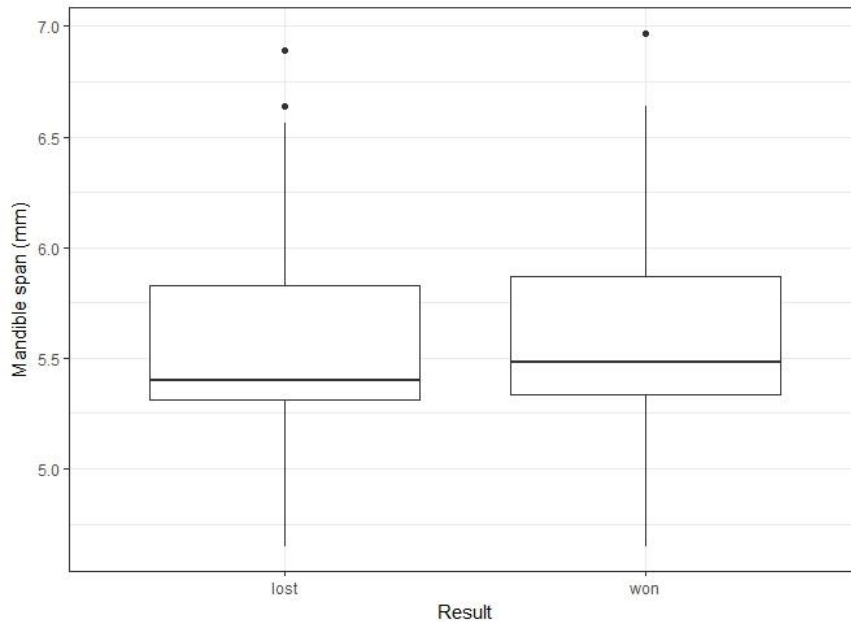


Figure 5.3a: Boxplot showing median and interquartile range of small male mandible spans of losers (n=24) and winners (n=14) of mandibular flaring.

Winners and losers of fights that ended in mandibular flaring did not significantly differ in head width ($t=0.028$, $d.f.=23.566$, $p=0.98$, Figure 5.3b).

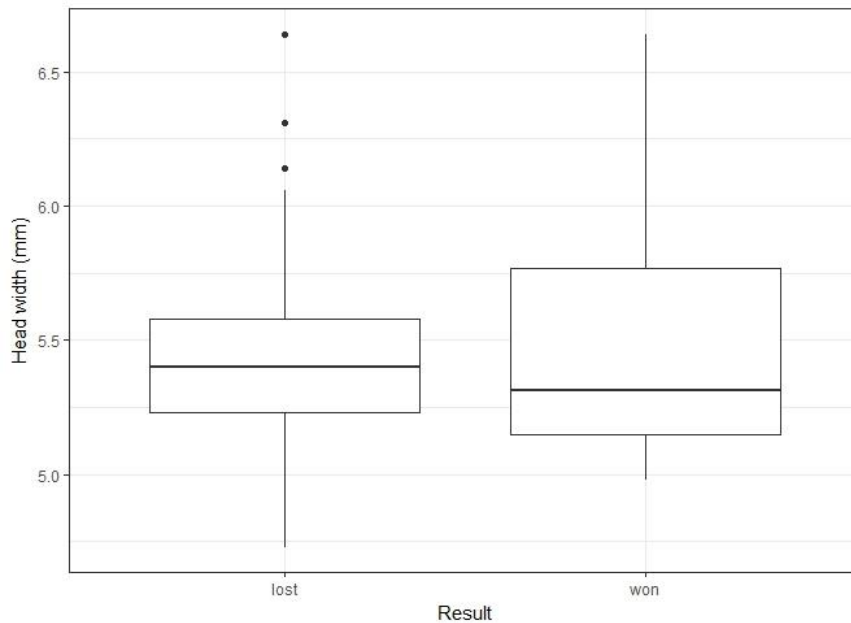


Figure 5.3b: Boxplot showing median and interquartile range of small male head widths of losers (n=24) and winners (n=14) of mandibular flaring.

Winners and losers of fights that ended in mandibular flaring did not significantly differ in their head/mandible width rf ($t=0.22$, $d.f.=23.692$, $p=0.83$, Figure 5.3c).

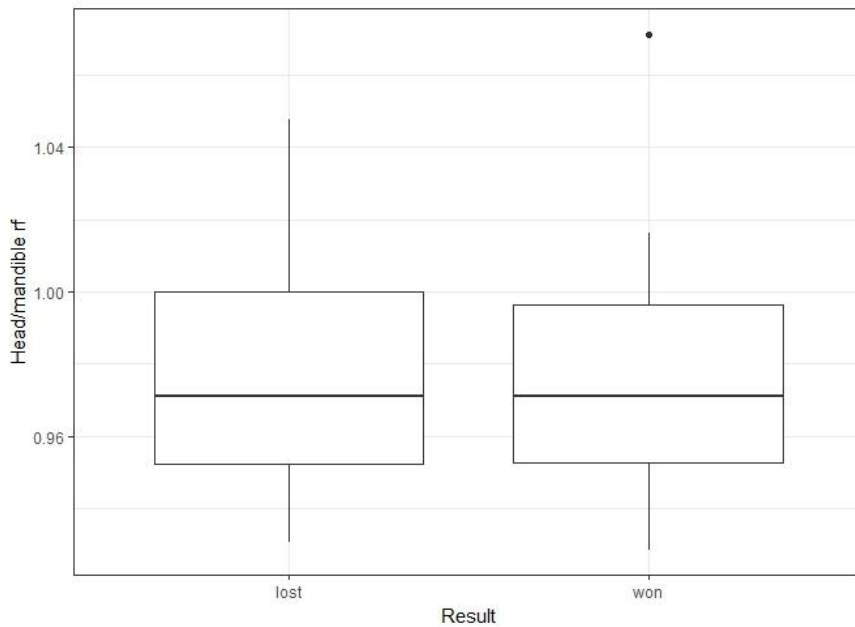


Figure 5.3c: Boxplot showing median and interquartile range of small male head/mandible rf of losers ($n=24$) and winners ($n=14$) of mandibular flaring.

Tests to see whether males were more successful if they were closer in weight to their large opponent also returned non-significant values ($t=0.46$, $d.f.=22.942$, $p=0.65$, Figure 5.4)

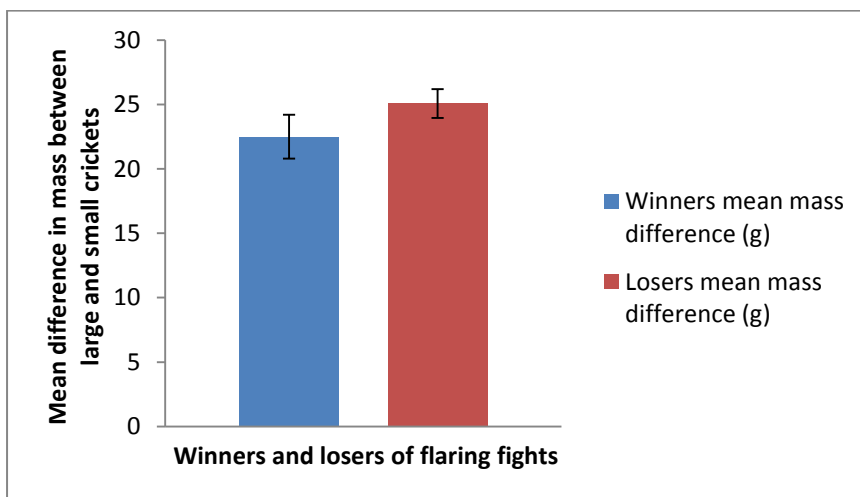


Figure 5.4: Bar chart of mean mass difference with standard error of small male winners and losers of fights that ended with jaw flaring.

A multiple logistic regression model with fight outcome at the flaring stage as the dependent variable was run using relative measurements of head width, mandible span, mass difference, and interactions of these measurements as independent variables. None of the interaction terms were significant so these were removed from the final analysis. None of the independent variables returned significant results (Head width: $\chi^2=0$, $p=0.98$; Mandible span: $\chi^2=0.104$, $p=0.75$; Mass difference: $\chi^2=2.309$, $p=0.13$). Further model reduction did not improve the fit of the model ($\Delta AIC < 2$).

5.3.3 Grappling fights, comparing small winners and small losers

As before, preliminary visual checks were used to check for normality and indicated normally distributed data from small winners and losers of grappling level fights. Winners and losers of fights that ended in grappling did not significantly differ in mandible span ($t=0.314$, $d.f.=9.791$, $p=0.76$, Figure 5.5a).

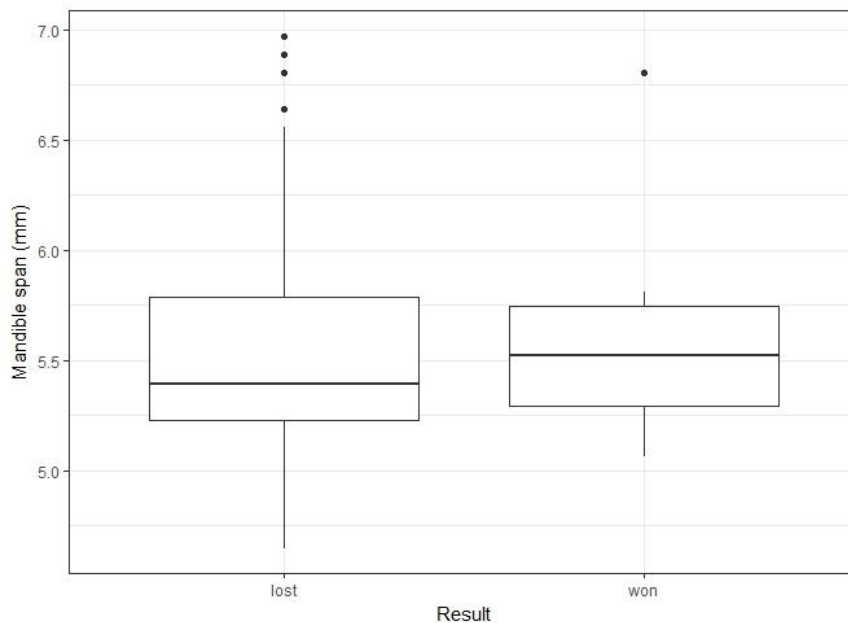


Figure 5.5a: Mandible spans showing median and interquartile range of small male winners (n=8) and losers (n=46) of grappling.

Winners and losers of fights that ended in grappling did not significantly differ in head width ($t=0.388$, $d.f.=10.496$, $p=0.71$, Figure 5.5b).

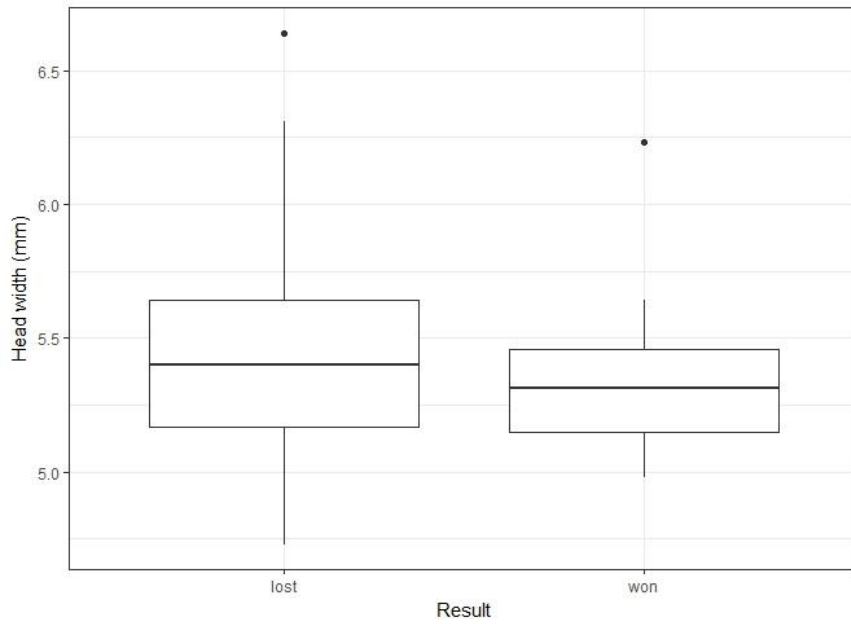


Figure 5.5b: Head widths showing median and interquartile range of small male winners (n=8) and losers (n=46) of grappling

Winners and losers of fights that ended in grappling did not significantly differ in their head/mandible width rf ($t=0.361$, $d.f.=9.914$, $p=0.73$, Figure 5.5c).

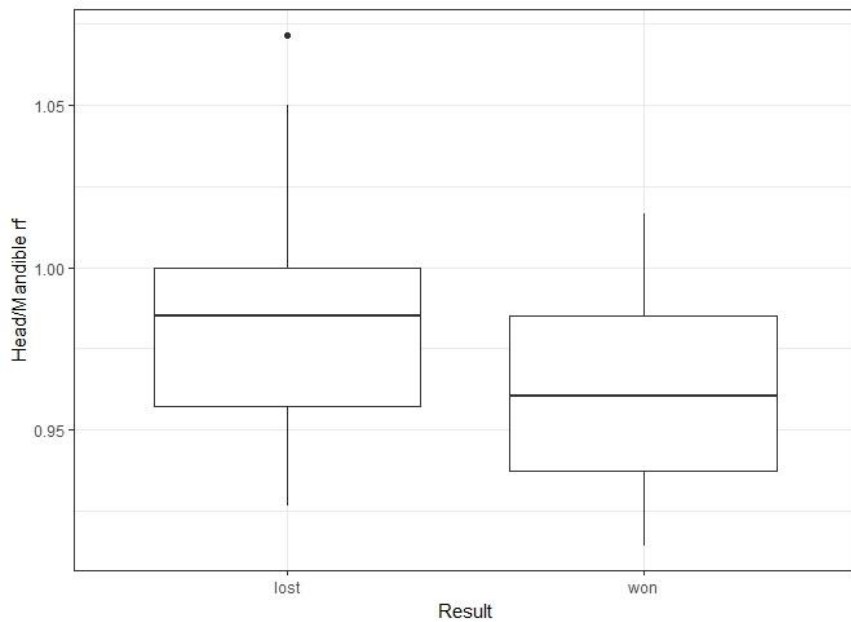


Figure 5.5c: Head/Mandible rf values showing median and interquartile range of small male winners and losers of grappling.

A test was carried out to see if the successful small males were significantly different in weight to those small males that lost grappling fights. This returned non-significant values ($t=-0.805$, $d.f.=13.078$, $p=0.44$, Figure 5.6)

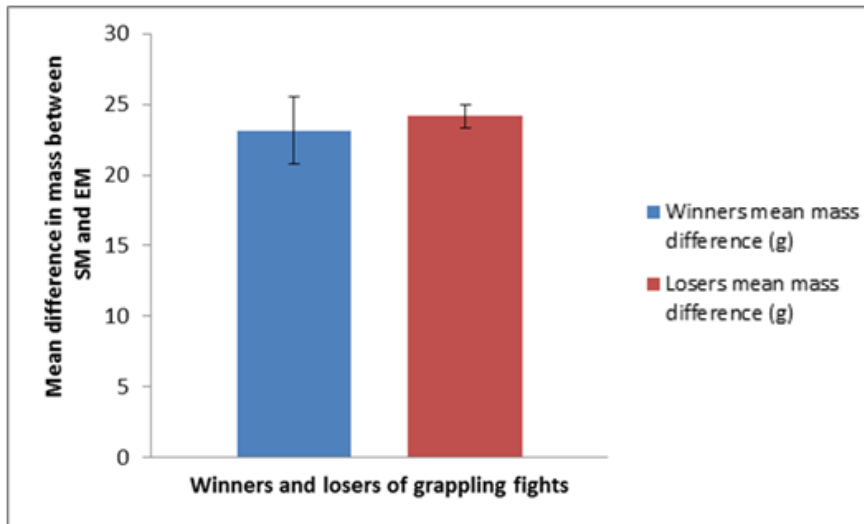


Figure 5.6: Bar chart of mean mass difference and standard error of small male winners and losers of grappling fights.

A multiple logistic regression model with small male victory in grappling fights as the binary dependent variable was run with relative measurements of head width, mandible span, mass difference, and interactions of these measurements as independent variables. None of the interaction terms were significant so these were removed from the final analysis. Neither mandible span (Wald $\chi^2=0.04$, $p=0.84$) or mass difference (Wald $\chi^2= 0.24$, $p=0.62$) had a significant effect on fight outcome. This analysis did indicate however that head width was a significant predictor of fight outcome (Wald $\chi^2= 5.36$, $p=0.02$). But, as only a small number of males (14.8%, $n=8$) were successful in grappling fights against their larger opponents, the importance of head width as an indicator of success could not necessarily be considered reliable. Boxplot and t-tests comparing winners and losers head width actually indicated that the head width of losers was higher than that of winners (Figure 5.5b). Further model reduction did not improve the fit of the model ($\Delta AIC < 2$).

