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Functional Ecology of Calling and Mating in the Cricket Species (Acheta domesticus and Telogryllus commodus)

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Dedicated to those who love to spread knowledge in unconventional ways.

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I would like to thank my advisor, lab mates, undergrad assistants, any and everybody who listened to me talk about my crickets in a casual setting with curiosity and wonder.

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Abstract:

Whole organism performance traits are measures of an organism's ability to complete dynamic functions relevant to fitness within the contexts of both sexual and natural selection. Performance capacities are subject to allocation based life history trade-offs, but are also frequently implicated in signaling via visual or auditory displays, many of which are sexually dimorphic. But although performance traits are known to influence the outcome of male combat interactions, the effect of performance on mating interactions is poorly understood. Recently, the invertebrate neurotransmitter octopamine has been shown to affect performance expression in house crickets independent of the underlying morphology, raising the possibility that performance traits can be manipulated by altering the pharmacological milieu by simple dietary supplementation or, in the case of crickets, by antennae removal. I used these approaches to test for and demonstrate functional role of a performance trait, bite force, in determining the course and outcome of mating interactions in *Acheta domestic* house crickets. I also found that blocking octopamine receptors with an antagonist (epinastine) significantly affects courtship call structure. Based on manipulation of the neurotransmitter octopamine, I found that dampening octopermegenic receptors affected male calling effort and the dominantly expressed frequency of courtship calls during mating interactions. Finally, I showed that antennae removal alters the relationships among overall calling effort; lifespan; and metabolic rate within *Telogryllus commodus* crickets.

Keywords: sexual conflict, locomotor performance, bioacoustics, life-history tradeoffs, fitness, metabolic rate and scope

Introduction:

Sexual selection can be broadly considered as intraspecific reproductive competition whereby certain individuals exhibit reproductive advantages over others of the same sex. Sexual selection arises from differences in mating success, and may exert stronger selective pressure than natural selection due to the frequency with which traits are driven beyond the optimum natural selection values (Hosken & House, 2011). Female choice specifically involves the utilization of internal and external mechanisms individuals may express to attract a mate. This choice occurs over differing stages of the mating process and may be based on either genotypic or phenotypic traits, and are thought to accrue either direct or indirect benefits to choosy females (Brown, 1999). In insects, various combinations of auditory, visual and contact-based displays and movements contribute to the duration of intersexual mating interactions and successful mating outcomes (Maklakov *et al.*, 2009). But although females show clear mating preferences in many animal species such that male mating success is often markedly skewed, the sources of variation in these displays and the male factors upon which females base their mating decisions are poorly understood (Zuk *et al.*, 2006, 2008; Chapman *et al.*, 2017).

Males are seldom passive participants in the female choice process, and female mating preferences can be altered, subverted, or entirely overridden by male traits or behaviors specifically selected for that purpose. This interlocus sexual conflict, whereby members of a given sex seek to bias the outcome of mating interactions in their own favor, stems from a mismatch between the female mating preference and the male phenotype, and is widespread throughout the animal kingdom. For example, male bedbugs practice traumatic insemination,

whereby sperm is injected directly through the female body wall to circumvent female control over parental identity (Kamimura *et al.*, 2014). In Malabar ricefish, males will rush to females very swiftly and strike them in the genital region with a complex clublike organ from below. If successful contact with the female body occurs, a modified anal fin releases a spermatophore with a dartlike spike. The spermatophore becomes firmly attached in the females flesh because of many recurved barbs at the spikes' tip. Females are adapted to these repeated assaults as the skin around their genital pore is markedly thickened, hardened, and many spermatophores are attached (Arnqvist & Rowe, 2005). Other male behaviors such as harassment and mate guarding, albeit less dramatic, nonetheless share the same goal of biasing fertilization in the favor of the harassing male. But despite a large literature on intersexual conflict, and in particular on the behaviors employed by coercing males, the proximate, functional mechanisms by which males are able to prevent female re-mating or otherwise ensure paternity during mating interactions are not well-studied.

Performance, sexual selection, and sexual conflict

Whole organismal performance describes an organism's ability to complete dynamic tasks relevant to fitness, and includes "athletic" traits such as jumping, running, and flying, amongst others (Bennett & Huey, 1987; Lailvaux & Irschick, 2006). Performance tasks have been implicated in sexual selection as important determinants of fitness in both vertebrates and invertebrates (Husak & Fox, 2008; Irschick *et al.*, 2008). For example, bite force predicts the outcome of male combat interactions in crickets (Hall *et al.*, 2010) and *Anolis* lizards (Lailvaux *et al.*, 2004; Lailvaux & Irschick, 2007), and also predicts male fitness in collared lizards (Husak,

2006). However, the evidence for performance as a target of female choice is mixed (reviewed in Lailvaux & Irschick, 2006), and the benefits to females of preferentially mating with high-performance males, if any, are unclear (see Nicoletto, 1995; Lailvaux & Kasumovic, 2011 for examples). Despite the clear importance of performance to male combat, the role of performance in mediating mating conflicts between males and females during the mating process is largely unexplored (Husak & Lailvaux, 2014). In particular, little is known about whether high performance allows males to override female mating preferences, or whether females who are good performers are better able to resist male coercion than females with poorer performance capacities.

Although subject to often strong selection, performance traits are not expressed in isolation and exist as part of the integrated organismal phenotype (Ghalambor *et al.*, 2007; Torres-Dowdall *et al.*, 2012). Consequently, performance is linked to other phenotypes due to either a shared genetic component or through their shared reliance on a common pool of energetic resources. Indeed, performance traits are expensive to express, maintain, and use (Garland, 1983; Lailvaux & Husak, 2017), and are thus subject to energetic based trade-offs with other traits that are closely related to fitness, especially life-history traits (Husak & Lailvaux, 2014; Lailvaux & Husak, 2017). Consequently, expression of certain traits might impinge on the expression of performance, and vice versa. For example, in *Teleogryllus commodus* crickets, males invest in reproduction through advertisement calling, an energetically expensive process that trades-off against other key life-history traits (Hunt *et al.*, 2004), and calling effort itself is affected by factors such as diet and the social environment (Maklakov *et al.*, 2009; Lailvaux & Kasumovic, 2011; Lailvaux *et al.*, 2011). At the same time, the aging trajectories of both jumping

and biting performance in *T. commodus* are affected by sex and mating frequency, suggesting plasticity in performance expression in this species as well (Lailvaux *et al.*, 2011). Although life-history trade-offs associated with calling have been well-studied, such research is largely correlative because calling effort is difficult to manipulate. Consequently, the relationship between male calling effort and performance expression has not been subject to rigorous experiment.

Performance motivation and octopamine

Octopamine is an invertebrate monoamine, structurally related to adrenaline and noradrenaline in vertebrates, which is responsible for adrenergic signaling and may be analyzed comparatively as an analogous system. Octopamine modulates a variety of behaviors, sensory organs and peripheral organs in invertebrates and affects the way insects may respond to an external stimulus. Along with tyramine, octopamine is the only nonpeptide neurotransmitter restricted to invertebrates. In many arthropod species octopamine is responsible in part for the control of aggressive behaviors (Bubak *et al.*, 2014), and high levels of octopamine are indicative of successful encounters in male combat scenarios for crickets (Roeder, 2005, 2020). The neurotransmitter octopamine affects arthropod movement, activity, and aggressive behaviors in males (Hoyer *et al.*, 2008). Condon & Lailvaux (2016) showed that bite performance, an important general determinant of male combat outcomes, decreases independent of bite morphology in male house crickets that have recently lost a fight versus fight winners or control males that did not fight, and suggested a role for octopamine in affecting bite force expression. Bubak *et al.*, (2022) subsequently confirmed the influence of

octopamine on maximum bite force expression independent of bite morphology in this same species via supplement of epinastine, a synthetic octopamine antagonist. But although intrasexual aggression is facilitated by octopamine, its effects on sexual conflict have not yet been investigated.

Energetic costs associated with locomotion and whole organism performance account for significant amounts of individuals' energy budgets, though the amount of energy expenditure it takes for an organism to perform tasks greatly vary. Energy expenditure is quantified through respirometry, where the amount of oxygen consumed versus carbon dioxide expelled are measured to determine metabolic rates. Within insect species locomotion and temperature affect metabolic rates through CO_2 expenditure or O_2 consumption typically measured in closed chamber environments. In Nespolo *et al.*, (2003) using *Hophlosphyrum griseus* cricket species the authors were able to demonstrate with repeatability, crickets had an increased rate of oxygen consumption when they were both larger and held at higher temperatures. Understanding and measuring the energetic costs underlying behaviors such as signaling is essential if we are to further understand trade-offs in other aspects of the organismal phenotype that might be linked to signal production.

In addition to confirming the role of octopamine in mediating maximum bite force, Bubak et al. (2022) also showed differences in maximum bite force when crickets have their antennae removed. Since octopamine affects aggression, locomotion, and other sensory modalities, the tradeoffs between calling effort and lifespan are subject to mediation by antennae removal via its effects on octopamine. Specifically, if removing antennae affects octopamine and octopamine affects performance, the link between antennae removal and

stridulation should reveal lifespan and life-history tradeoffs. Such an intervention would be novel because, despite the large literature on the evolutionary ecology of calling in model species such as the Australian black field cricket, no studies thus far have manipulated muscular activity or stridulation to test for resultant energetic trade-offs that might be caused by the mechanism of calling itself. (Bentsen *et al.*, 2006; Judge *et al.*, 2008; Drayton *et al.*, 2010; Callander *et al.*, 2013).

In this dissertation, I directly manipulate calling effort; courtship call structure; and bite force in the house cricket species *Acheta domesticus* and the black field cricket *Teleogryllus commodus* by altering octopamine levels (Hoyer *et al.*, 2008). By doing so, I rigorously test how performance expression impinges upon the expression of other key life-history traits such as calling effort, and whether performance affects male ability to coerce females; females' ability to withstand male coercion; or both.

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CHAPTER I: Bite force, body size, and octopamine mediate mating interactions in the house cricket (*Acheta domesticus*)

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Abstract

Mating interactions are rife with conflict because the evolutionary interests of males and females seldom coincide. Intersexual conflict affects sexual selection, yet the proximate factors underlying male coercive ability and female resistance are poorly understood. Male combat outcomes are often influenced by bite force, with superior biters being more likely to achieve victory over poorer biters in a range of species, including crickets. If good performers also achieve mating success through sexual coercion, then bite force might play a role in intersexual conflict as well. We tested the capacity of bite force to influence mating interactions in house crickets both directly by measuring bite forces of males and females, and by altering male bite capacity through neuropharmacological manipulation. In addition, the invertebrate neurotransmitter octopamine both mediates aggression and underlies motivation to bite in male house crickets. By blocking octopamine receptors through the application of an antagonist, epinastine, we tested the effects of reduced bite force on male mating success. Our results show that male bite capacity, in combination with body size, influences both the likelihood and the outcomes of mating interactions, whereas treatment of males with epinastine eliminates motivation to mate. Our results suggest a functional role for bite force in affecting both sexual conflict and sexual selection, and expand our knowledge of the influence of biogenic amines on reproductive behavior.

Introduction

Reproductive interactions between males and females can give rise to conflict if the evolutionary interests of the sexes are incompatible. In mating systems that are dominated by female choice, males may attempt to subvert female mating preferences, often resulting in females mating with non-preferred males (Parker, 1979; Arnqvist & Rowe, 2005). Although the evolutionary consequences of this interlocus sexual conflict have received a great deal of attention (Hall *et al.*, 2010; Lailvaux *et al.*, 2010; Bonduriansky, 2011; Edward *et al.*, 2014), we currently lack a general understanding of the proximate mechanisms by which males are able to coerce matings from uncooperative females. Understanding these mechanisms is necessary if we wish to gain insight into whether selection commonly acts on similar mechanisms in females to resist unwanted matings, or if female resistance and male coercion are functionally distinct.