5.3.3.1 Residual analysis comparing weapon size of winners and losers

The unstandardized residuals from a linear model of mandible span modelled on head size were extracted and were tested to determine whether relative weapon size differed between winners and losers. A Kolmogorov Smirnov test indicated that the data was non-parametric so a Mann-Whitney U test was used to compare the residuals. The analysis showed there was no significant difference between winners and losers in relative weapon size (Mann-Whitney $U=1435.5$, $p>0.05$).

5.3.4 Aggressive behaviour observations

Fifty-seven percent of encounters between asymmetrically weighted pairs reached grappling intensity at least once but an unexpected number of small males engaged in combat at grappling level in their very first meeting with their large opponent. A logistic model of the fight data with escalation to grappling as a binary variable indicated that the more asymmetric the weights of the opponents, the more likely it was that a fight would escalate to grappling at first contact (Figure 5.7). The model output was only close to significance, however ($\chi^2=3.41$, $p=0.065$).

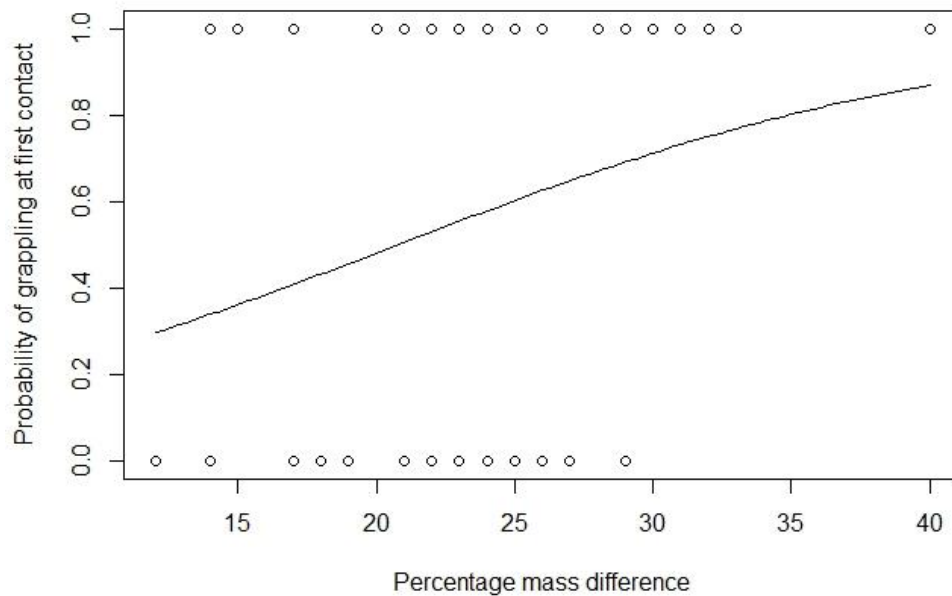


Figure 5.7: Binary regression plot-curve of the probability of the first fight in an encounter escalating to grappling as weight asymmetry between opponent's increases.

Further investigation of fighting behaviour indicated that the small male's performance in his first fight with his larger opponent within each trial had a significant impact on the final outcome. A binary model with final victory status as a binomial variable and first fight win as a categorical showed a highly significant relationship. The result of the initial contact between asymmetrically weight opponents was highly predictive of overall encounter outcome: of the small males that won their first fight ($n=15$), 11 of them went on to finish their encounter as the dominant male against their larger opponent ($\chi^2=26.434$, $p=2.727e-07$, Figure 5.8). Number of fights between the asymmetrically matched opponents ranged from one to ten fights in the five minute encounter time. The mean number of fights was 5.18.

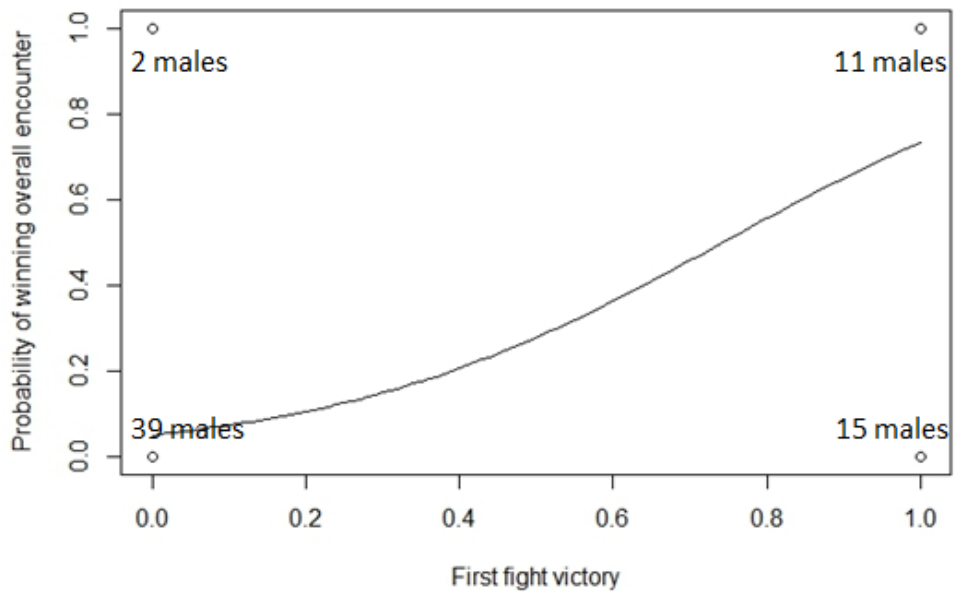


Figure 5.8: Binary regression plot-curve of the effect of the small male winning the initial fight and ending the encounter the winner.

5.4 DISCUSSION

By pitching asymmetrically weighted pairs of naïve, adult, male *G. bimaculatus* against each other, and observing their fighting behaviour in a neutral environment, this study aimed to determine how and why a smaller male might emerge victorious when behavioural theory predicts he will likely lose to his larger opponent (Dixon and Cade, 1986; Simmons, 1986a; Simmons, 1995; Hofmann and Schildberger, 2001; Nosil, 2002; Buena and Walker, 2008; Reaney et al., 2011). Throughout the experimental trials, the large males did win their fights significantly more often than their smaller opponents, as would be expected given the importance of mass asymmetry as a predictor of fight outcome in the Orthoptera (Simmons, 1986a; Brown et al., 2006; Briffa, 2008; Judge and Bonanno, 2008; Reaney et al., 2011). This allows for the acceptance of Prediction 1. It was important to be able to demonstrate this aspect of fight outcome, as it showed that the laboratory raised crickets used in this study were behaving normally, and therefore allowed for accurate testing of the other hypotheses that formed a part of this work.

5.4.1 Jaw flaring

Weapon size has been shown to act as a visual cue of RHP between weight-matched males in *G. bimaculatus* (see Chapter 4 of this work) resulting in some fights ending before physical grappling could even start. Of the thirty-eight encounters that included fights that ended with displays of mandibular flaring, fourteen were won by the smaller male but statistical analysis of morphological data from the small winners and small losers indicated that the two groups did not exhibit significant differences in their mandible size. As mandibular flaring can be considered a direct visual cue, used by opponents in order to assess each other's relative weaponry as a component of RHP, there could be other components of this display that have not been accounted for by this study, though what these are is not clear. Previous studies that have manipulated information exchange during assessment stages of cricket fighting have blinded male crickets to show that mandibular flaring does function as an assessment cue that can end a fight, and that fights escalated to grappling more often between blinded crickets than control pairs (Hofmann and Schildberger, 2001). Similarly, fights between asymmetrically weighted males escalated higher if the smaller male was blinded (Rillich et al., 2007). The lack of a significant difference in mandible spans between

the small males that won or lost mandibular flaring displays does not allow for the acceptance of Prediction 2.

5.4.2 Grappling

Fights that escalated to grappling were observed in sixty-seven percent ($n=37$) of all pairs during their five minute encounter period. Agonistic conflict theory states that escalation to physical contact in many species should only be seen in situations where RHP asymmetry between opponents is low, i.e. between conspecifics that are relatively well matched, as fights that escalate this high require the most energy expenditure and, in some species, carry a concomitant risk of serious injury (Parker, 1974; Parker and Rubenstein, 1981; Hofmann and Schildberger, 2001; Kelly, 2006; Batchelor and Briffa, 2010; Vieira and Peixoto, 2013). A standard form of control in studies on agonistic conflict between males specifically makes use of size or weight-matching as a component of their experimental design in order to trigger high escalation levels in the study species (Hack, 1997; Rillich et al., 2007; Judge and Bonanno, 2008; Hall et al., 2010; Judge et al., 2010). However, in the present study grappling (the highest level of escalation) not only occurred across a range of mass asymmetries from fourteen to forty percent, but it accounted for fifty-seven percent of first fights when opponents initially met. Given that all males were naïve and therefore not under any form of winner/loser effect, nor had they been exposed to any other stimulus that could cause a major shift in motivational asymmetry such as chemo-tactile contact with a female immediately prior to fighting (Simmons, 1986a; Tachon et al., 1999; Killian and Allen, 2008) (also see Chapter 3 and 4 of this work), this observed behaviour was unexpected. Whilst this does demonstrate that some small males ($n=31$) will display what might be construed as overly aggressive decision making when initially confronted by a larger opponent, it does not fulfil the requirements of hypothesis three as even though many small males did escalate the first fight to grappling, they were rarely successful ($n=6$). As weight asymmetry approached forty percent (the upper limit in this work) the more likely a small male would be to escalate his first encounter to grappling. Under normal circumstances a smaller male will not engage an opponent if he has little chance of success. While some studies have forced aggressive interactions by manipulating fight parameters (see above), for the naïve small males in the present study to engage with an opponent that was up to forty percent heavier than themselves potentially points to components of innate

genetic aggression or aspects of decision making akin to the desperado effect that have not been explored elsewhere. The desperado effect would normally be used to explain those scenarios in which males take the option of standing and fighting rather than retreating; either because winning the contest may provide high value benefits that are worth potentially high risks (including death), such as reproductive success (Grafen, 1987; Elias et al., 2010; Rypstra et al., 2016), or because there is no other choice but to fight, as seen in male *Gryllus texensis* that have lost the power of flight and cannot escape as easily, and so engage in escalated fights with other males (Guerra and Pollack, 2010). None of those scenarios are applicable to the current study, however, and further investigation into desperado effects in divisively asymmetric first contacts would be an interesting line of further investigation.

5.4.3 First fight precedent

Finally, it was found that first fight success for the small males, regardless of escalation level, set a significant precedent for overall encounter result. While a total of thirteen small males were victorious at the end of their encounter, eleven of those had beaten their larger opponent at first contact. Interestingly, the first contact did not always escalate to grappling. The mean escalation level of these first fights was 3.67, meaning that small males were/are capable of winning at varying levels of escalation. As weaponry, weight asymmetry, and motivational effects have been ruled out as reasons for small male victory as far as the methodology of this study and the scope of its analysis is concerned, the reasons for such a result are speculative at this time. Studies have shown that aggression levels can vary within a population, and that certain individuals that are more likely to demonstrate higher risk behaviour (such as latency to emerge from shelter in a new environment) also win more fights against weight-matched opponents that showed lower aggressive tendencies (Kortet and Hendrick, 2007). Some large males in the present study actually left the fight at low escalation levels, meaning that very little energy had been spent. Therefore, another possibility is that the larger male may not have considered the resource (in this case the combat arena) as valuable enough to warrant spending the energy on fighting, even against a smaller opponent, and thus return to exploring the environment instead of escalating the fight (Morrell et al., 2005), which also would be an example of what Maynard Smith and Parker (1976) called a 'paradoxical solution' to why small males

with lower RHP might win. In this scenario, the small male would then be a winner of a contest against a larger opponent, despite the fight not escalating to a high level. The significance of winner and loser effects and their impact on aggression, fight performance, and willingness to engage in further fights, especially against a previously dominant opponent, has been well documented (Khazraie and Campan, 1997; Killian and Allen, 2008; Reaney et al., 2011; Condon and Lailvaux, 2016). Whether the large male considers himself a loser in this context is not clear, but the effect it has on the small male could explain the relationship between first win and final victory seen in these results. Further investigation of how small male aggression is mediated by prior fight performance could be accomplished via manipulation of fight outcomes to promote the formation of an artificial winner effect. How the small male then fairs in combat against a larger opponent could then provide insights into how an opponent reads aggressive signalling, and whether a genuine victory is required to generate a winner effect in a small male.

CHAPTER 6. THE EFFECT OF MATING AND/OR FEMALE CONTACT PRIOR TO COMBAT ON MALE AGONISTIC BEHAVIOUR IN *GRYLLUS BIMACULATUS*

6.1 INTRODUCTION

Competition over resources is a primary aspect of aggressive interactions, but the value of any given resource can vary between individuals, such that we can distinguish between absolute and subjective resource value (RV). The more importance the value of a resource has to any given competitor, the more they will invest in claiming it (Enquist and Leimar, 1987; Hurd, 2006; Arnott and Elwood, 2008). This can potentially override normal predictors of fight outcome, such as body weight or weapon size (Hofmann and Schildberger, 2001; Gruber et al., 2016; O'Connor et al., 2015). Context dependent motivational asymmetry can therefore act as an extremely important component of fight escalation, modifying thresholds of acceptable loss and increasing risk taking behaviour that can have a major effect on fight outcome (Maynard Smith and Parker, 1976; Arnott and Elwood, 2009). For many species, competition arises over access to mating opportunities, either by competition for territory that receptive females will likely be found in (Siva-Jothy, 1987; Jang et al., 2008; Bergman et al., 2010; Santos and Peixote, 2017), or fighting over access to females already present (Kelly, 2006; Casey et al., 2015).

6.1.1 Mate guarding

Following mating, a male cricket will normally stand motionless in close proximity to the female while maintaining antennal contact, but will engage in aggressive behaviour toward nearby males if they attempt to approach the female (Frankino and Sakaluk, 1994). This combat readiness will normally last at least as long as it takes for the male to generate a new spermatophore (known as the sexual refractory period and lasts approximately fifty-five minutes in *Gryllus bimaculatus* (Ureshi and Sakai, 2001; Parker and Vahed, 2009)), at which point he may mate again with the same female. This repeated mating/guarding behaviour is seen in many commonly studied species including the Anostostomatid *Libanasidus vittatus* (Bateman and Toms, 1998) and the Gryllids *Gryllus bimaculatus* (Simmons, 1986a; Wynn and Vahed, 2004), *Gryllodes sigillatus* (Bateman and MacFayden, 1999), and *Gryllus pennsylvanicus* (Souroukis and Murray, 1994). Post-mating aggression

may be driven by the need to ensure that the female remains uncompromised, that is that the spermatophore attached to her is not knocked off by the actions of a competing male during sperm transfer, a process taking around 60 minutes to complete (Simmons, 1986b), and that the actions of rival males or the female herself will not adversely affect spermatophore retention by trying to remove it (Sakaluk, 1991; Souroukis and Murray, 1994; Bateman and MacFayden, 1999; Wynn and Vahed, 2004; Parker and Vahed, 2010).

6.1.2 Female contact and increased male aggression

An increase in aggression associated with female presence has been demonstrated in many species of field crickets (Dixon and Cade, 1986; Simmons, 1986a; Tachon et al., 1999; Brown et al., 2006; Brown et al., 2007; Loranger and Bertram, 2016; Montroy et al., 2016). The effect of mating itself and spermatophore transfer on aggressive behaviour is not explicitly clear, however (Killian and Allen, 2008; Judge et al., 2010). The effect of female contact on male aggression, specifically against a previously dominant opponent was shown by Killian and Allen (2008). The authors conducted bouts between pairs of naïve adult male *Acheta domesticus* to the point of established dominance (the subordinate male consistently evades confrontation with his opponent i.e. level 1 on the scale used in Hofmann and Schildberger (2001)), separated the males, and then allowed the subordinate males contact with a female. Post-treatment, the previously fought pairs of males then refought the same opponent. Previously subordinate males that had mated with a female before the rematch showed willingness to engage in aggressive confrontation, with >79% of bouts reaching at least level 3 on the scale in Hofmann and Schildberger (2001). In contrast, control pairs in which neither male received treatment but were only separated for a set time, saw escalation to at least level 3 in only 26% of bouts. The study went on to show that the act of mating was not necessary to promote this change in motivational state and its effect on aggression. Groups of experimental subordinate males that only achieved chemo-tactile contact with a female or failed to secure a full mating showed levels of aggression not significantly different to those seen in the mated subordinates: level 3 or higher in 57% of chemo-contact only, and in 63% of failed matings. This conclusion fits neatly with that of Khazraïe and Campan (1997), who showed that established dyadic hierarchies will generally not alter until the motivational statuses of the involved males change.