The unfettered expression of female mating preferences is associated with higher female fitness in a variety of species and contexts (e.g. Havens et al., 2011; Iyengar & Eisner, 1999), despite the costs incurred by choosiness (Vitousek *et al.*, 2007; Forstmeier *et al.*, 2021). However, when female choice is subverted by males, females commonly suffer a variety of additional costs associated not only with the act and consequences of mating (Morrow & Innocenti, 2012), but also with the evolution of resistance to unwanted matings, particularly in low-resource environments (Rostant *et al.*, 2020). Males can potentially thwart female mating preferences in a variety of ways. In crickets, for example, sexual conflict occurs over the attachment of the male spermatophore to the female reproductive tract. Unattractive males might take longer to solicit matings from females (i.e. exhibit longer latency to mate), and may also attempt to override female mating preferences by coercing her into allowing attachment of the spermatophore during mating, altering overall mating time. Once attached, the female can attempt to remove the spermatophore, and unattractive males might harass her to prevent her from doing so, thus ensuring that more ejaculate is transferred than she might like due to longer

attachment times. Although these behaviors are well-characterized, relatively little attention has been paid to the role of physical, functional traits such as whole-organism performance capacities (defined as the ability to perform dynamic, physically challenging and ecologically relevant tasks such as jumping, running, or biting; (Lailvaux & Irschick, 2006; Irschick *et al.*, 2008) underlying those behaviors in facilitating either male harassment and coercion or female resistance to unwanted matings (Watson *et al.*, 1998; Husak & Lailvaux, 2014).

Performance is relevant to both male-male combat (Lailvaux & Irschick, 2007; Hall *et al.*, 2010; McLean *et al.*, 2020) and female competition (Bywater *et al.*, 2008) in that better performers tend to be victorious over poorer performers in aggressive intraspecific interactions with other individuals of the same sex (reviewed in Husak & Fox, 2008; Lailvaux & Irschick, 2006). For example, in crickets, maximum bite force has been positively associated with the likelihood of winning male-male fights both by itself (Condon & Lailvaux, 2016) and in combination with other performance traits, such as jumping ability (Hall *et al.*, 2010). However, those performance traits that are associated with success in male combat situations are typically not the same traits that are preferred by females (Lailvaux *et al.*, 2010; Okada *et al.*, 2014; McCullough & Simmons, 2016), and consequently the utility of performance traits might be sex-specific, and thus subject to conflict over either their expression or use in males and females (reviewed in Husak & Lailvaux, 2014; e.g. Tarka *et al.*, 2014). If performance also mediates reproductive conflicts between males and females during mating, then whole-organism performance capacities could be targets of selection within the contexts of male coercion or female resistance, or both.

In addition to measuring performance of individuals involved in reproductive interactions, insight into the effect of performance capacities on reproductive outcomes might be gained via performance manipulation. Octopamine and its precursor tyramine are the invertebrate analogs of adrenergic neurotransmitters (Farooqui, 2007). Octopamine affects arthropod movement, including muscle performance and respiration, activity, and aggressive behaviors in males along with the fight or flight

response and motivation (Roeder, 2005; Hoyer *et al.*, 2008). In addition, octopamine can also affect the expression of whole-organism performance traits. For example, *Achaeta domesticus* crickets treated with a synthetic octopamine antagonist, epinastine, exhibit a significant decrease in maximum bite force expression compared to control animals (Bubak *et al.*, 2022). A similar neuropharmacological intervention affects courtship call structure in this same species (Adeola *et al.*, 2022). Furthermore, in crickets levels of octopamine increase in the hemolymph after courtship, fight encounters and flying (Adamo *et al.*, 1995; Rillich *et al.*, 2019). Despite the role of the biogenic amines in affecting aggression and male combat (Rillich *et al.*, 2019; Palavicino-Maggio & Sengupta, 2022), previous studies administering octopamine antagonists have shown that octopamine does not affect courtship behaviors in those insects studied thus far, including the cricket *Gryllus bimaculatus* (Zhou et al. 2008; Rillich et al. 2019). Collectively, these findings suggest that octopamine is a promising candidate for modifying bite force expression, and thus for experimentally testing the role of bite force in mediating male-female conflict during mating interactions.

To test the hypothesis that bite force mediates reproductive interactions in *A. domesticus* house crickets, we measured maximum bite force both before and after trials and estimated the utility of bite force to predict mating outcomes and to affect the time course of mating interactions. Specifically, we predicted that differences in bite force and body size between males and females will affect not only the probability of successfully mating, but also the latency to mate; time it takes to mate; and the attachment times of the spermatophore packets. We then tested the secondary hypothesis that altering octopamine levels through oral supplementation of epinastine prior to mating trials affects both bite force expression and consequently the timing and outcomes of mating interactions. We predict that treatment groups will differ in these same four variables, namely mating success, latency to mate, mate time, and spermatophore attachment time.

Methods and Materials

The *A. domesticus* crickets used for this experiment were laboratory descendants of 1000 count cricket stocks originating from Fluker's Cricket Farm, Baton Rouge, Louisiana. We stored crickets, at similar densities, in 66-quart storage containers with mesh nets in the lid for ventilation. We monitored food and water provisions bi-weekly, similar to previous studies (e.g. Condon & Lailvaux, 2016; Adeola *et al.*, 2022). Once crickets reached maturity, distinguishable by eclosure where the final molt results in wings, we randomly allocated 300 male and female crickets to either control (n=200) or treatment (n=100) groups. We separated males into containers to ensure virginity before the start of the trials, but we kept females in containers with non-experimental males. Therefore, females had the option to mate before the start of the trial, so that when paired they would not mate due to lack of options as females engage in polyandry in this species (Mautz & Sakaluk, 2008; Rillich *et al.*, 2009).

We fed males in the treatment group a pureed sweet corn and epinastine mixture at a concentration of 15mg/ml (as in Bubak *et al.*, 2013, 2022; Adeola *et al.*, 2022). Control males were fed similar amounts of pureed sweetcorn without epinastine. We allowed males 2 hours to feed and returned them to the 8x8 cubic centimeter containers, after which we measured the bite force of each isolated cricket using standard methods. Briefly, we encouraged crickets to bite down on an Economical Load and Force System printed circuit strip calibrated in Newtons. We collected readings three times per cricket to ensure the maximum reading was recorded (Hall *et al.*, 2010; Lailvaux *et al.*, 2011; Condon & Lailvaux, 2016; Bubak *et al.*, 2022). Different sensors were used for males and females to avoid female ingestion of trace amounts of neurotransmitters from possible regurgitation of sweet corn blends.

For mating interactions, the numbers assigned to the crickets were placed in an array and randomly permutated to choose pairings. We placed cricket pairs in 17x11.5x6 centimeter containers with clear lids and damp paper towels at the base of the containers to provide favorable mating conditions (Hall *et al.*, 2008; Adeola *et al.*, 2022). We allowed the pairs an average of approximately 2.5

hours to initiate mating. We defined the latency to mate as the time in seconds from when males and females were placed in the same container to the initial start of mating where backward slipping of males under the female occurs to engage in copulation. We defined mate time as the time the male took to attach the spermatophore packet once back slipping under the female began. If the mating attempt was successful (i.e. a spermatophore packet was produced and attached to a female during copulation), the bite force for both sexes was measured. We defined attachment time as the time it the spermatophore packet was attached to the female post copulation till the time it was discarded from the female external genetalia. If unsuccessful, the same pairs were re-mated within 48 hours with their same partner. Of the 100 control pairs, 11 mated successfully on the first pairing, and 12 mated only on the second pairing. There were 45 pairs of crickets in the epinastine group, however none of them mated. Before re-mating, male crickets were additionally supplemented with treatment dosages. Once re-mated we again measured the bite forces.

Statistical Analysis

We conducted all analyses using R version 4.0.4 (R Development Core Team, http://www.R-project.org). We tested the influence of bite force on mating interactions by measuring the difference between the maximum bite forces of the male and female that constitute a given mating pair. We also measured thorax size differences between males and females to account for allometric effects of body size on bite force. Within the control trials, we tested the influence of size and bite force on the probability of mating by fitting a logistic mixed model using binomial errors and Adaptive Gaussian Quadrature (Pinheiro & Bates, 1995) to the binomial mating outcomes. Because cricket pairs that did not mate on the first pairing were given a second opportunity to do so the next day, we coded grouping as a random effect to control for any associated variance and included this in our probability of mating mixed model. Fixed effects in this model were the differences between both bite force and thorax size between the

male and female involved in each interaction, as well as the interaction between size and bite force differences. We fit this logistic mixed model using the GLMMadaptive package.

For latency to mate, mate time, and attachment time, we fit separate generalized linear models to test for effects of thorax size differences and bite force differences between males and females on the respective dependent variables. We used Poisson distributions to model the amount of time seconds it took for crickets paired in their containers to start mating (i.e. latency to mate); the amount of time in seconds it took crickets to mate (i.e. mate time); and the amount of time the spermatophore packet was attached to each female (i.e. spermatophore attachment time) (Dobson & Barnett, 2008). In addition to testing for effects of size and bite differences and the interaction between them, we also fit a nonlinear term for bite force difference in all of the saturated models to all for the possibility that such differences might disproportionately affect the timing of these key events depending on their relative magnitudes. In all cases we used log-likelihood ratio reduction tests to find the minimum adequate model for each dependent variable (i.e. the simplest model explaining the greatest amount of variation for each instance)(Crawley, 1993). We note that we did not conduct any statistical analyses on the epinastine treated crickets because, out of the 45 pairs of epinastine treated crickets, none successfully mated, and including these data in the mixed model logistic regression skewed the dataset and introduced singularity errors which prevented model convergence.

Results

Males within the control group of this study had a greater mating success rate when their recorded maximum bite force measurements were stronger than the paired female (Table1, Fig 1). The minimum adequate models retained significant interactions between thorax size differences and bite force differences for mating success, latency to mate, mate time and spermatophore attachment time. Males

in the epinastine group did not mate; consequently, all of the subsequent analyses pertain to the 100 control mating pairs.

Within the control group, larger males that bit harder than the females with which they were paired were more likely to successfully mate, even after accounting for mating group (i.e. whether pairs mated on the first or second attempt) (Table 1; Figure 1). Our model for mate time showed males who had weaker bite forces than females and were smaller in thorax width took a longer time to initiate mating (i.e latency to mate) compared to males that were larger and stronger biters than the paired female (Table 2a; Fig. 2). Larger crickets that bit harder were also more likely to have both shorter mating times and longer spermatophore attachment times (Table 2b, c; Fig. 3, Fig.4). Mating times were also prolonged in cases where females were larger, but males had bite force differences of greater than ~0.15 N. (Fig 3).

For all three of these variables, the minimum adequate models were the saturated model, retaining not only interactions between size differences and bite force differences, but also nonlinear effects of bite force differences on all of the time variables (Table 2). These results indicate that not only are latency to mate, mate time, and spermatophore attachment time significantly affected by both size and bite force differences between males and females, but that the effects of bite force become ever more pronounced as the differences between male and female biting become larger. The nonlinearity is particularly manifest in the case of overall mate time, in the sense that the surface for mate time shows two distinct peaks, with mate times being especially prolonged in both males with both weak and strong bite forces relative to females, depending on the specific combination of bite force and size differences (Figure 3).

Discussion

Whole organism performance abilities such as the ability to exert forceful bites affect male combat outcomes in a variety of vertebrate (Lailvaux *et al.*, 2004; Husak, 2006) and invertebrate taxa (Hall *et al.*, 2010; Condon & Lailvaux, 2016). However, the functional factors bolstering males' coercive abilities are not as well understood from a performance perspective. We measured maximum bite force for both males and females to determine the potential for bite force to mediate conflict between the sexes, and to test the hypothesis that reducing bite force via octopamine manipulation would affect reproductive interactions.