Conversely, however, a paper by Judge et al., (2010) entitled “A lover, not a fighter: mating causes male crickets to lose fights” that used *Gryllus pennsylvanicus*, concluded that mating actually reduced a male’s own fighting ability when fought against a naïve male. Mated males won less often compared to unmated males, or males with only female contact experience. However, the study itself did not always allow a male to maintain contact with his mate after spermatophore transfer, potentially removing the motivation for the aggressive behaviour that mate guarding triggers as the male enters the sexual refractory window for spermatophore regeneration. The authors highlighted this drop in aggression post-mating by comparing mated male combat performance with males who were mounted by a female but did not transfer a spermatophore. These experience-only males won their fights significantly more often than mated males or naïve males and showed increased aggression, indicating that their motivational status was changed.

The two original studies Killian and Allen (2008) and Judge et al.(2010) ended up demonstrating some aspects of the motivational effect that mating and female contact can have on aggression but they did it in different ways. For the reader this presents a problem. Mating was shown to cause males to both win **and** lose fights. This obviously causes some degree of confusion, though it is also important to note that Killian and Allen (2008) utilised a different experimental design and species of cricket to Judge et al. (2010).

6.1.3 Aims

The aim of this study was to investigate the effect of mating, denied mating (i.e. separation prior to spermatophore transfer), and near-mating opportunities (i.e. female contact), on male aggression.

Because the results of Judge et al. (2010) and Killian and Allen (2008) were, on the face of it, contradictory, I will avoid making *a priori* directional predictions about the effect of mating on aggression in *G. bimaculatus*.

As this study was itself a unification and consolidation of two contradictory behavioural works, the methods of Study 1 and Study 2 reflect those used by the original authors. As both original studies used different species of cricket to come to their conclusions, *Gryllus pennsylvanicus* in Judge et al. (2010), and *Achetus domesticus* in Killian and Allen (2008) the

decision was also made to remove this issue as well. Both study recreations therefore made use of *G. bimaculatus* as the test species.

6.2 METHODS PART ONE

6.2.1 Study 1 Recreation of “A lover, not a fighter: mating causes male crickets to lose fights”

The methods of this experiment were based on Judge et al. (2010), but used *G. bimaculatus* as the test species to investigate the effect of female contact with an experimental male (EM) prior to agonistic conflict with a naïve stimulus male (SM). Four treatments based on the methodology from Judge et al. (2010) in their original research paper were carried out following their protocol.

6.2.1.1 Preparation and treatment of crickets

All male crickets (n=200 in weight-matched pairs i.e. 100 pairs) were weighed, matched, marked for experimental use, and singly housed prior to use in this experiment as outlined in section 2.2.1. All female crickets (n=100) were selected and treated as outlined in section 2.2.2.

Pairs of previously weight-matched (to within 2%) and marked male crickets were individually transferred to neighbouring plastic holding tanks measuring 200 x 130 x 70mm (see Figure 6.1), over which a heat lamp maintained a temperature of 27-28°C. To minimise stress, at no point were the animals physically handled. One of the males was randomly nominated to be the Experimental Male (EM) via a coin toss. His counterpart thus became the Stimulus Male (SM). Both the SM and the EM were given two minutes to acclimatize to the holding tanks before the treatment stage began. A virgin, naïve female *G. bimaculatus* was then added carefully to the holding tanks housing the EM in Treatment 1.1 and 1.2.

It is possible that the behaviour of the stimulus male could also be altered by the scent of the female. However, all stimulus males in these trials were taken from the husbandry room in which cages containing females were also present. Therefore, although the stimulus males came from single sex tanks, they would all have had previous experience of female scent.

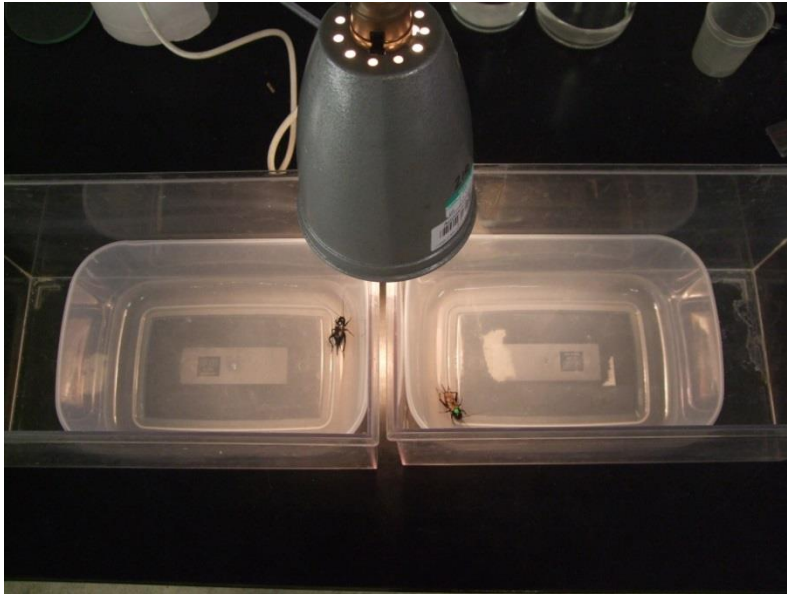


Figure 6.1: The pair of holding tanks used for Treatment groups 1.1 and 1.2 (photo credit: DG).

Note: the holding tanks were in turn held inside larger plastic aquaria to prevent males from jumping and escaping during the treatment phase

6.2.1.2 Female selection for male treatments

Both studies recreated below require female contact and, in some cases, successful mating behaviours from experimental males. The female used for each treatment stage was selected from a cultured single sex population of virgin adults based on her body length as to ensure she was neither too short nor too long to successfully mate with the experimental male if required. No female was ever reused with any other male.

6.2.1.3 Treatment Group Specific Details

Block 1

Treatment 1.1: Mounted only, female removed

The EM was allowed to court the female until she initiated mounting behaviour. Once the male was in place to transfer a spermatophore, the mating was interrupted by gently knocking the female from the male with a small paintbrush. The female was then carefully removed from the holding tank (n = 25 EM males).

Treatment 1.2: Mated, female removed

The EM was allowed to court the female and transfer a spermatophore. Once the spermatophore had been transferred the female was carefully removed from the holding tank (n = 25 EM males).

6.2.1.4 Treatment Group Specific Details

Block 2

Treatment 1.3: Mated and mate guarding

Both the designated EM and the SM were placed into the arena on either side of the dividing wall and allowed to acclimate for 2 minutes. As in the previous set, a size appropriate female cricket was then gently added to the side of the arena containing the EM (as shown in Figure 6.2). The EM was allowed to court the female and transfer a spermatophore. Once the spermatophore was successfully transferred the fight stage was started (n = 25 EM males).



Figure 6.2: The female was gently placed within a few cm of the EM inside the arena for Treatments 1.3 and 1.4 (photo credit: DG).

Treatment 1.4: Mated, female covered

This treatment follows the protocol of Treatment 1.3 but differs in that the mated female was then covered over with an opaque lab bottle lid to prevent contact with the EM or SM (see Figure 6.3, n= 25 EM males).



Figure 6.3: The female was covered with a robust, opaque blue bottle lid after the male had mate-guarded for two minutes (photo credit: DG).

6.2.2 The fight stage for all groups in recreation of ‘*Lover, Not a Fighter*’

The SM and EM from Block 1 Treatments 1.1 and 1.2 were gently moved in their holding tanks and transferred carefully into the fight arena measuring 130 x 130 x 180mm with a dividing wall running through the centre (see Figure 6.4). The transfer process took approximately 60 seconds. The two males were placed one on either side of the removable dividing wall to acclimatize for two minutes.



Figure 6.4: SM and EM males in groups 1.1, and 1.2 were placed on either side of the arena wall (photo credit: DG).

Males in Block 2 Treatments 1.3 and 1.4 were already in the arena as this was where they had received treatment.

Once all transfer or post mating requirements were complete, the dividing wall was removed and a 5 minute timer was started.

All fights between opponents were watched and accurately scored with the level of escalation and the winner in each case using the escalation scale in Figure 2.7 and following the fight protocol laid out in sections 2.8 and 2.9 of the General Methods. Judge et al. (2008) notes that fight winning *G. pennsylvanicus* crickets often tremulate and sing loudly while chasing the loser of aggressive encounters; a behaviour that is also demonstrated by *G. bimaculatus*. Following Judge et al. (2010), any pairs of males that did not engage in any level of aggressive interaction (intensity level=0) were removed from the study. Judge et al. (2010) did not place a time limit for conducting the fight encounters between males. Therefore, for this study, a fight was treated as concluded when intensity level 1 (Established Dominance) was consistently observed for a period of 2 minutes.

The whole arena and dividing wall were wiped down with cotton-wool balls and 70% ethyl alcohol between bouts of fighting. This was in order to remove any residual pheromone traces from previous trials that may have impacted future data collection.

6.2.3 RHP (Resource Holding Potential) data collection

Analysis for this work included consideration of weapon size as a potential factor in fight outcome and took a measure of maxillae span as an indicator of weapon size. As such, after their experimental fight was concluded, all males were euthanized by freezing and then stored in 70% ethanol as per section 2.4 of the General Methodology. Maxillae measurements were made with the use of a dissection microscope and an eye-piece graticule calibrated to a stage micrometer. The maxillae span was measured across the basal articles of the maxilla (Figure 6.5) as per Judge and Bonanno (2008), and relative weaponry size for analysis was calculated as EM-SM.

Measurement of maxillae span as used in:

Judge, K.A. Bonanno, V.L. 2008 Male weaponry in a fighting cricket. *PLoS One* 3(12) pp.1-10

Content removed for copyright reasons

Figure 6.5: Ventral aspect of the head showing measurement points for the maxillae span, MxS. Picture adapted from Judge and Bonanno (2008).

6.2.4 Statistical Methods

The effect of female contact treatment on contest intensity (binary response; whether the fight reached Level 5) in both experimental groups of 'Lover, Not a Fighter' was tested using a logistic binary regression with treatment, absolute relative weaponry (EM-SM) and the interaction between these as independent variables.

All analysis in this recreation was conducted using Rstudio running R v3.4.1

6.3 METHODS PART TWO

6.3.1 Study 2 recreation of “Mating Resets Male Cricket Aggression”

The set of experiments in study 2 were based on Killian and Allen (2008), but using *Gryllus bimaculatus* as the test species instead of *Acheta domesticus*.

To determine the effect that contact with a female cricket would have on a male cricket's aggressive state, previously subordinate crickets (having just lost a bout of fighting with a conspecific) were allowed to interact with a female. Any resulting change in aggressive behaviour, possibly due to the female contact affecting his motivational state was recorded by then refighting the male against his previously dominant opponent.

This study required that male pairs in this study fought each other twice. The initial encounter was used to establish a dyadic hierarchy between the two, with the dominant male becoming the Stimulus Male (SM) and the subordinate male (EM) his opponent. The males were then refought after the EM had undergone a specific level of contact treatment. This study compared 4 treatment groups. The differences between treatments relate specifically to the degree of female contact the EM male received.

6.3.1.1 Changes to methodology

There are a few aspects of this recreated study that have purposely been altered, deemed unnecessary, or excluded as their components have been examined elsewhere. The most significant of these points are:

- Killian and Allen (2008) required that the crickets be acclimated for 30 mins pre-study in a dimly lit room. As *Gryllus bimaculatus* are behaviourally robust, a reduced resting period was used in my work.
- Killian and Allen (2008) only required that one cricket in each pair be marked on the pronotum with nail varnish. Both crickets used in my trials were marked.
- Killian and Allen (2008) paired crickets up by eye, to be “approximately size matched”. Crickets in my study were paired based on weight in grams to 2 decimal points.

- Killian and Allen (2008) made use of a video camera to record fights from above for fight data. All individual fights in my study were watched by eye making use of different perspectives to accurately note behavioural cues that are not easily viewed from above e.g. mandible flaring in small males is more subtle than when performed by a larger male.

6.3.1.2 Preparation and treatment of crickets

All male crickets were weighed, matched, marked for experimental use, and singly housed prior to use in this experiment as outlined in the General Methods sections 2.1 and 2.2. All female crickets were selected and treated as outlined in the General Methods 2.2.2.

6.3.2 Fight: Establishing a dyadic hierarchy

A pair of weight matched males was transferred into the fight arena, with one male placed either side of the dividing wall. Heat lamps were placed above the arena to maintain a temperature of 27-28°C. The two males were allowed to acclimatize for two minutes before the wall was removed. As the males began to move around the arena, a stopwatch was started as soon as they made contact with each other (usually via antennae) in order to record specific points in the ensuing fight. All fights between opponents were watched and accurately scored with the level of escalation and the winner in each case using the scale in Figure 2.7 and following the fight protocol laid out in sections 2.8 and 2.9 of the General Methods.

Once an observed dyadic hierarchy (Level 1) was in effect, the males were separated to either side of the arena and the dividing wall was replaced. The dominant male was then designated SM (Stimulus Male) and the subordinate male EM (Experimental Male) for use in the next experimental phases.

6.3.3 Treatment Group Specific Details

Within one minute of the male crickets being separated, a randomly assigned treatment was chosen from the 4 possible treatments and a suitably sized (see 6.2.1.2), naïve, virgin female *G. bimaculatus* was placed in the enclosure on the same side as the EM (no female

was used in the Control pairs). The four experimental group Treatments, as used in Killian and Allen (2008) were:

Treatment 2.1: Successful spermatophore transfer.

The EM was allowed to elicit a mating response from the female, via mating call, which resulted in transfer of a spermatophore to the female. The time to successfully secure a spermatophore was noted. This was based on visual confirmation that the male had successfully transferred the spermatophore. The male was then allowed to engage in normal mate-guarding behaviour for a further 2 minutes before the female was carefully removed.

Treatment 2.2: Interrupted copulation attempt

The EM engaged in normal courtship behaviour including singing and was mounted by the female. A soft, clean paintbrush was used to gently move the female whenever she attempted to mount the male, thereby preventing successful spermatophore transfer.

Note: Killian and Allen (2008) allowed this stage to be self-selecting i.e. the male could not secure a mating himself, despite trying for 15 minutes. After pre-experiment trials using *G. bimaculatus*, I considered this to be too unreliable as a method. Actual failed matings between virgin, size-matched crickets accounted for less than 10% of test pairs. One might infer that a male that fails to mate is biologically defective in some capacity that might affect more than just spermatophore transfer. The decision to intervene in this process removed the self-selectiveness of this stage, but also allowed for a good sample size. The original study only produced 19 data points of this stage, or approximately 16% of their female contact outcomes.

Treatment 2.3: Chemo-tactile contact with the female.

Once the EM attempted courtship behaviour after making physical contact, specifically singing, the female was removed from the enclosure.

Treatment 2.4 Control: No female contact.

The pair of males was left for 10 minutes on either side of the dividing wall.

All female-treatment pairs were given a 5 minute break after their contact with the female, as outlined in the original study by Killian and Allen (2008).

6.3.4 Fight 2: Testing the established dyadic hierarchy post-treatment

After the treatment and 5 minute rest stages, the dividing wall of the arena was removed and the EM and SM were observed. As before, a timer was started once contact was made. All fights between opponents were watched and accurately scored with the level of escalation and the winner in each case using the scale in Figure 2.7 and following the fight protocol laid out in sections 2.8 and 2.9 of the General Methods. In this data collection, whether the EM became dominant by the end of the fight period was also noted.

The second round of combat was timed for 3 minutes as this was deemed all that was necessary to observe the effect of treatments on previously subordinate male behaviour. Killian and Allen (2008) did not state a specific length of time for this stage.

6.3.5 Statistical Methods

Logistic models were built with treatment as categorical variables and fighting behaviours in each test e.g. whether the fight escalated to level 3+, as binomial variables. Note: Killian and Allen (2008) set their aggression threshold for analysis as level 3+ (unilateral mandibular flaring). As in the original paper by Killian and Allen (2008), statistical testing of differences in fight escalation using Wald's chi-square analysis, results were considered significant at the $P < 0.0083$ (0.05/6) level in order to control for type I errors.

Kruskal-Wallis test for non-parametric data was used to compare frequency of behavioural responses between groups. As the behavioural treatments were applied in order to promote aggression between groups, a non-parametric test was suitable as non-normally distributed data were an expected response. All statistical work was carried out using R Studio, R version 3.4.1. GGplot2 and car packages.

6.4 RESULTS

6.4.1 Results: Lover, not a fighter

6.4.1.1 Experiment 1: The effect of mating experience on aggression

Eighty-five percent of weight-matched *G. bimaculatus* pairs escalated the fight to its highest level of intensity (Level 5), involving locking of the jaws and grappling, regardless of the experimental treatment that had been applied (Table 6.1). Analysis of fight data indicated that neither treatment in Experiment 1 (i.e. mated versus mounted only) affected the probability of fights between the experimental male (EM) and stimulus male (SM) escalating to Grappling (Chi-squared for given probabilities: $\chi^2=0.44$, d.f.=1, N=50, $p=0.51$, Figure 6.6).

Table 6.1: Fight escalation figures from both treatments, Mated or Mounted only, in each experiment. The majority of fights escalated to Level 5, Grappling.