The difference in bite force and thorax size between males and females engaging in reproductive interactions consistently predicted both the outcomes and the time course of those interactions. First, that males were able to successfully mate with females at all depended significantly on both the difference in body size as indicated by the size of the thorax, and the difference in bite force between males and females in a given interaction. Because bite force has an interaction with thorax size we cannot independently determine the likelihood of mating success based on thorax size difference or bite force difference alone (see Engqvist, 2005), but only in combination with each other, such that males who were both larger and stronger biters than their respective females exhibited higher mating success. The mechanism by which bite force influences the probability of successfully mating is not apparent from our current dataset, but could be determined via more detailed behavioral observations than we employed here. However, our results are consistent with the interpretation that sexual conflict in this species has a functional basis that involves biting as a means of either coercion or resistance to unwanted matings (Watson et al. 1998)

We define success in this context as males that were able to successfully attach a spermatophore packet to females (Fig. 1) (Richmond, 2014). It is of note that only >25% of mating interactions in our control group resulted in successful matings on either the first or second mating

trials. But more marked even than this was the effect of epinastine administration on the likelihood of mating. We had expected that treatment of males with an octopamine inhibitor would reduce bite force, thus affecting the time course of each of the mating variables of interest; what we found instead is that not even a single male in the epinastine treatment group mated successfully. Thus, epinastine treatment eliminated mating entirely in this experiment, and indeed during mating trials treatment males seldom moved or engaged with the females. Previous behavioral work on octopamine and its antagonists have demonstrated a key role for octopamine in moderating male aggressive behavior in insects (Hoyer *et al.*, 2008; Bubak *et al.*, 2014), but no effects on courtship or mating success. For example, octopamine depletion reduces the intensity of aggressive behavior and reduces the likelihood of male contest escalation in *Gryllus bimaculatus* crickets (Stevenson *et al.*, 2000, 2005). But although Zhou et al. (2008) found that mutant *Drosophila* flies lacking octopamine entirely were nonetheless able to express normal courtship behaviors, and Rillich et al. (2019) found that epinastine did not significantly affect key aspects of male courtship in *G. bimaculatus*, our results show a drastic effect of blocking octopamine receptors on male courtship. These results point to some potential differences in the octopaminergic systems of these different cricket species. For example, Rillich et al. (2019) also found that treatment with epinastine did not affect courtship call production in *G. bimaculatus*, yet courtship call structure is significantly altered by epinastine in *A. domesticus* (Adeola *et al.*, 2022). The cause of these differences is not apparent from our current dataset, although it could be the case that certain traits are affected differently by the various biogenic amines in different species, as both courtship behavior and courtship calls are affected by dopamine and serotonin in *G. bimaculatus* (Rillich *et al.*, 2019). However, it could also be the case that these differences stem from the epinastine treatment protocol we employed. Specifically, we administered epinastine orally at a concentration of 15mg/ml (as in Bubak *et al.*, 2013, 2022; Adeola *et al.*, 2022), whereas other studies have used injections of various concentrations of saline and epinastine, particularly to test aggressive encounters in male crickets

(Rillich *et al.*, 2011, 2019; Stevenson & Rillich, 2012). It could be that bulk administration of epinastine as in this study entirely saturates the octopamine receptors, resulted in differences in behavior that are not observed in studies using more fine-grained epinastine manipulation.

The results of our epinastine treatment notwithstanding, our results demonstrate clear effects of bite force on all stages of the *A. domesticus* mating interactions. However, it is important to note that these effects are, in all cases, significant in combination with that of overall body size. In interactions where males were both larger and bit harder than females, males were able to initiate mating more quickly. The spermatophores also remained attached for longer in such pairings, implying potential fitness benefits to the males in these interactions because longer attachment times allow for more sperm to be transferred to the females in cricket species such as *Teleogryllus commodus* (Bussière *et al.*, 2006; Hall *et al.*, 2010). Although previous studies have pointed to aggressive harassment and mate guarding behaviours on the part of the male as instrumental in prolonging spermatophore attachment (Bussière *et al.*, 2006), our results strongly imply, but do not directly demonstrate, a role for bite force in enabling harassment behavior as well. This suggests a common role for functional capacities such as bite force in enabling male aggression towards both males and females, but also raises the possibility that the failure of males who are smaller and bite less forcefully than females are less successful in manipulating mating interactions to their advantage at least in part because of their relatively poorer functional abilities. Indeed, the inclusion of the nonlinear term for bite force differences in all of our models suggests that such differences become even more important as the gap in male and female functional capacities increases, such that males with especially strong bite force relative to female mating partners should have enhanced capacity to manipulate mating outcomes. Future studies that combine performance measurement with detailed behavioral studies of male-female mating interactions, in the same way that researchers have previously done to test the role of performance in

male combat, would be useful for understanding exactly how these capacities are used to affect the outcomes of those interactions.

Studies of interlocus sexual conflict have demonstrated the importance of both pre- and postcopulatory male-female interactions in driving and altering evolutionary trajectories in crickets (e.g. Hall et al., 2013). Our results extend previous findings of this phenomenon in two important ways. First, we show that octopamine is necessary for successful mating in *A. domesticus* in a way that does not appear to be the case for other cricket species studied so far. Second, we show that the relative performance capacities of males and females, in combination with their respective differences in body size, exert a strong influence on both the timing, latency to mate, and outcomes of mating interactions in this same species. These findings demonstrate that animal functional capacities are relevant to sexual conflict, just as they are to sexual selection, and suggest that selection might act on such capacities in both males and females in *A. domesticus*, not only males.

Conflict of interest

The authors declare no conflict of interest.

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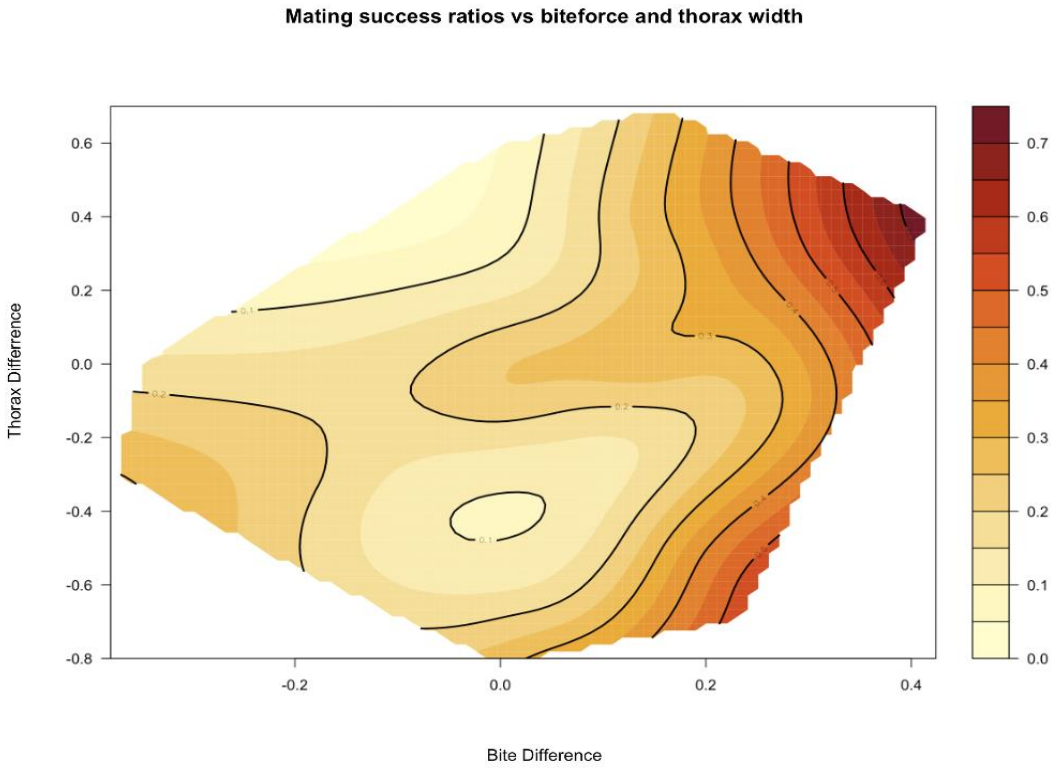

Fig 1: Males within the control group of this study enjoyed greater mating success when they were both larger and exhibited higher maximum bite forces than the specific females they were paired with, whether they mated on the first or second pairing. Males in the epinastine group did not mate.

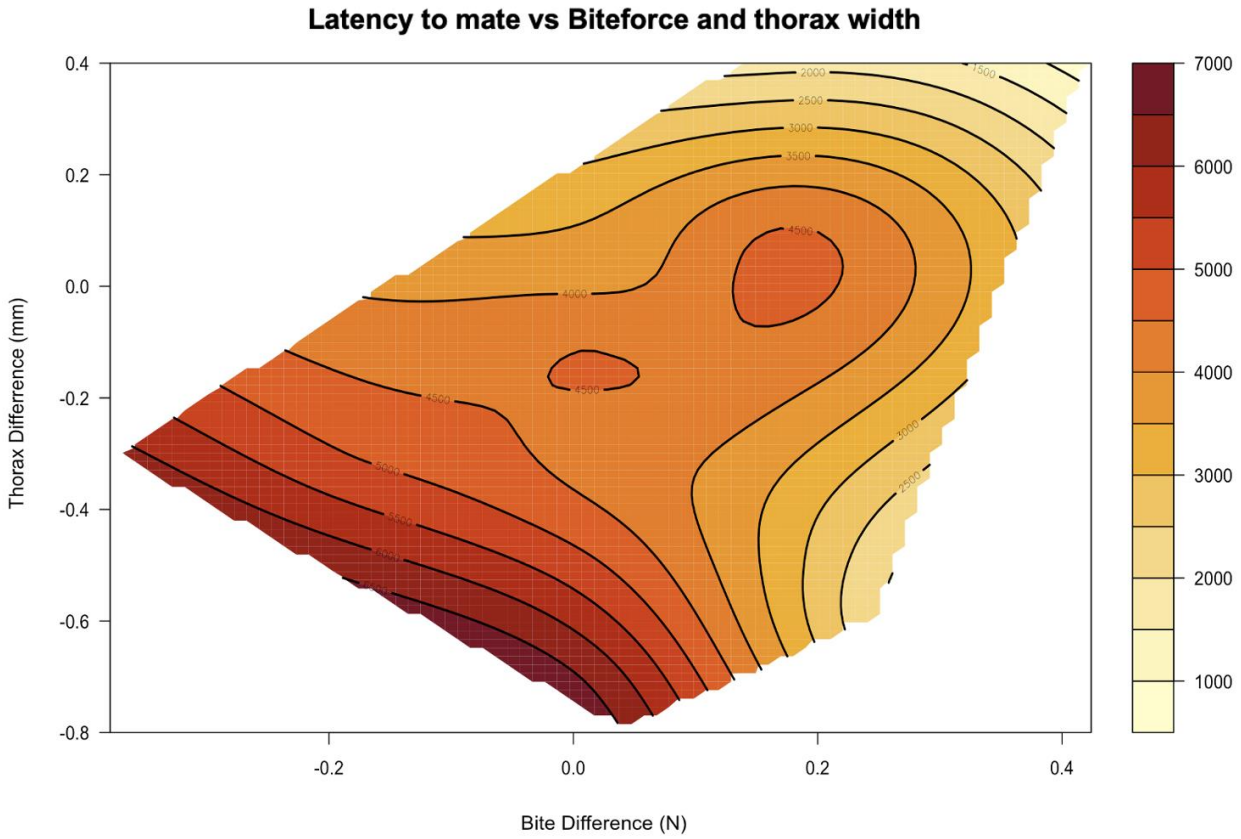

Fig. 2: The minimum adequate model for latency to mate retained a significant interaction between thorax size differences and bit force difference in males and females, such that these times (seconds) were longer when male crickets were smaller and exhibited weaker bite forces than females.