<u>Intensity</u>	<u>Experiment 1</u>		<u>Experiment 2</u>	
	<u>Mated</u>	<u>Mounted only</u>	<u>Contact</u>	<u>No contact</u>
0	0	0	0	0
1	0	1	0	1
2	1	3	0	0
3	0	2	0	0
4	4	3	0	0
5	20	16	25	24
Total=	25	25	25	25

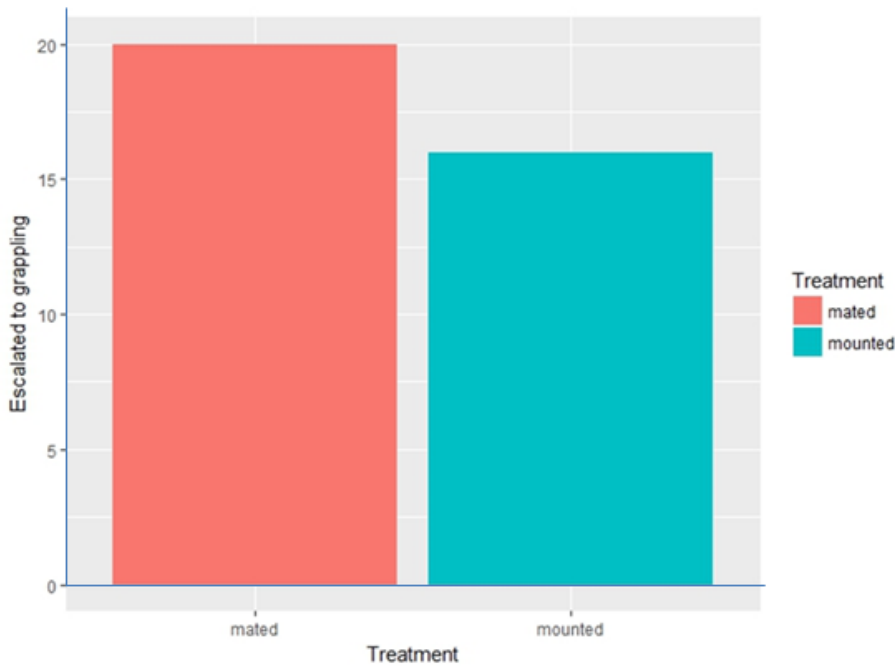


Figure 6.6: Fights escalating to grappling by treatment (Mounted or Mated to the female, n=25 in each group).

A multiple logistic regression model was run with escalation to Grappling as a binomial dependent variable, and treatment, weapon difference and their interaction as independent variables. There was no significant interaction between treatment and weapon difference (Wald $\chi^2=0.694$, $p=0.41$) and no effect of either treatment (Wald $\chi^2=1.367$, $p=0.24$), or weapon difference (Wald $\chi^2 = 0.105$, $p=0.75$) on fight escalation. Removing the non-significant interaction term did not improve the fit of the model nor did further model reduction ($\Delta AIC < 2$).

The effect of mating treatment on the probability of the experimental male (EM) winning the fight indicated that mated males were no more likely to win their fights than males that had only experienced contact with a female (Chi-squared for given probabilities: $\chi^2=0.27$, d.f.=1, N=50, $p=0.6$, Figure 6.7).

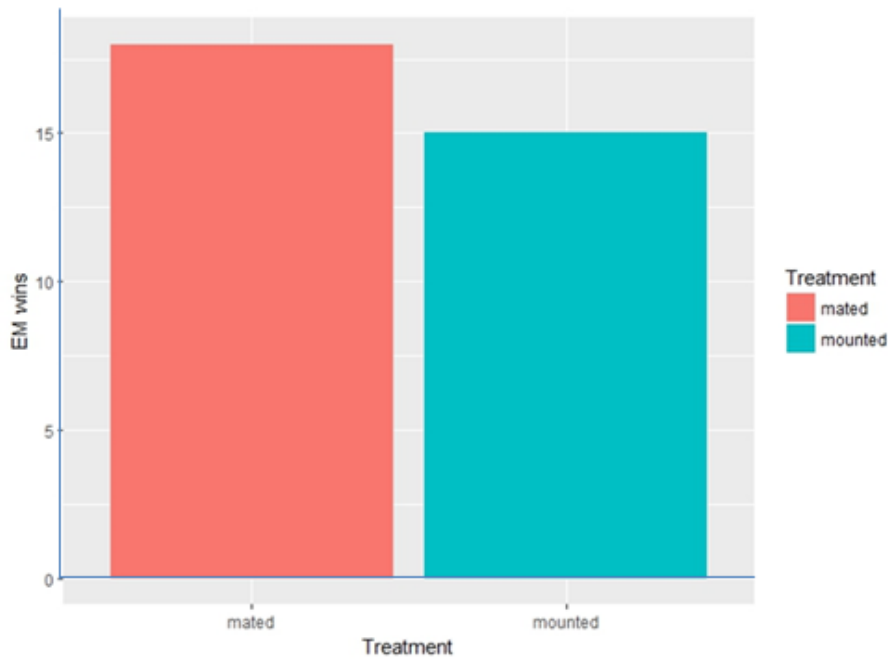


Figure 6.7: Fights won by the experimental male (EM) by treatment (Mounted or Mated to the female, n=25 in each group)

A multiple logistic regression model was built with fight outcome as the binomial dependent variable and treatment, weapon difference, and their interaction as independent variables. The model showed no significant interaction ($n=50$, $d.f.=1$, Wald $\chi^2=0.0001$, $p=0.99$), nor any effect of treatment (Wald $\chi^2=0.382$, $p=0.54$), but a significant effect of weapon difference (Wald $\chi^2=4.433$, $p=0.035$, combined model AIC=66.325). Following model reduction, only weapon difference had a significant effect on fight outcome (Wald $\chi^2=4.433$, $p=0.035$, model AIC=63.591, Figure 6.8).

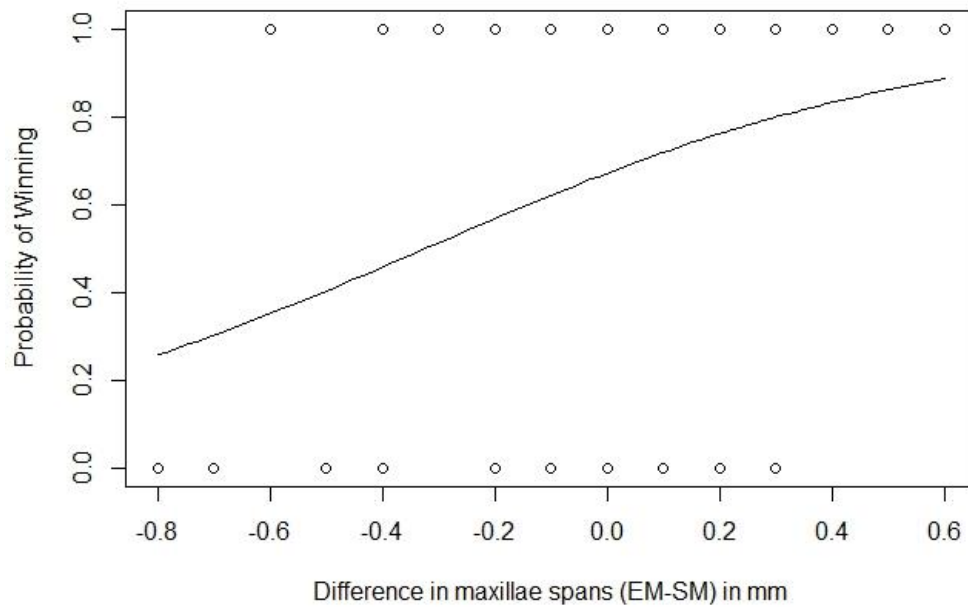


Figure 6.8: The effect of weapon difference on the probability of the experimental male (EM) winning his fight against the stimulus male (SM) (in experiment 1).

6.4.1.2 Experiment 2: The effect of maintaining female contact on aggression

The probability of fights escalating to grappling was not significantly different between treatment groups (Chi-squared for given probabilities: $\chi^2=0.02$, d.f.=1, N=50, $p>0.05$, Figure 6.9).

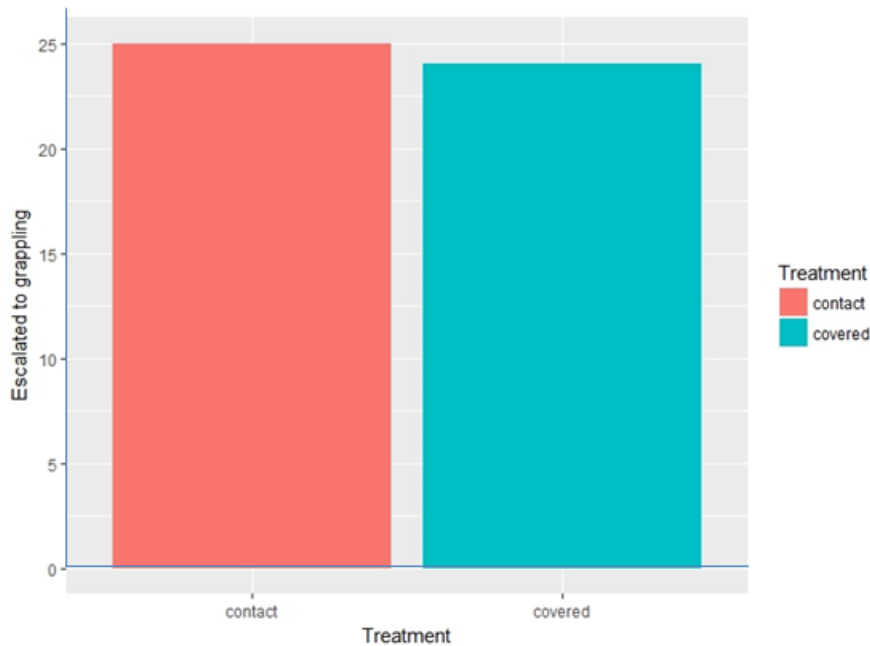


Figure 6.9: Fights escalating to Grappling by treatment (Mated, with female contact, or, Mated, with female then covered, n=25 in each group).

A multiple logistic regression model was run, with fight escalation (i.e. probability of the fight escalating to grappling) as the dependent variable, and weapon difference, treatment group, and their interaction as independent variables. This showed that there was no significant interaction (n=50, d.f.=1, Wald $\chi^2=0$, $p=0.99$), and no significant effect of either treatment (Wald $\chi^2=0.972$, $p=0.99$) or weapon difference (Wald $\chi^2=0$, $p=0.76$, combined model AIC=16.289) on escalation. Removal of the non-significant interaction improved the fit of the model (AIC change=2) but neither treatment nor weapon difference achieved significance. Further model reduction leaving only weapon difference as a predictor variable improved the fit of the model but still returned non-significant results (Wald $\chi^2=0.063$, $p=0.8$, model AIC=13.738).

Experimental males were not more likely to win the fight if they maintained contact with the female than if she was covered (Chi-squared for given probabilities: $\chi^2=0.26$, d.f.=1, N=50, $p=0.61$, Figure 6.10).

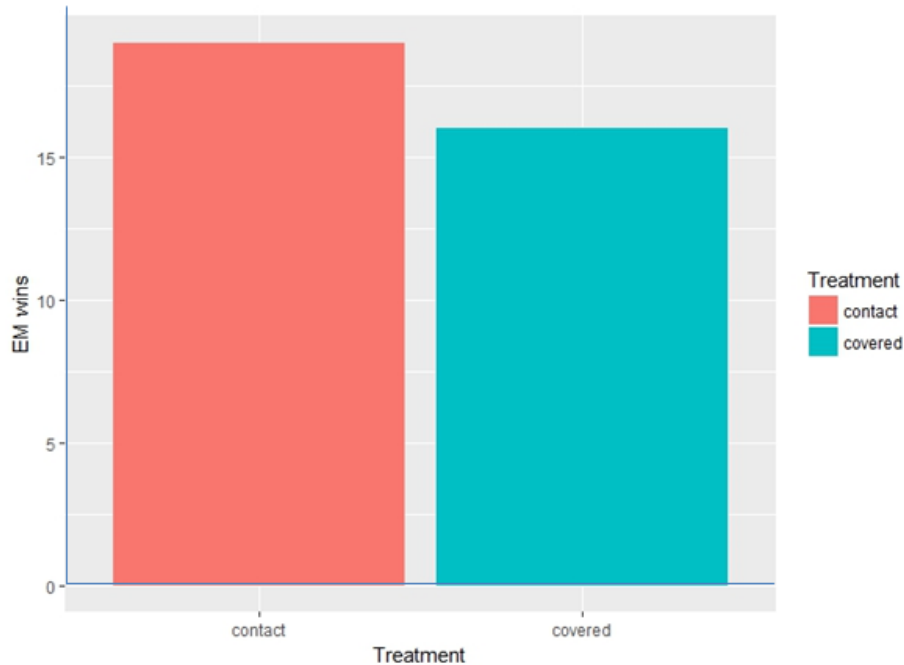


Figure 6.10: Fights won by the EM by treatment (Mated, with female contact, or, Mated, with female then covered, n=25 in each group).

A multiple logistic regression model was run with fight outcome as the dependent variable, and weapon difference and treatment and their interaction as independent variables. There was no significant interaction (n=50, d.f.=1, Wald $\chi^2=3.076$, $p=0.08$) and neither treatment (Wald $\chi^2=0.519$, $p=0.47$) or weapon difference (Wald $\chi^2=1.871$, $p=0.17$, combined model AIC=53.897) were significant predictors of fight outcome. Following model reduction weapon difference emerged as the only significant predictor of fight outcome (Wald $\chi^2=6.876$, $p=0.009$, model AIC=13.738).

6.4.2 Results: Mating resets aggression

6.4.2.1 Encounter 1 and treatment

When pairs of weight-matched, sexually naïve *G. bimaculatus* males were allowed to fight in the arena, they formed a dominant-subordinate dyadic hierarchy within the arena environment. The majority of pairings in each pre-treatment group reached an escalation of at least Level 3 (unilateral mandibular flaring). This included 90% of control pairs, 97% of contact only pairs, 100% of failed mating pairs, and 96% of mated pairs. The mean escalation level of the fights was not significantly different between the four groups (Kruskal-Wallis $\chi^2=2.79$, $p=2.97$, Figure 6.11).

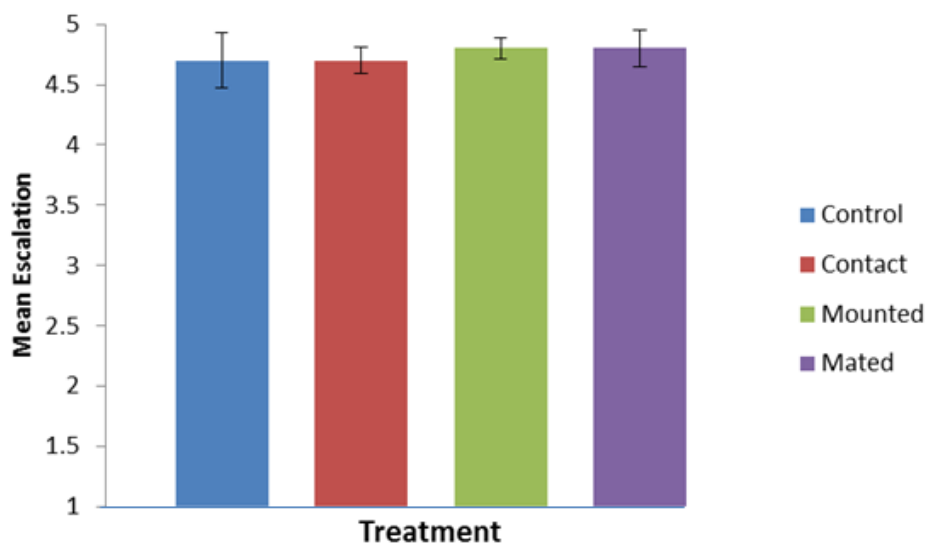


Figure 6.11: First encounter mean fight escalation with standard error bars across groups **before** treatment (n=25 in each group)

6.4.2.2 Encounter 2: The effect of treatment on escalation and fight level

Males in the mated treatment group were far more willing to enter into combat with their previously dominant opponent, with 100% of confrontations between the pairs reaching at least Level 3 during their second encounter (compared to 38% of the control group (Wald's $\chi^2=11.95$, $p=0.0008$). The probability of mated males escalating to level 3+ in the second encounter was not significantly different to the males in the contact treatment (61%, Wald's

$\chi^2=3.41$, $p=0.009$) or the males in the mounted treatment group (81%, Wald's $\chi^2=2.43$, $p=0.119$).

Female contact eliciting a positive effect on male motivation during follow up fights was not only seen in fight escalation but also in mean fight level across treatment groups (Figure 6.12). The mean fight level reached by mated males was 4.8, compared to control males averaging 2.2 (Kruskal-Wallis $\chi^2=27.52$, $p=1.552e-07$). A mean of 3.2 in the contact only group was also significantly higher than controls (Kruskal-Wallis $\chi^2=4.48$, $p=0.034$), and significantly lower than mated males (Kruskal-Wallis $\chi^2=14.25$, $p=0.0002$). Finally, the mean fight level reached by males that were mounted by the female but were prevented from transferring a spermatophore was 4.3, which was significantly higher than that of control males (Kruskal-Wallis $\chi^2=15.88$, $p=6.76e-05$).

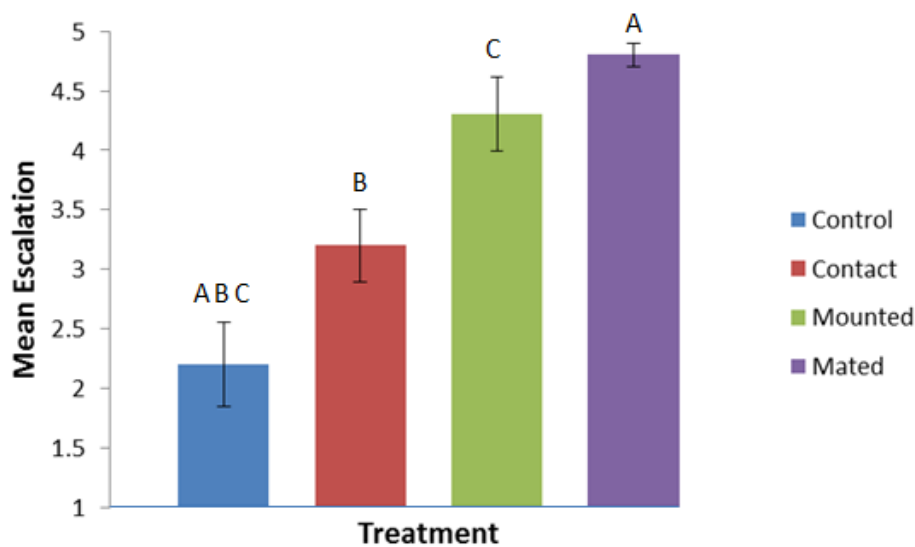


Figure 6.12: Second encounter mean fight escalation with standard error bars across groups **after** treatment (each group $n=25$). *Matching letters* indicate a significant difference between groups.

6.4.2.3 Encounter 2: Pre-established dominance and rank switch

Less than 2% of male pairs exhibited pre-established social rank at the start of encounter 1. After the treatment phase when pairs were refought in encounter 2, 28% of males across all treatments retained their subordinate states within the established dyadic hierarchy and did not attempt to reengage their opponent. In the mated male treatment, no pairs exhibited

pre-established dominance in the second encounter, compared to 62% of control pairs (Wald $\chi^2=11.3$, $p=0.0008$). 9.5% of males in the mounted-only group resumed a subordinate relationship with their opponent, which was significantly lower than the control group (Wald $\chi^2=9.93$, $p=0.0016$). 39% of males in the contact only group resumed their subordinate position compared to control males but the result was not significant (Wald $\chi^2=2.76$, $p=0.097$). A comparison of contact only and mated males showed a marginally non-significant difference in the probability that pairs showed pre-established dominance (Wald $\chi^2=6.82$, $p=0.009$) (NB: the threshold level of significance for Wald's chi square tests used in this study was $p<0.0083$, in order to reduce type I errors – see methods) (Figure 6.13).

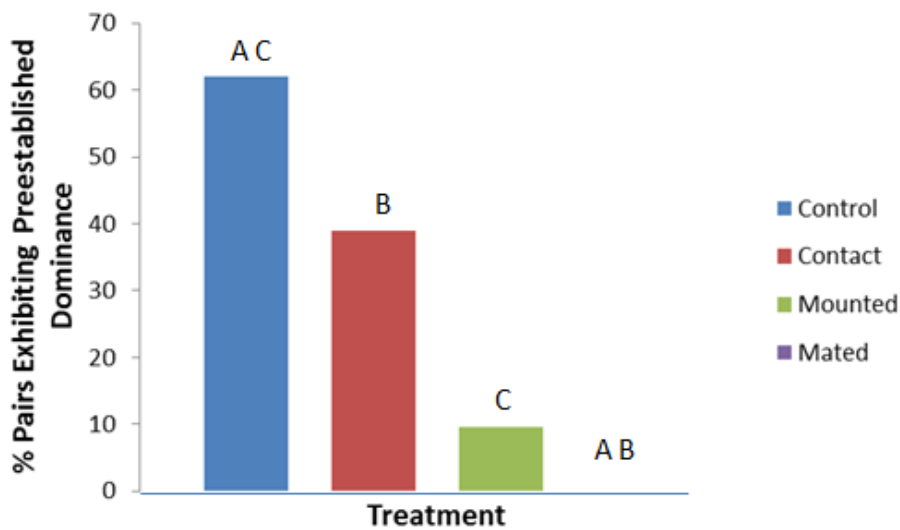


Figure 6.13: Percentage of pairs showing pre-established dominance by the EM in the second encounter (each group $n=25$). *Matching letters* indicate a significant difference between groups.

The potential for a reversal of rank between a pair of crickets, with the previously subordinate EM winning against his opponent in their second encounter, was most pronounced when comparing mated males and control males: 33% of mated males became dominant, compared to 4.7% of controls, though this figure was not statistically significant (Wald $\chi^2=4.36$, $p=0.037$, Figure 6.14) (NB: the threshold level of significance used in this study was $p<0.0083$, in order to reduce type I errors – see methods). While both other treatment groups did show a positive effect of treatment on tendency to rank switch, neither the mounted only group (19% switched rank, Wald $\chi^2=1.77$, $p=0.18$) or contact only

males (17% switched rank, Wald $\chi^2 = 1.54$, $p=0.22$) results were significantly different from the control group.

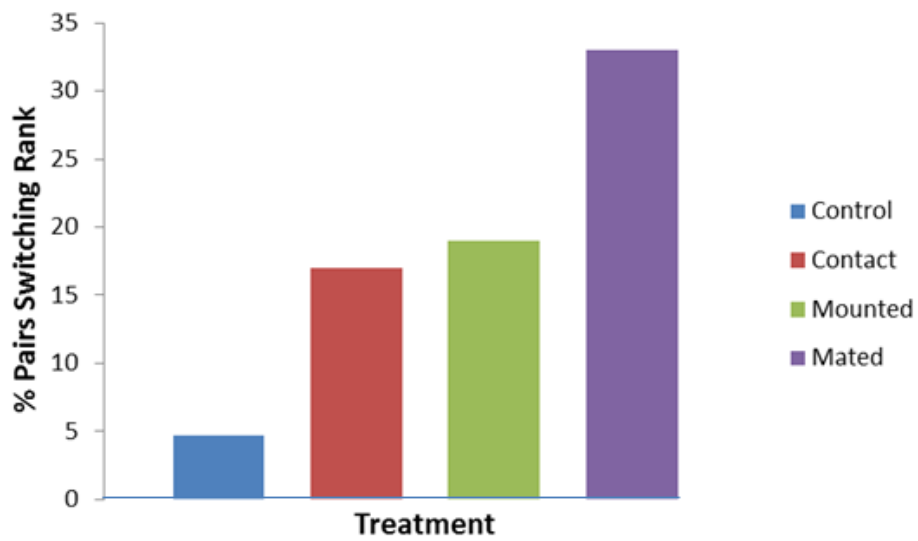


Figure 6.14: Percentage of EM males switching from subordinate to dominant position in encounter 2 in each treatment group (each group n=25).

6.5 DISCUSSIONS

6.5.1 Discussion: A Lover, Not a Fighter

This study aimed to assess the impact of female contact and mating experience on the likelihood of fight escalation and fighting success by altering the experimental males (EMs) motivational state before he fought his opponent, whilst also accounting for the RHP of each fighter. However, there was no significant difference between treatment groups in the probability of escalation to grappling or of the EM winning in either Experiment 1 in which experimental males were either mated to a female or only mounted before fighting, or in Experiment 2 in which experimental males were mated to a female and 50% were allowed to engage in mate guarding during their fight, whilst the other 50% were not allowed to maintain contact. The majority of fights across both recreated experiments from Judge et al. (2010) escalated to the maximum level, and weapon difference was the only consistent predictor of EM fight success across all treatment groups (but had no significant effect on the level of aggression in terms of whether or not the fight escalated to grappling). In contrast, Judge et al. (2010) found that the mated *G. pennsylvanicus* males in Experiment 1 were less likely to win fights than those that had only experienced female contact. This was the only major difference between the 2 species, though, and may just represent a species-specific threshold of acceptable loss. With all other results being fairly similar between treatments, it is reasonable to consider that female contact and/or spermatophore transfer, at least within the context of this experiment, is not enough to illicit a significant change in behaviour in this species.

Gryllus bimaculatus is an aggressive species of field cricket, and the males readily engage in agonistic displays over territory and mating opportunities with conspecifics. An escalation in fight intensity between a pair of naïve males that are meeting for the first time in a novel environment will normally be dependent upon asymmetry in body size and weight, with escalation level increasing as the degree of asymmetry between the opponents decreases (Dixon and Cade, 1986; Hack, 1997a; Hofmann and Schildberger, 2001; Brown et al., 2006). This study found that 85% of weight-matched *G. bimaculatus* pairs would escalate a fight to the highest level, involving locking of the jaws and grappling with the opponent, regardless of the experimental treatment that had been applied. The comparable study using *G.*

pennsylvanicus found that 64% of fights between weight-matched males escalated to this level of intensity (Judge et al., 2010). This is understandable given that the males were matched for weight.

Mating opportunities are a high value resource and the presence of a female can be an influencing factor on the length and costliness of escalated displays of aggression between males (Fitzsimmons and Bertram, 2013a; Loranger and Bertram, 2016; Montroy et al., 2016). Energetic investment in courtship and securing successful copulation is predicted to be a major source of motivation in terms of promoting aggressive behaviour, especially in order to prevent a rival male from dislodging a recently attached spermatophore and negatively affecting fertilization success (Simmons, 1986b; Simmons, 1987; Wynn and Vahed, 2004), or to prevent the female from removing it herself as she tries to mate with other males (Simmons, 1986a; Hockham and Vahed, 1997; Bateman et al., 2001a; Bussière et al., 2006). Yet, despite the high costs associated with losing contact with a mated female, there was no significant drop in aggression and the EM did not lose fights more often than a male who maintained contact with the female. One potential reason for the lack of significant difference in aggressive behaviours between the EM and SM when testing the effect of retaining female contact is the time scale involved, specifically in relation to the refractory period- the spermatophore renewal process. The fight stages of this work took place approximately 4 minutes after treatment, including mating treatments, and this short turnaround time might account for the minimal differences in behaviour seen between EMs that maintained female contact and those that did not. The refractory period is a time-fixed physiological mechanism in the male cricket, taking approximately one hour to complete and for the male to be ready to mate again (Ureshi and Sakai, 2001) that leads to renewal/replacement of the spermatophore, through a redirection of the male's physical resources, and could cause a drop-off of aggression and suppression of general courtship behaviours over time (Nagao and Shimowaza, 1987; Parker and Vahed, 2009). This means that once a male field cricket has transferred a spermatophore and engages in mate guarding behaviour in order to protect his investment against potential competitors, his ability to maintain an aggressive state over time could be limited as his body enters the refractory period, thereby directing physiological resources to producing a new spermatophore. Research examining ejaculate transfer rate in *G. bimaculatus* show that the

first ten minutes after spermatophore transfer sees optimal sperm movement (Parker and Vahed, 2009). It is logical to assume that the ten minute period immediately after mating requires the mated male to maintain his aggressive stance prior to aggression levels dropping as the refractory process begins.

Judge et al. (2010) examined aggression in *G. pennsylvanicus*, and this recreation used the closely related *G. bimaculatus*; both studies involved subjecting female-experienced males to combat against a naïve male within approximately 4 minutes of the female mating treatment, and both found no statistical difference in fight escalation or result whether the female was covered or not. This time window is well within the post-mate period that precedes the effect of refractoriness (predicted lowering of aggression) and could account for the lack of significant differences between treatment groups. This idea has never been tested scientifically but a study structured around measuring aggressive responses at set points within the refractory period would perhaps shed some light on how the refractory process affects, and eventually lowers, the aggressive behaviour of a mate guarding cricket. Other components of female presence affecting fight outcome could be seen in the data; males that were allowed continued access to their mate when the fighting started fared slightly better than their naïve opponent, winning 76% of the time, compared to 64% of males whose female was covered. A lack of statistical significance when comparing fight results between males that lost female access and those that maintained contact supports the notion that contact with a female does increase aggression in males, but also that the time gap between mating and fighting may have played a role in allowing males to maintain their aggression levels. The refractory period had possibly not had time to come into effect in the males that lost female contact, meaning that aggression across groups was comparably high.

6.5.2 Discussion: Mating Resets Aggression

This study found that in the absence of female contact or mating, male *G. bimaculatus* that had previously been beaten in combat were most likely to exhibit pre-established subordinate behaviour when they re-met their opponent. Experimental males (EM) were more likely to reengage in agonistic display with that same previously dominant opponent if they had received a treatment involving female contact in between the two fights. Contact

only and failed copulation males all escalated significantly more often than control animals. Males that were allowed to copulate with a female and mate guard her for two minutes before she was removed were more aggressive in their following encounter and showed a tendency to escalate a fight more often than a comparable male that had only made antennal contact with a female. Mated males also never immediately resumed a subordinate status when they first re-met their opponent, compared to 62% of control males. The tendency for the EM to resume subordinate behaviour upon re-facing their opponent dropped off as degree of female contact increased, with contact only groups and failed mating groups displaying increased probabilities of re-engaging, and with mated males showing the most significant change of behaviour. These results closely reflect those of *A. domesticus* in Killian and Allen (2008) with the exception that whilst they found that failed copulation group males tended to exhibit pre-established subordinate behaviour more often than mated males, this study found that in *G. bimaculatus*, this was not the case. The present study therefore supports Killian and Allen's (2008) claim that mating resets aggression.

The observed changes in behaviour in experimental males in this study indicates that the motivational status of the experimental male has changed, and that whilst his opponent had previously beaten him in a fight based on mutual comparison of physical traits such as weapon size traits (Emlen, 2008; Judge and Bonanno, 2008; McCullough et al., 2016), the treatment had affected his behaviour in such a way that the original asymmetry in fighting ability was no longer the only factor affecting decision making when the opponents refought. Previous fighting experience can affect male decision-making in both positive and negative ways (Rutte et al., 2006; Stevenson and Rillich, 2013b); losing a fight can influence the outcome of subsequent confrontations for up to several hours and a loser male that is confronted by a previously dominant opponent is far more likely to lose, or choose to flee from the fight (Khazraie and Campan, 1997; Khazraie and Campan, 1999; Iwasaki and ast et al., 2006), though he will also be more likely to lose against novel opponents as well (Rutte et al., 2006). To cause a shift in behaviour that overcomes the 'loser effect' requires that the male overcome his decision to flee when he next meets his opponent, and this requires motivation in the form of a high value resource that forces the opponents to reassess their dyadic status. Theory suggests that the relationship between two opponents will not change

unless something in their environment does; a male will not re-engage a dominant opponent unless the risk/cost is acceptable (Khazraïe and Campan, 1997). Within the context of this experiment, the valuable resource that the EM was given access to was a virgin female, though in other studies food and shelter ownership have also been shown to promote similar responses as they are both important factors in achieving lifetime reproductive success (Simmons, 1986a; Bateman, 2000; Nosil, 2002; Jang et al., 2008). This change in motivational state, brought on by subjective resource value can be a powerful leveller that has been shown to overcome physical asymmetries such as body size/mass or weapon size that would normally be considered significant predictors of fighting success (Hofmann and Schildberger, 2001; Hurd, 2006; Brown et al., 2006; Brown et al., 2007). Note: Killian and Allen (2008) did not control for the mass of *A. domesticus* males that fought in their original study. This study using *G. bimaculatus* controlled for this parameter as it was desirable to limit the traits that could influence fight outcome.

As females were used as the high value resource in this study, the effect of subjective resource value on the EM can be assumed to revolve around mating opportunities and perceived sperm competition. The EM previously lost a fight in an empty arena but by allowing him access to a virgin female, a significant source of motivation, the potential reward for winning is higher, as is the cost of losing. A receptive, virgin female cricket will preferentially mate with dominant males (Kortet and Hendrick, 2005; Shackleton et al., 2005; Bretman et al., 2006), so males who were not allowed to transfer a spermatophore during their treatment (Contact and Mounted groups) would be expected to demonstrate a heightened degree of aggression as they would now be aware that a female is part of their environment (Tachon et al., 1999; Brown et al., 2006; Brown et al., 2007). This increase in aggression was observed in both unmated groups, but Mounted group EMs escalated to higher fight intensities on average than Contact EMs and showed a lower tendency to resume a subordinate position. This difference could be due to the increased female contact time (15 minutes of failed mating attempts, compared to a matter of seconds for the contact only males) during the treatment phase. It should also be acknowledged that fifteen minutes of courtship behaviour would represent a high degree of energetic investment from the courting male, so to immediately resume a subordinate position when facing his opponent might be considered behaviourally maladaptive. For the males that had

successfully transferred a spermatophore and been allowed to initiate mate guarding, the most significant change in behaviour was observed when the second encounter began, and this may be because of the two minute mate guarding portion of his treatment time. Experimental evidence suggests that mate guarding behaviour in field crickets acts to maximise reproductive returns by preventing early spermatophore removal either by the female (Hockham and Vahed, 1997; Bussière et al., 2006) or another male (Bateman and MacFadyen, 1999; Bateman et al., 2001a; Wynn and Vahed, 2004), although nobody has specifically investigated aggressiveness post-mating. Mate guarding intensity (in terms of body rocks and antennal rotations) drops as the animal enters a refractory period (Parker and Vahed, 2010), when a new spermatophore is produced, and this drop in intensity could be linked to the redirection of energy reserves to physiological processes involved in spermatophore formation. The ability to maintain aggressive post-copulatory mate guarding behaviour as long as possible into the refractory period would be considered adaptive from the male point of view, as the longer the spermatophore is in place the more ejaculate can be transferred (Bateman et al., 2001a), thus rewarding an extended period of aggression.