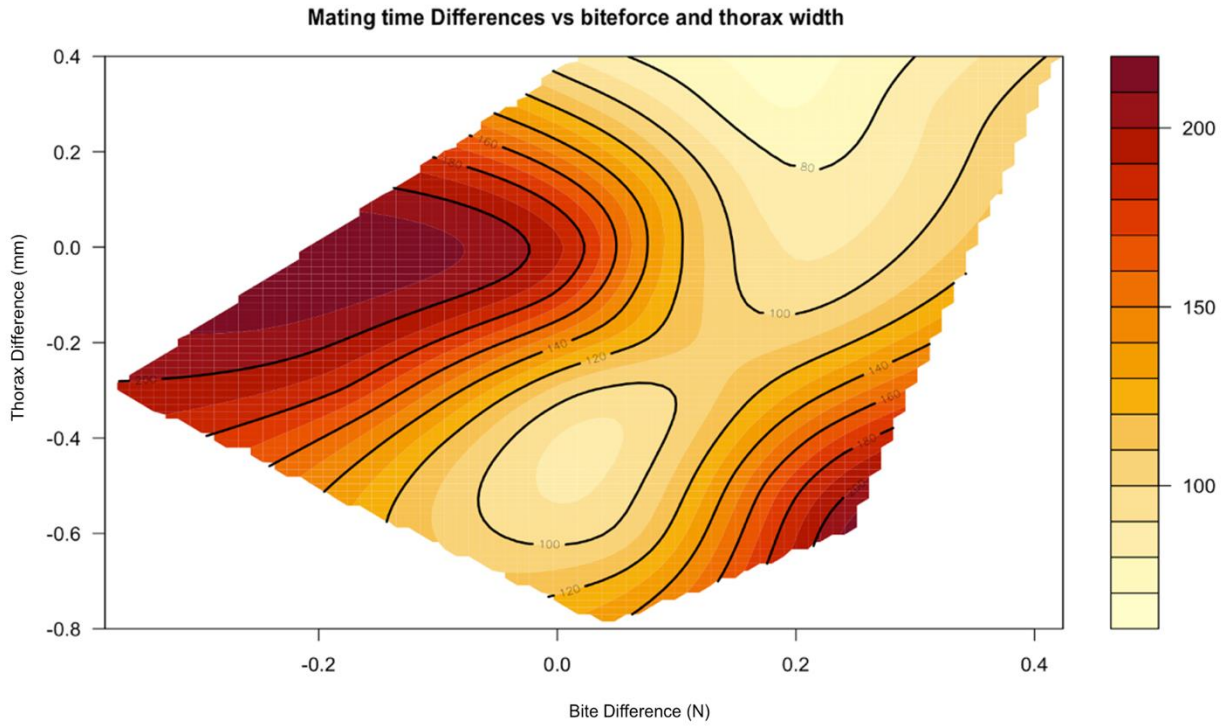

Fig. 3: The minimum adequate model for mate time retained a significant interaction between thorax size differences and bite force difference between male and female mating pairs such that mate time (seconds) was the longest both when females mated with larger males with weak bite forces, and when females mated with smaller males with stronger bite forces.

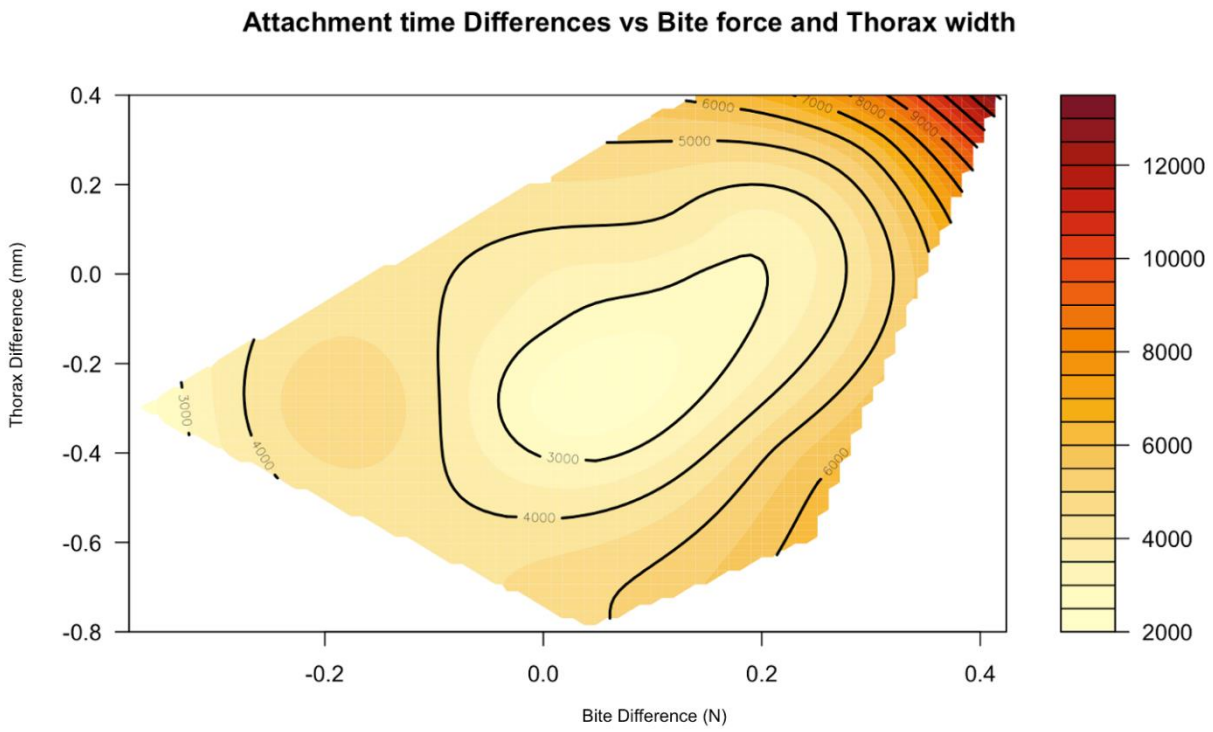

Fig. 4: The minimum adequate model for spermatophore attachment time (seconds) shows that females were slowest to remove attached spermatophores of males that were both larger and bite harder than females. The nonlinear nature of these effects is manifest as the marked peak for such males as these differences become ever larger.