6.5.3 Joint Discussion

To compare the “lover not a fighter” and “mating resets aggression” results directly is not straightforward. Both studies investigated the effect of mating on a male field cricket, and how that experience affected his subsequent aggressive interaction with an opponent, but Judge et al. (2010) and Killian and Allen (2008) used different species in their methodologies (*G. pennsylvanicus* and *A. domesticus*, respectively), albeit closely related species that are both well studied. The use of a single species in the present study was intended to overcome this complication. As methodologies do differ between the two studies, it was necessary to look for parallel themes that aimed to examine similar aspects of behaviour. Both studies included a treatment component that aimed to assess the effect on male aggression of being mounted by a female but not transferring a spermatophore (‘mounted’ in Lover, Not a Fighter and ‘Failed Copulation’ in MRA, now referred to as ‘mounted’) and how that compared with another group that successfully transferred a spermatophore (‘Mated’ in both studies).

Judge et al., (2010) found that mated male *G. pennsylvanicus* were more likely to lose their fight than males that had only been mounted by a female and had not transferred a spermatophore. Killian and Allen (2008) found that mated *A. domesticus* were significantly more likely to re-engage in combat and escalated fights significantly higher than controls and contact only groups, but not significantly higher than males in the mounted treatment group. This recreation of both studies, using *G. bimaculatus* in place of both other species, found that mated males were as just as likely to win their fight had they been mated or mounted, unlike *G. pennsylvanicus* (Judge et al., 2010). Mated males were more likely to engage in fighting behaviour, and less likely to adopt a subordinate position than males that only experienced contact or mounting from a female, which is line with *A. domesticus* (Killian and Allen, 2008). Both original studies and this recreation showed that female contact can have a significant effect on aggressive behaviour in field crickets, although it could be argued that the degree of behavioural change is clearer in Killian and Allen (2008) due to the multistage design of the methodology used.

Judge et al., (2010) did not use a control group i.e. pairs of males that did not experience female treatment prior to fighting, to compare with treatment groups. Judge et al. (2010) also centred on a single fight, post-treatment, in order to gauge the effect of female treatment on aggression, and found that both treatments resulted in high levels of aggression that did not vary significantly between groups. Had they used a control group, as Killian and Allen (2008) did, they might have found reduced aggression levels that led to a different conclusion. As all experimental males, across both stages, in Judge et al., (2010) had experienced female contact prior to fighting; it may be that their aggression levels were already extremely high. Killian and Allen (2008) did use a control group, though, and they demonstrated very different escalation and fight behaviours in *A. domesticus* when no female treatment was applied. This difference between control and treatment groups was also demonstrated in *G. bimaculatus* in the current study. Additionally, Judge et al. (2010), by only making use of a single fight design could not demonstrate the change in motivation and aggression that female contact produced in Killian and Allen (2008). Both Killian and Allen (2008) and Judge et al. (2010) were interested in using motivation (through contact with a female) as a key factor in promoting aggression, and both showed that high levels of escalation were associated with female contact. Killian and Allen (2008) were able to show

that the degree of female contact could be important (contact only, mounted or mated), and that applying female contact to males that had lost a fight could change their motivational state leading them to reengage in escalatory aggression significantly more often than control males. This effect was also shown when the study was recreated using *G. bimaculatus*.

Judge et al. (2010) and Killian and Allen (2008) came to different conclusions about the effect of mating on aggression, and the reason for this may also be down to their methodologies. In addition to not using a control group, Judge et al. (2010) progressed to the fighting components of their studies as soon as the female contact treatment had been applied. As there are many instances in the scientific literature of female contact increasing aggression in field crickets both before (Simmons, 1986a; Tachon et al., 1999; Brown et al., 2006; Brown et al., 2007) and after mating (Bateman and MacFayden, 1999; Bussièrè et al., 2006; Parker and Vahed, 2010), beginning the fighting trials straight after female contact may have confused some of their findings. In the first round of their experiment, Judge et al. (2010) showed that mated *G. pennsylvanicus* were more likely to lose fights than if they had only been mounted by the female. The difference may be that losing the female immediately after spending energy courting and mating and to then be faced by an opponent represented too great a loss for *G. pennsylvanicus* in Judge et al. (2010), although this would need to be investigated further. The recreated study using *G. bimaculatus* showed mated and mounted males were equally aggressive, so this may indicate that the two species differ in their assessment criteria, and/or that *G. bimaculatus* stays aggressive for longer than *G. pennsylvanicus* after spermatophore transfer.

Mated *A. domesticus* males in Killian and Allen (2008) were far more aggressive and won more fights than a control group or males that had only experienced contact with a female. Mated males in Killian and Allen (2008) were also given time to mate guard after transferring a spermatophore. They showed that mated males could exhibit heightened aggression, even against a previously dominant opponent, and this effect was also seen when recreating the study using *G. bimaculatus*. When comparing the two original studies, the effects of female contact on aggression can be seen in both works, but Judge et al. (2010) made the difference between mated and mounted treatments harder to distinguish. They essentially showed that mated *G. pennsylvanicus* males were **less** aggressive, but not

that mounted males were more aggressive. Due to their experimental design that did not account for the loss of the mated female as a reason for a lack of aggression in *G. pennsylvanicus*, the conclusion that mating makes males lose fights could be somewhat erroneous.

An investigation into the level of aggression that a male will demonstrate if he has a set period to enter his mate guarding/refractory period with or without the presence of a female, controlling for whether she is or is not carrying his spermatophore may provide further insight into how cricket behaviour is affected by mating status, and what cues are used in the mediation of aggressive behaviour post-mating. This is the subject of the next study.

CHAPTER 7. THE EFFECT OF BOTH THE MODE OF SPERMATOPHORE TRANSFER (NATURAL OR ARTIFICIAL) AND FEMALE PRESENCE ON AGGRESSION IN MALE *GRYLLUS BIMACULATUS*

7.1 INTRODUCTION

Prior fighting experience and the change it can evoke in a male cricket in his next encounter with a rival, specifically with regard to his aggressive state and decision-making processes, is known as the 'winner/loser effect' and can have a strong, but not permanent, effect on how a male approaches subsequent confrontations (Adamo and Hoy, 1995; Khazraïe and Campan, 1997; Khazraïe and Campan, 1999; Killian and Allen, 2008). Any given contest between two rival males will be decided by a combination of the fundamental components of resource holding potential, RHP, that are positively correlated with success in competition such as body size/weight (Dixon and Cade, 1986; Hofmann and Schildberger; Brown et al., 2006; Briffa, 2008) and superior weaponry (Rillich et al., 2007; Emlen, 2008; Judge and Bonanno, 2008) and the sum of each male's experience and current motivational state. Khazraïe and Campan (1997) demonstrated very clearly that pairs of male *Gryllus bimaculatus* that have established a dyadic hierarchy by engaging in physical combat will maintain that dominant-subordinate relationship if their environment does not alter i.e. they will not continue fighting as neither their ability to take control of the resource (RHP) nor their motivational status relative to their opponent has changed. The loser effect, that is the early withdrawal from subsequent fights after a recent defeat, can be considered adaptive as it gives the male time to recover and thereby minimise injury (Adamo and Hoy, 1995).

The loser effect in *Gryllus bimaculatus* demonstrated in Khazraïe and Campan (1997) was replicated as part of a study examining the effect of prior fighting experience and motivation on reengaging an opponent in *Acheta domesticus* by Killian and Allen (2008). They showed a similar knock-on effect of losing a fight on future fighting behaviour but they also went on to show that male subordinates fought with renewed intensity against a previously dominant opponent after being allowed access to a female to which they were allowed to mate and assume a mate guarding position. I found very similar results using *G. bimaculatus* (see Chapter 5). Judge et al. (2010) showed that virgin male *G. pennsylvanicus*

given contact-only access to a female before fighting took place were more aggressive than males that had been allowed to mate with the female but not allowed to mate guard her. The mated males that were not allowed to mate guard showed a severe reduction in aggression and a tendency to flee from an aggressor (in my recreation of these experiments using *G. bimaculatus*, however, I found no significant reduction in aggression levels in this group).

7.1.1 Motivation

The role of motivation, and its ability to affect aggression and decision-making, is hard to predict but can be extremely important in conflict resolution (Nosil, 2002; Brown et al., 2007; Arnott and Elwood, 2008). It is also very difficult for other males to determine what current motivational effects an opponent may be carrying as motivation is, by its very nature, cryptic. For example, when male field crickets sing from their burrow, they advertise their RHP, their stamina, their ability to fight (Dixon and Cade, 1986; Hack, 1997a; Brown et al., 2006; Buena and Walker, 2008). They do not broadcast their motivational state, as informing local conspecifics of the value of the resource they are holding would be maladaptive (Brown et al., 2006). Motivation and its effect on aggressive behaviour, the fact that it can potentially overcome RHP flaws that would not normally correlate with success, make it a vital aspect of aggressive interactions (Tachon et al., 1999; Hofmann and Schildberger, 2001; Hurd, 2006; Buena and Walker, 2008) and is the effect that Khazraïe and Campan (1997) and Killian and Allen (2008) showed to be so important. It may also be why perceived asymmetries in RHP lead to males underestimating the fighting ability of a smaller, or previously unsuccessful, opponent (Just and Morris, 2003; Hurd, 2006; Reaney et al., 2007).

Competition theory states that male decision making is based on assessing an opponent and making predictions of odds of winning and acceptable loss, thresholds of energy usage based on RHP asymmetries (Maynard Smith, 1973; Hammerstein, 1981). Motivation, however, can make accurate assessment of an opponent's RHP difficult (see section 1.3.5) and can therefore see a male incurring greater costs than expected when he initially encountered his opponent and made the decision to engage in competition with him (Hofmann and Schildberger, 2001; Brown et al., 2007; Briffa and Lane, 2017).

7.1.2 Mating and aggression

Prior to mating, antennal contact with a female conspecific is required for a male to adopt courtship behaviour. Cuticular pheromones are used to identify the potential mate before standard courtship behaviour is attempted (Tregenza and Wedell, 1997). Antennation in this manner always precedes a courtship or aggression behavioural response (Nagamoto et al., 2005). Willingness to mate by the female is followed by mounting behaviour prior to spermatophore transfer. This mounting behaviour and genital contact with the female induces a behavioural change in the male during which all movement, save for the rolling of his antennae, ceases. Once coupling of the genitals is complete, protrusion of the male's spermatophore appears to be a reflex response, as opposed to a decision by the male (Kumashiro and Sakai, 2001). The nature of this automated nervous response, and the part it plays in mating, had previously been highlighted by Sakai et al. (1995). They showed that reflex movements including thrusting and pulsating of the abdomen, in partly dissected live males to simulated female stimuli, occurred at the local level of the terminal abdominal ganglia (TAG). The same study also showed that decerebrated males, when given female stimuli, were still capable of spermatophore protrusion as it was controlled locally by the TAG.

Once they have successfully mated with a female, male *G. bimaculatus* enter a refractory period wherein a new spermatophore must be fully manufactured internally in a process that takes approximately 50 minutes (Hall, et al. 2000). This process is neurohormonally regulated and accompanies a change in the animal's behaviour in which he no longer produces courtship song (Nagao and Shimiwaza, 1987), but instead actively engages in mate-guarding behaviour. The male will engage in aggressive behaviour towards approaching rival males who in their efforts to mate with the female may dislodge the original spermatophore. The male will also attempt to maintain contact with the female and display repeated body rocks and antennal rotations. He may also react aggressively to the female herself if she attempts to remove the spermatophore. It is thought that the male is essentially protecting his own investment in his ejaculate whilst another spermatophore is being produced (Alcock, 1994; Wynn and Vahed, 2004; Parker and Vahed, 2010). The present study centred on the increase in male aggressive behaviour associated with female contact, specifically the aggressive behaviours associated with post-copulatory mate

guarding (see Killian and Allen 2008; Chapter 6). The methodology was developed in order to test the circumstances that were necessary to trigger such a response from a male cricket.

The 'mated' treatment group in Killian and Allen (2008) allowed natural mating of an experimental male to a female, two minutes of retained contact with the female, followed by five minutes of rest, followed by a follow up fight with a previously dominant opponent. After the female was removed, two minutes following spermatophore transfer, she was not replaced for the follow up fighting stage of the experiment. Taken individually, each of the components in the mated treatment found in Killian and Allen (2008) could produce an increase in aggressive behaviour when the male refaced his previous opponent: female contact has been shown to raise aggression levels in many species (Simmons, 1986a; Tachon et al., 1999; Brown et al., 2007; Judge et al., 2010), previously beaten males will reengage a previously superior opponent if they experience a change in motivation (Adamo and Hoy, 1995; Khazraïe and Campan, 1997; Khazraïe and Campan, 1999), and mate guarding behaviour itself is characterised by a period of increased aggression immediately after spermatophore transfer (Wynn and Vahed, 2004; Sakai et al., 2017). It is therefore difficult to determine the variables that could have acted as triggers for the increase in aggression in the 'mated' females in Killian and Allen (2008, see also Chapter 6).

7.1.3 The effect on male cricket aggression of maintaining or losing female contact after spermatophore transfer

A primary aim of this study was to examine the effect of maintaining female presence post-mating on levels of aggression by mating subordinate experimental males to a female and then allowing them to retain contact with the female for a set period of time before refighting their previously dominant opponent. A second group would lose contact with the female as soon as spermatophore transfer was complete and they would also be left for a set period of time before refighting their opponent. As mate guarding is thought to have evolved in order for the male to maximise his reproductive fitness by protecting his attached spermatophore by deterring rival males from mating the female (Alcock, 1994; Hockham and Vahed, 1997; Wynn and Vahed 2004), males that are allowed to guard their mate should engage in more aggressive behaviour, more often, than males in the comparative

group without a female to guard. The methodology developed for this study improved upon Killian and Allen (2008) by giving males the chance to mate guard for five minutes (as opposed to 2 minutes used by Killian and Allen 2008), or to leave them alone for five minutes with no female contact, before a second fight and by having the female present (or absent) during the whole of the second fight. In addition to improving the mated treatment approach in Killian and Allen (2008), the method adopted in the present study also addresses an issue with Judge et al. (2010), whose methodology required males to transfer a spermatophore and immediately engage in fighting. My recreation of that study using *G. bimaculatus* showed no difference in aggression between males that had contacted a female and those that had transferred a spermatophore (see Chapter 6) and so a more efficient experimental design was needed in order to identify the actual effect of female contact on male aggression. By implementing a fixed time period between fights and by either removing the female from the male after mating or allowing him to mate guard her throughout the break period and into the next fighting stage, this new methodology overcomes shortcomings in Killian and Allen (2008) and Judge et al., (2010). If male aggression post-mating is triggered by the maintained contact of the mated female when the fight stage begins and results in more instances of high intensity fighting, then males that lose female contact after mating and do not have a mate to guard in the fight stage should behave less aggressively and avoid high level fight escalation. This experimental design allows for this prediction to be tested accurately.

7.1.4 The effect on male aggression of the mode of spermatophore transfer and whether the presence of the spermatophore on the female is required for males to begin mate guarding behaviour

A secondary aim of the present study was to examine the role of spermatophore transfer in triggering mate guarding aggression and whether its attachment to the female is necessary in order to see post-copulatory mate guarding behaviour. Mate guarding theory states that male aggression during this phase functions to maximise paternity by preventing spermatophore removal for as long as possible (Alcock, 1994; Hockham and Vahed, 1997; Wynn and Vahed 2004). Sakai et al. (1995) and Kumashiro and Sakai (2001) showed that spermatophore transfer during mating occurs as a reflexive action to stimulation at the TAG. I wanted to ascertain whether full transfer of the spermatophore to the female was

essential for the male to initiate mate guarding behaviour or, because the transfer is controlled locally at the genitals, whether the male would guard the female because his spermatophore was gone and the female was in close proximity to him. By conducting a second set of fight-treatment-fight trials, but with the spermatophore removal performed mechanically and removed from the treatment area, male behaviour in follow up fights with or without retained female contact could be used to clarify what the mate guarding male is using as his cue to aggressively defend the female. If the absence from the male of a fully formed spermatophore, together with the presence of a female are the triggers for mate guarding behaviour, then manual spermatophore removal and female presence should result in high levels of aggression from the mate guarding males, and fight avoidance and low aggression in males without female contact. In creating a set of males that do not have a spermatophore ready to transfer, the manual spermatophore treatment mirrors a situation that occurs frequently in nature: field cricket males of several species have been observed to frequently remove their own spermatophore from their spermatophore pouch if they have been carrying it for a few hours (Reinhardt and Siva-Jothy, 2005). This appears to be a strategy of maintaining young sperm in the spermatophore, which may have a competitive edge in sperm competition (Reinhardt and Siva-Jothy, 2005). Furthermore, it is not uncommon in field crickets for the spermatophore to fail to attach to the female properly and to fall off (Wynn and Vahed 2004). The manual spermatophore removal group with the female present therefore, to some extent, also mirrors a failed mating attempt.

7.1.5 Aims and predictions

Using a 2x2 factorial design, this study aimed to determine the effect of both the mode of spermatophore transfer (by mating versus manual removal) and the presence (versus absence) of a female following spermatophore transfer on levels of aggression when a pair of males were refought after forming a dyadic hierarchy of dominance and subordination. The following predictions were tested:

- a) Levels of aggression are predicted to be higher if the female is present following spermatophore transfer (i.e. if the male is allowed to guard her) than if she is absent.
- b) If the absence of the spermatophore from the male's spermatophore pouch triggers increased aggression associated with mate guarding, then there should be no

significant difference in levels of aggression between males in which the spermatophore is removed manually versus those in which the spermatophore is removed via mating.

- c) If the presence of the male's spermatophore on the female is a trigger for increased aggression associated with mate guarding then female presence should have a stronger effect on the level of aggression in males that transfer their spermatophore via mating (as opposed to males that have had their spermatophore removed manually).

7.2 METHODS

Pairs of male crickets were prepared as per section 2.2 of the General Methods in preparation for this study.

7.2.1 Encounter 1, establishing a dyadic hierarchy

A pair of virgin, naïve, weight-matched and marked male crickets was carefully placed in an experimental arena on either side of a removable dividing wall (see General Methodology for notes on construction, dimensions, and parameters of the arena). A female cricket was also removed from a population of virgin female crickets and kept in a sampling cup next to the arena ready to use after the initial stage had concluded. The female used for each treatment stage was selected based on her body length as to ensure she was neither too short nor too long to successfully mate with the experimental male if required. No female was reused in any other experiment. Once the pair of male crickets were on either side of the dividing wall, a two minute timer was started to give the crickets time to settle after being moved. After the two minute acclimation period, the dividing wall of the arena was removed to allow the males to move around the whole arena. A 10 minute timer was started when the pair of males made antennal contact. All fights between opponents were watched and accurately scored with the level of escalation and the winner in each case using the scale in Figure 2.7 and following the fight protocol laid out in sections 2.8 and 2.9 of the General Methodology. After 10 minutes the wall was replaced with one cricket on either side.

The cricket that was displaying established subordinate behaviour to his dominant opponent (Level 1 on the Hoffman and Schildberger Scale) at the end of the 10 minute session was considered subordinate and given a designation of experimental male, EM. His dominant opponent was designated as the stimulus male, SM.

One of four separate treatments was then randomly selected via a double coin toss and used on the experiment male (EM) cricket after the initial encounter (Table 7.1).

Table 7.1: A summary of the four experimental groups. The presence or absence of a female was combined with normal or mechanical spermatophore removal

Treatment code	Initial fight	Spermatophore removal method	Follow up fight	Female
1	Loser = EM	Tweezers (50% of EMs)	EM (previous loser) V SM (previous winner)	Absent
2	Loser = EM	Mated (50% of EMs)	EM (previous loser) V SM (previous winner)	Absent
3	Loser = EM	Tweezers (50% of EMs)	EM (previous loser) V SM (previous winner)	Present
4	Loser = EM	Mated (50% of EMs)	EM (previous loser) V SM (previous winner)	Present

7.2.2 Introduction of the female to the EM

After the fight stage had concluded and the arena dividing wall had been replaced, a female cricket was gently released from her holding cup into the side of the arena holding the EM, no more than 5 cm from his position. A 10 minute timer was also started at this point*.

The EM, upon making antennal contact, was observed until he began courtship behaviour, specifically singing to the female. When the EM began courtship singing and the female reciprocated by moving behind the male to mount him, treatment codes as described in Table 7.1 were implemented. Males in the tweezer treatment groups (Treatment code 1 and 3 in Table 7.1) were treated with the method described below in 7.2.2.1, whereas males in the mated treatment groups (Treatment code 2 and 4 in Table 7.1) were allowed to court the female, be mounted by her and transfer a spermatophore to her, with the point of transfer described below in 7.2.2.2.

*Had the EM not attempted to court the female within 10 minutes, it was assumed that he did not have a spermatophore in place. EM crickets that did not sing at this time were removed from the study, along with their corresponding SM, and encounter one fight data were not used.

7.2.2.1 A method for the manual removal of a spermatophore from the EM in treatments 1 and 3

After initiating courtship behaviour and after the female cricket started to mount him, the EM was carefully scooped up from the arena floor with a clean 100ml sampling cup. The female was then scooped up in a second sampling cup.

The EM was gently removed from the cup by hand. The index and middle finger were held against the dorsal surface, running parallel to the wings, and the thumb was used to hold the cricket in place on the ventral surface of the abdomen. The cricket's head therefore faced inward towards the palm. Very gentle pressure was applied to the dorsal and ventral abdomen surfaces causing protrusion of the spermatophore. Once protruding, the spermatophore was removed from the male with clean, fine tip tweezers. As soon as the spermatophore was removed, the female cricket was quickly removed from her tub and her head placed next to that of the EM, ensuring that antennal contact was achieved. This contact was carried out to mimic aspects of natural spermatophore transfer. The EM was then returned to the side of the arena he was removed from.

The SM was also then removed from his side of the arena in a sampling tub, held in the same manner as the EM (minus female contact), and then replaced in his side of the arena. This was to replicate any minor handling stress that the experimental male may have experienced during his treatment. This entire process took approximately 60 seconds. The crickets were then left for five minutes.

7.2.2.2 Determining the point of spermatophore transfer in treatments 2 and 4

Spermatophore transfer point can be deduced either directly if the crickets are in full view of the observer, or by watching the antennal movements of the animals. During mounting behaviour, the male and female make wide sweeps and tight circles with their antennae. As the spermatophore is taken by the female, all antennal motion by the male either stops or becomes noticeably reduced. That is taken to be the point of transferral for this experiment*.

* If females were removed from the arena post-mating (as in Treatment 2 in Table 7.1), she was removed specifically at this point so as not to adversely affect experimental timing or

trigger any possible mate guarding behaviours. Some females remain mounted on the male for up to several minutes, whilst others climb off quickly. This method controlled for that potential uncertainty in Treatment 2 groups.

7.2.2.3 Treatment code 1 and 2: Female contact lost post-treatment

After the EM had either had his spermatophore manually removed (Treatment 1, n=16) or had transferred to the female (Treatment 2, n=16), the female was removed from the experiment at this point. She was NOT present for the second fight.

7.2.2.4 Treatment Code 3 and 4: Female contact maintained post-treatment

After the EM had either had his spermatophore manually removed (Treatment 3, n = 24) or had transferred it to the female (Treatment 4, n = 22), the EM was allowed to maintain contact with the female for 5 minutes. The female WAS present during the next fight.

7.2.3 Encounter 2, refighting the SM

Once 5 minutes had passed since spermatophore transfer/removal, the arena dividing wall was removed. Once the males made antennal contact, a 10 minute timer was started. As in Encounter 1, all fights were monitored and their escalation level and the winner of each fight were noted. After the fight, both males (and the female if the males were in Treatment 3 or 4) were released into a communal breeding tank. No crickets were reused.

7.2.4 Statistical methods

The 78 pairs of males used in this study engaged in 794 aggressive interactions over the course of their second encounters.

A Generalised Linear Model (GLM) with escalation to level 5 grappling as a binomial variable, and with treatment (method of spermatophore removal) and female presence as categorical variables was built to examine whether method of spermatophore removal and female presence that would affect the probability of an encounter escalating to this level of intensity. The effect of a potential interaction between the categorical variables was also tested by including the interaction in one model, and then without it.

A comparison of the total number of physical contacts and the number of contacts that escalated to level 5 grappling by treatment group was then conducted. Generalised linear models with a poisson link function were built to compare count data between treatment groups, followed by Bonferroni post-hoc tests to determine in which groups fighting behaviours were most affected by the treatments. Rstudio was used for all statistical work, and 'ggplot2' was used to produce the bar chart.

7.3 RESULTS

7.3.1 Encounter 1

Initial encounters between weight matched crickets resulted in 85% (n=66) of all pairs reaching Level 5 Grappling behaviour at least once during the encounter, with no significant difference between groups (Kruskal-Wallis $\chi^2=2.1891$, d.f.=3, $p=0.53$)

7.3.2 Encounter 2

7.3.2.1 *Re-meeting a previously dominant opponent*

After experimental males had received treatment and the second encounter began, 1.3% (n=1) of experimental males retained a subordinate behavioural position relative to their previous opponent. All other males engaged with their previously dominant opponent, with 85% (n=66) of experimental males across all four treatments escalating to level 5 grappling at least once.

7.3.2.2 *The effect of treatment on escalation to grappling during encounter 2*

One hundred percent of mated males that retained female contact after spermatophore transfer and into encounter 2 (Treatment 4) escalated to grappling at least once in their follow up encounter with their previously dominant opponent, compared to 81% of males that lost their female immediately after mating (Treatment 3). Males that underwent tweezer treatment for spermatophore removal and maintained contact with the female (Treatment 2) escalated to grappling in 83% of pairs, compared to 69% of tweezer pairs that lost female contact (Treatment 1).

Logistic regression with escalation to grappling as a binomial variable indicated that the presence of the female was a significant predictor of escalation ($\chi^2=4.15$, $p=0.042$), and spermatophore removal method was close to being statistically significant ($\chi^2=3.33$, $p=0.06799$). There was no significant interaction effect between spermatophore removal method and female presence ($\chi^2=2.55$, $p>0.05$). Further model reduction did not improve the fit of the model ($\Delta AIC < 2$).

7.3.2.3 Frequency of grappling

Tweezer and mated males in the alternative treatments (Treatments 1 and 2) that lost female contact engaged in fights escalating to grappling in 14.6% and 15.7% of total fights, respectively (Figure 7.1). Maintained female contact resulted in mated males (Treatment 4) engaging in grappling behaviour in 30.5% of their total fights, compared to 22.5% of their tweezer treatment male counterparts (Treatment 3). A generalised linear model with a poisson link function was built with number of grappling fights as the dependent variable, and spermatophore removal method, female presence, and their interaction as independent variables. There was no significant interaction term ($\chi^2=3.06$, $p=0.08$), but there was a significant positive effect of female presence ($\chi^2=22.32$, $p=2.304e-06$). The effect of spermatophore removal method indicated a trend towards being significant ($\chi^2=3.45$, $p=0.064$). Further model reduction did not improve the fit of the model.

Bonferroni pair-wise comparisons of groups in the model indicated that the most significant difference in grappling frequency was between normally mated males with (Treatment 4) and without (Treatment 2) female contact ($p=0.0065$). A significant difference in grappling frequency was not found in the manual spermatophore removal groups; males maintaining female contact (Treatment 3) were not statistically different to those that lost female contact (Treatment 1) ($p=1$). There was also no significant difference in grappling frequency between males that had their spermatophore removed manually as opposed to by mating either in the presence of a female ($p=0.29$) or in the absence of a female ($p=1$), (Figure 7.1)

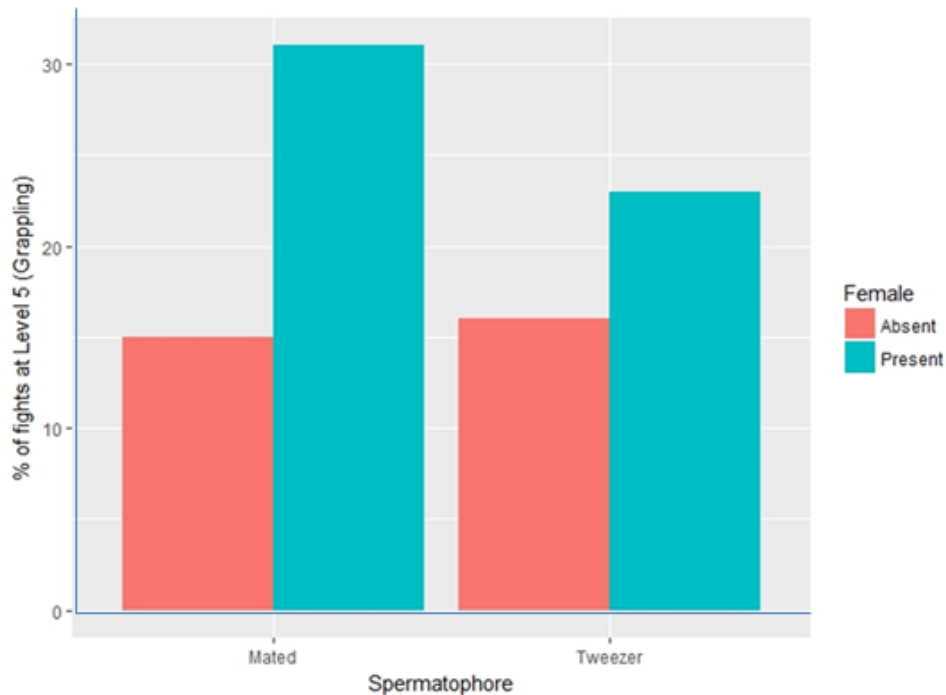


Figure 7.1: Percentage of fights that escalated to grappling by manner of spermatophore removal and female presence or absence.

7.3.2.4 Total contacts

Maintaining female contact resulted in significantly more contacts (taken as at least level 1 interaction i.e. required antennal contact for opponent recognition and reaction) between the experimental male and his opponent. A generalised linear model with poisson link function was built with the number of total contacts that took place in post-treatment encounters as the dependent variable, and spermatophore removal method, female presence, and their interaction as independent variables. There was no significant interaction term ($\chi^2=2.59, p=0.11$), and no significant effect of spermatophore removal method ($\chi^2=0.33, p=0.56$) but female presence was associated with significantly more contacts ($\chi^2=8.28, p=0.004$). Removal of the interaction term and removal method both improved the fit of the model, leaving female presence as highly significant ($\chi^2=8.35, p=0.003$).

Although maintaining female contact was a significant factor affecting contact frequency, post-hoc Bonferroni pair-wise comparisons of groups in the model indicated that there was no significant difference between groups in the number of contacts. Males that lost female

contact after manual spermatophore removal (Treatment 1) or after mating (Treatment 2) showed no significant difference in contact frequency ($p=1$). Males that maintained female contact after manual spermatophore removal (Treatment 3) or after mating (Treatment 4) showed no significant difference in contact frequency ($p=1$). A comparison of mated males that lost (Treatment 2) or retained (Treatment 4) female contact was also non-significant with regard to contact frequency ($p=0.27$) (Figure 7.2).

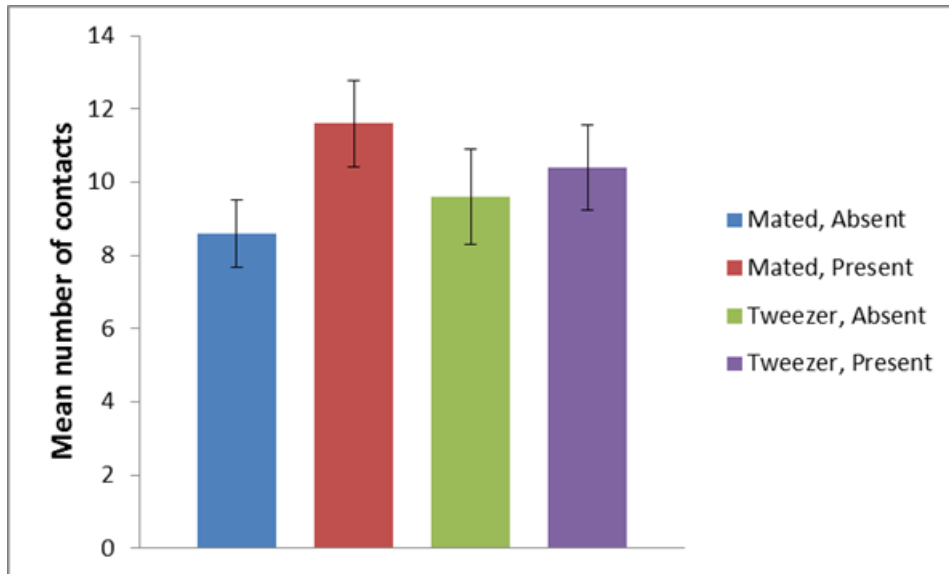


Figure 7.2: Mean contacts with standard error in each treatment group. “Absent” and “Present” refer to the presence or absence of the female; “Mated” and “Tweezer” refer to the mode of spermatophore removal from the male. No significant differences between any of the 4 treatments.

7.4 DISCUSSION

This study aimed to determine whether the level of aggression shown by a previously subordinate male field cricket was affected by the mode of spermatophore removal (by mating versus manual removal) and the presence versus absence of the female following the spermatophore treatment. Almost all (99%) of the experimental males used in this study went on to engage in combat with a previously dominant opponent, regardless of which treatment group they were in. As previous research has shown that males require a change in motivational state in order to reengage an opponent in such a scenario (Adamo and Hoy, 1995; Khazraïe and Campan, 1997; Khazraïe and Campan, 1999 and Chapter 6 of this work), it is reasonable to assume that contact with the female during the treatment period had changed the subordinate male's motivational state as predicted by theory. While Killian and Allen (2008) similarly demonstrated that previously subordinate males will increase levels of aggression in the presence of a female, the present study sought to investigate how longer periods of female contact changes the behaviour of a mated male, and how that behaviour is triggered.

Killian and Allen (2008) removed the female after just two minutes of contact with their experimental male. In the "female present" treatment in the present study, however, females were present for the full five minute rest period after spermatophore transfer and were also present during the subsequent fight with the previously dominant opponent. The present study showed that maintained female contact after spermatophore transfer promoted mate guarding behaviour in terms of an increase in the level and frequency of aggression, and that removing the female immediately after mating led to a significant drop in aggression. There is far less ambiguity here (in comparison with Killian and Allen 2008) by virtue of the experimental design. By maintaining female contact after spermatophore transfer for the five minute break period between fight stages and into the second fight stage (Treatment 4), when the second fight began the mated experimental male was displaying characteristics of mate guarding – body rocking, antennal rolling, and aggressively engaging with the stimulus male when he approached the female. This behaviour is thought to maximise reproductive fitness by preventing premature removal of the spermatophore by rivals or the female herself (Hockham and Vahed, 1997; Bateman and MacFayden, 1999; Wynn and Vahed, 2004). Other males that had also transferred a spermatophore to a

female but had the female taken away had spent five minutes alone (Treatment 2). When the second fight stage began they did not display aspects of mate guarding and were significantly less aggressive. This indicated that they had in fact entered their refractory period for spermatophore renewal. The statistical significance of the results supported the original predictions that female contact after spermatophore transfer triggered mate guarding behaviour in male *G. bimaculatus*.

The effect of female contact on the level of aggression in the males that had their spermatophore removed manually with tweezers (rather than by mating) was not as straightforward. Female contact in the tweezered male test group was also associated with increased aggression: tweezer males that maintained female contact (Treatment 3) throughout their treatment phase and into their second encounter escalated more fights to grappling than the comparable tweezer group with no female access (Treatment 1), though the differences were not statistically significant (in the post-hoc tests). This suggests that mate guarding aggression had not been triggered fully by female contact in males that had their spermatophore removed manually, unlike in males that had transferred their spermatophore to the female (although note that the interaction term was non-significant in any of the GLM analyses, which does not support this interpretation). It may be that the presence of the male's spermatophore on the female is necessary in order to trigger mate guarding aggression fully. Given that visual cues play a role in fight escalation in many Gryllids (Hofmann and Schildberger, 2001; Rillich et al., 2007; Judge and Bonanno, 2008) and mandible flaring displays have been shown to settle fights in *G. bimaculatus* (see Chapter 4), we can assume that their visual capabilities are functional at least at short range. However, crickets also receive a considerable amount of information about their surroundings and the identification of conspecifics: their sex, their sexual history and their fight record- through the use of the antennae in data collection. Pheromonal cues are used to assess mating opportunities and may play a key role in pre-fight decision making as regards local sperm competition (Mallard and Barnard, 2003; Nagamoto et al., 2005; Thomas and Simmons, 2009b; Thomas, 2011). As this experimental work was carried out in a well-lit laboratory, and used males that had complete antenna, it cannot be stated categorically whether males use visual or pheromonal confirmation that their spermatophore is attached to the female or not. Given the extensive use of pheromonal cues in the Gryllidae, especially with regard

to mating status of conspecifics (Adamo and Hoy, 1995; Thomas and Simmons, 2009), it would be fair to assume that spermatophore attachment is confirmed by pheromonal cues. However, a further investigation to demonstrate this experimentally would be required to confirm this.

Males in Treatment 3 (spermatophore removed with tweezers, female contact maintained) and Treatment 4 (spermatophore transfer to female by mating, female contact maintained) were not significantly different in the proportion of fights that escalated to grappling. This cross-group similarity between males that differed in spermatophore removal (manual versus by mating) could suggest that it is the removal of the spermatophore from the male that triggers mate guarding aggression, irrespective of the mode of spermatophore removal. However, this does not necessarily imply that males in both treatments were exhibiting aggression for the same reason. A potential reason for increased levels of aggression from males in Treatment 3 (manual spermatophore removal, female present) linked to the presence of a female is that rather than displaying post-copulatory mate guarding behaviour, the males were in fact engaging in **pre-copulatory** mate guarding. This behaviour is seen in many arthropod groups, in which the male attempts to defend the female while a new ejaculate is being produced by his body or until the female becomes receptive (Jormalainen, 1998; Parker and Vahed, 2010; Elias et al., 2014). In nature, male field crickets routinely remove their own spermatophores from their spermatophore pouch if they have been carrying them for several hours (Reinhardt and Siva-Jothy, 2005). If a male field cricket encounters a female and does not have a spermatophore ready to transfer, it might benefit him to engage in pre-copulatory mate guarding to allow him time to form another spermatophore (a process which takes about 50 minutes) (Parker and Vahed, 2010). As yet, no study has compared the relative aggression levels of pre- and post-copulatory mate guarding in field crickets. One might assume that post-copulatory behaviours would be more intensively aggressive, as a successfully mated female carrying the male's spermatophore is a more valuable resource than one that has not yet mated with him. This would be an interesting line of investigation for future work.

The present study showed that the presence of a female following spermatophore transfer led to increased levels of aggression. The lack of a significant difference in levels of aggression produced by manually removing the spermatophore with tweezers (as opposed

to by mating) suggests that male mate guarding behaviour is triggered by spermatophore loss from the male, combined with the presence of the female. However, the effect of the presence of the female on aggression was strongest in males that had transferred their spermatophore through mating (as opposed to by manual removal). This could suggest that the presence of the male's spermatophore on the female may be necessary to trigger full mate guarding aggression.

Future work to understand the cues a male uses to trigger mate guarding behaviour could follow a line of investigation based on the methodology and findings of this chapter. Experimental manipulation of spermatophore attachment time post-transfer could give insights into what cues a male uses to begin mate guarding. The removal of attached spermatophores from normally mated females and the corresponding behavioural responses from guarding males could then be used to determine how male aggression is maintained into the refractory period. It would also be interesting to compare the intensity of aggression when males are engaged in pre-copulatory mate guarding (i.e. in males that encounter a female when they do not have a spermatophore ready) versus males engaged in post-copulatory mate guarding.

CHAPTER 8. GENERAL DISCUSSION/ CONCLUSIONS

This work has investigated the factors that affect the outcome of agonistic encounters in adult male *Gryllus bimaculatus*, focussing on the effects of morphological variation (especially in mandible size and characteristics of tooth processes) and female contact experiences. With direct physical conflict between males playing such a central role in competition for resources in this highly aggressive species, looking to see if there was sexual dimorphism in the structures that would logically play a role in such fights was an important first step in this study. This thesis (Chapter 3) was the first study to confirm that male *G. bimaculatus* do have significantly larger mandibular weaponry than their female counterparts, indicating that these structures are acted upon by sexual selection (see also Judge and Bonanno (2008) for a similar finding in *G. pennsylvanicus*). However, in other species in which weapons play a major role in resource holding such as beetles and wetas, we often see cases of developmental trade-offs between weapon size and investment in sperm production (ie. testes size) (Moczek and Nijhout, 2004; Pomfrett and Knell, 2006; Simmons and Emlen, 2006; Kelly, 2008b). This can result in massive weapon investment and positive allometric development of weapons being associated with proportionately smaller testes, or conversely, lesser male weapon traits being associated with greater ejaculate investment i.e. strategies focussing on either pre- or post-copulatory intrasexual selection. With *G. bimaculatus* there was no indication of such a developmental trade-off. This suggests that their behaviour reflects a blend of both pre- and post-copulatory investment, with aggression both before and after mating, including mate guarding, playing a key role in reproductive fitness (Bateman, 1998; Bateman et al., 2001a; Wynn and Vahed, 2004; Parker and Vahed, 2009; Sakai et al., 2017).

Contests between males can end at a visual mandible flaring display or escalate to grappling. This implies that there is a component of RHP visible during flaring that is used during assessment between opponents. It also means that variation in weapon structure could play a role in fight performance. In addition to finding that a relatively wider mandible span was a significant predictor of success during mandible displays, I found that specific components of tooth structure, namely the length of the incisor and length to distal tip,

were significantly associated with victory at the jaw flaring stage (Chapter 4). This is the first time that this relationship had been identified in the Gryllidae. As to what role this visual cue plays in combat is speculative, but it may point to an aspect of jaw strength that, if the fight was to escalate to grappling, could prove more injurious than normal. Beetles with enlarged mandibles often exhibit longer muscle attachment points to allow them to exert power despite short lever input (Goyens et al., 2015a; Goyens, 2015b). Goyens et al. (2014b) found that stag beetles were able to exert 18% more bite pressure through tooth structures on the middle of the mandible (compared to the mandible prongs), which is in approximately the same position as the incisor tip in *G. bimaculatus*. If this effect carries over to *G. bimaculatus*, further experimentation with trait modification could shed more light on what this mandibular trait actually does. If the incisor and distal tip are indicators of bite pressure capability that are enough of a threat to warrant retreat behaviour from an opponent, an experimental treatment that removes the processes from males that have been shown to win in flaring displays could be implemented in order to see how opponent males respond when what we assume to be the visual cues are not present. Other studies have used trait modification to monitor male performance in a number of species and have included changing the degree of asymmetry in earwig forceps (Munoz and Zink, 2012) and reduction of horn lengths in rhinoceros beetles (Madewell and Moczek, 2006). A minor procedure using a Dremel-type tool with a micro drill bit could be used to perform light ablation work in order to reduce process length of *G. bimaculatus* jaws, and an experimental design based around this procedure could provide some very interesting results.

In the experiments presented in Chapter 5, asymmetrically weighted male crickets were pitted against each other to test the prediction that small males that won fights against a larger opponent would have larger jaws than small males that lost against a larger opponent. I found no difference in weaponry between small winners or losers of fights that escalated to mandibular flaring or grappling. The results did confirm, however, that the larger male had a greater chance of winning. The results also indicated that there was a far more aggressive approach to first contact escalation by the smaller males than the current literature would have predicted. The mean first fight escalation score was 4.10 between males that were asymmetrically matched for weight by between 12 and 40%. In

comparison, the weight-matched males used in the previous study (Chapter 4) showed a first fight mean escalation score of 4.38. Choosing to fight in an out-matched position, where costs associated with fighting may be high, is possibly an aspect of the desperado effect (Grafen, 1987). In the literature it is most often applied to scenarios in which a loss is terminal, the effects of which cannot be (or are unlikely to be) recovered from, but the rewards mean that the risk is acceptable. For example elephant seal harem loss may represent a complete loss of reproductive fitness for older males who may not be able to take ownership of another (Haley, 1994; also see section 1.3.5.2 on the Residency and territoriality effect). In some circumstances, actively displaying for females in key sites means increased predation risk, but is shown to increase mating opportunities and reproductive success in *Pardosa milvina* wolf spiders (Rypstra et al., 2016), while potentially fatal fighting is deemed an acceptable risk for the Salticid *Phiddipus clarus* as they attempt to secure better mating sites (Elias et al., 2010). The experimental design of Chapter 5 did not invoke any of these conditions, the males were simply matched asymmetrically for mass and were one week mature adults, but produced aggressive escalation of level 3+ in 81% of first fights between naïve males. Although it is only speculation at this time, further work in this area may shed light on a hitherto unknown aspect of the desperado effect.

This raises several key questions about the rigidity of assessment methods used by *G. bimaculatus*: firstly, whilst weight-matched males will retreat from an opponent with higher perceived RHP (see Chapter 4), is there a cut-off point, or threshold, at which opponent size is no longer a factor? And second, does a hyper-aggressive behavioural response exist in this species that is adaptive, because small males did win some of these first fights and ended their whole encounter victorious? As high aggression does seem to be a ubiquitous trait for this species, it would be interesting to see just how far this aggressive first contact goes in terms of willingness to engage with a larger opponent. An expanded experimental setup using a fixed set of large males (>1 gram), fought once each day (any more would incur winner/loser effects) against novel smaller opponents of various weights could be used to build a bank of data to determine whether a threshold point for hyper-aggression based on a certain degree of asymmetry does exist.

Chapter 6 showed that male willingness to fight was highly flexible and that aggression levels could be changed based on motivational effects from female contact treatment. Chapter 6 part 1 recreated a set of mating treatment experiments by Judge et al. (2010) and showed that female contact was consistently associated with aggressive behaviour from males. But, as I highlighted in the discussion, there was no control group with which to draw a comparable figure. However, the reworking of the original study did show that *G. bimaculatus* appears to be more aggressive than the species that was originally used, *G. pennsylvanicus*. Judge et al. (2010) had stated that once males lost female contact they were more likely to lose fights. This recreation showed consistent fighting behaviour from *G. bimaculatus* that did not vary in intensity whether or not the male maintained female contact.

Chapter 6 part 2 gave more of an insight into the effect of motivation in promoting aggression in *G. bimaculatus*, and how that motivational shift can impact subsequent fighting behaviour. My findings using *G. bimaculatus* were very similar to those of Killian and Allen (2008) who used *Acheta domesticus*, and found that simple chemotactile contact with a female was enough to change the aggressive state of a previously subordinate male, and caused him to actively engage in an escalatory contest with a previously dominant opponent. This study did utilise a control, which was no female contact, and so the results from the three types of female treatment could be compared. Control males maintained a subordinate stance, as would be predicted by theory (Khazraie and Campan, 1997) whilst contact, mounted and mated males showed increasingly aggressive behaviour towards their opponent, with mated males showing the greatest change in aggression levels. As my results were very similar to those of the original authors, knowing that *A. domesticus* and *G. bimaculatus* are similar in their use of aggression is very interesting and should allow for a cross-species investigation of comparable mandible structures. Briffa (2008) stated that *A. domesticus* mandibles only played a marginally significant role in male-male agonistic conflict, so this would be a good opportunity to apply the procedure developed in Chapter 4 to see how male fighting behaviour, mandible use and mandible shape varies between the two species.

The results of Chapter 7 suggested that the aggressive behaviour exhibited by mate guarding males is dependent on the presence of the female and the spermatophore she carries. While the previous studies have shown male *G. bimaculatus* to be highly aggressive, with a tendency to escalate fights to high levels (Chapters 4 and 5), and that female contact can produce a resurgence of fighting enthusiasm (Chapter 6) they still limit their agonistic responses when the situation requires it. When males lost contact with their mate after spermatophore transfer (in the “female absent” treatment in Chapter 7), they fought less often and retreated from fights earlier, presumably because there was no adaptive reason to fight. This contrasts with Judge et al (2010) and the recreation of that work in Chapter 6 part 1 that found no difference between males that did or not retain female contact after mating. However, see sections 6.6.1 and 7.4 for an explanation of why time between fights and the onset of refractory period impacts upon aggression levels.

Furthermore, while other studies have shown that crickets can identify the species and sex of individuals (Nagamoto et al., 2005; Thomas and Simmons, 2008), recognise previous opponents (Khazraïe and Campan, 1997; Killian and Allen, 2008), previous mates (Ivy et al., 2005), and relatives (Simmons, 1989), this work has been able to show that males appeared to be able to detect whether the female they were in contact with was carrying their spermatophore or not. Importantly, this shows that males can, and do, make context-specific decisions regarding their fighting behaviour. A direct comparison of aggression levels during pre- and post-copulatory stages of mate guarding to assess how males assess the value of a potential mate compared to a mated female would be an interesting next step in an investigation of how males make their decisions regarding priorities when engaging in mate guarding behaviour.

In conclusion, this thesis has demonstrated that there is sexual dimorphism in mandible size in *G. bimaculatus* (Chapter 3) and that aspects of mandible morphology are associated with an increased chance of winning, even at the mandible-flaring stage of combat (Chapter 4). Male body size is also a key predictor of fighting success, but heightened aggression in small males could represent an alternative approach to contest strategy that can end in success despite high levels of RHP asymmetry, although the exact reasons why are not clear

(Chapter 5). Finally, the presence of a female, especially following successful mating, can overcome the loser effect and cause a male to re-engage with a previously dominant opponent (Chapter 6 and 7). Fighting behaviour in this species is therefore highly flexible and factors affecting the outcome of contests are complex. There is much scope for further studies on this topic.

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