Mate Success Generalized Linear Mixed Effects Model				
Fixed Effects:	Estimate	Std.Err	Conf.low	Conf.high
(Intercept)	-1.7244	0.3161	-2.344	-1.1048
bitediff	4.2070	2.0021	0.283	8.1310
thoraxdiff	-0.7565	0.7617	-2.249	0.7363
bitediff:thoraxdiff	6.8016	5.5277	-4.033	17.6357

Table 1: Generalized linear models explaining mate time with interactions between bite force and differences in thorax size between males and females. The method used was the adaptive Gauss-Hermite quadrature rule

A

Latency to Mate Generalized Linear Mixed Effects Model			
Fixed Effects:			
	Estimate	Std. Error	Z value
(Intercept)	8.373455	0.004442	1884.90
thoraxdiff	-0.800833	0.011149	-71.83
bitediff	-0.610972	0.026005	-23.49
I(bitediff^2)	-7.145587	0.088831	-80.44
thoraxdiff:bitediff	5.062986	0.086031	58.85

B

Table : Mating Time			
Coefficients:			
	Estimate	Std. Error	Z value
(Intercept)	4.93716	0.02162	228.313
thoraxdiff	0.51133	0.06080	8.411
bitediff	-2.39595	0.13279	-18.043
I(bitediff^2)	5.99539	0.37984	15.784
bitediff:thoraxdiff	7.74370	0.42169	-18.364

C

Table : Attachment Time			
Coefficients:			
	Estimate	Std. Error	Z value
(Intercept)	7.958917	0.005518	1442.33
thoraxdiff	-0.591354	0.016365	-36.13
bitediff	2.031494	0.028259	71.89
I(bitediff^2)	2.597051	0.095966	27.06
thoraxdiff:bitediff	2.594166	0.085568	30.32

Table 2: Generalized linear models explaining initiation time (A); mate time (B); and attachment time (C). All models retained interactions between bite force and thorax size differences between males and females, as well as a nonlinear effect of bite force difference in each case, as indicated by log-likelihood ratio tests.

Chapter II: Octopamine affects courtship call structure in male *Acheta domesticus* crickets

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Abstract

Secondary sexual displays vary considerably in both type and structure both within and across animal species. Although such variation is of keen interest to evolutionary biologists, the functional factors driving variation in male displays are poorly understood. In crickets, acoustic calls are produced by muscular contractions via stridulation of file and scraper wing components. We tested the effect of varying octopamine, an important biogenic amine neurohormone in invertebrates, on call production in male *Acheta domesticus* house crickets by blocking the octopamine receptors that influence skeletal muscle function with epinastine, a synthetic octopamine antagonist. We then measured male courtship calls and analyzed the call structure to quantify the differences in call structure based on the changes in carrier frequency, and whether chirps or ticks are a more prevalently expressed frequency in treated vs untreated males. Males treated with epinastine exhibited clear differences in call structure compared to untreated controls, such that epinastine-treated males were more likely to produce simpler calls and to exhibit their carrier frequencies as ticks rather than chirps. Thus, we were able to directly modify male courtship calling performance during mating interactions by altering the neuropharmacological milieu, demonstrating the potential role of biogenic amines in contributing to the diversity of call types in nature.

Keywords: *Acheta domesticus*, auditory performance, courtship calls, sexual selection

Introduction

Secondary sexual signals and displays are common throughout the animal kingdom and are key determinants of reproductive success (Andersson, 1994). For this reason, a large literature exists documenting the consequences of such displays in ecological contexts ranging from predator-prey interactions (e.g. Leal, 1999) to male combat (e.g. Brandt, 2003; Jenssen et al., 2005) and female choice (Brooks and Endler, 2001; Candolin, 2003; Hunt et al., 2004). Although researchers have long sought ultimate explanations for the evolution of male displays, the proximate mechanisms affecting the structure, variation, and degree of stereotypy exhibited by such displays, as well as the intrinsic factors influencing display production, remain poorly understood.

A theme common to most animal displays is conspicuousness, and examples exist of species enhancing the conspicuousness of their displays via each of the main sensory modalities (Searcy and Nowicki, 2005). Visual and auditory displays specifically are frequently characterized by dynamic, often spectacular movements that either constitute the display itself in the former case, or serve to produce sounds of a particular frequency and temporal structure in the latter (Johnstone, 1998). Because such dynamic displays are often mediated by locomotor activities, potentially allowing signal receivers to assess aspects of the display for errors and irregularities in motor skill (Johnstone, 1998), recent research on the mechanical and physiological basis of signalling has focused on aspects of the musculoskeletal system that bolster signal production within vertebrates in particular. For example, Fuxjager et al. (2013) showed that androgen receptors and their activation play an important role in sustaining abilities necessary for performing elaborate high-speed displays in the golden-collared manakin, *Manacus vitellinus*. However, despite recent interest in the mechanisms underlying motor skill competency, these studies remain largely limited to vertebrate species.

Invertebrates exhibit a variety of visual and auditory signals and displays that function within the contexts of female choice or male combat. Similar to vertebrates, these displays are often linked to dynamic movements involving rapid muscular contraction and are thus subject to similar mechanical constraints. In invertebrates specifically, there is evidence that muscle function can be regulated by various intrinsic pharmacological agents (Evans and Maqueira, 2005). Octopamine (OA), a biogenic amine, functions as a neurotransmitter, neurohormone, and neuromodulator and along with tyramine, an OA precursor, regulates physiological and behavioral processes such as courtship, locomotion, cognition, and reproduction in invertebrates ranging from crustaceans to arthropods (Hana and Lange, 2017). Octopamine also has regulatory effects on energy metabolism and homeostasis (Fields and Woodring, 1991; Roeder, 2020) and aggression (Bubak et al., 2014; Hoyer et al., 2008). Consequently, behaviors altered by manipulation of OA include the modulation and initiation of locomotor patterns, and the ability of individuals to engage in complex social interactions (Rillich and Stevenson, 2015). In addition to affecting function within specific ecological and behavioral contexts, OA also affects muscle function directly; in the desert locust, *Schistocerca americana gregaria*, for example, twitch and tetanic tensions in flight muscles of adult males treated with OA yielded an increase in mechanical power output, a result which was reversible with the application of an OA antagonist (Malamud et al., 1988). Given this array of effects, OA is a likely candidate agent for exerting regulatory control over the emergent properties of muscle function, such as signal production, as well.

Crickets are a useful model system for understanding both the evolutionary and functional ecology of calling. Males of most species call in both male-male and male-female interactions, and the structure of cricket calls is subject to both sexual and natural selection (Gray and Cade, 1999a, 1999b). Male *Teleogryllus commodus* crickets, for example, experience multivariate stabilizing selection on the properties of the advertisement call, used to attract females over long distances, that is driven by female preferences for specific call components (Bentsen et al., 2006; Brooks et al., 2005). By contrast,

advertisement calls in the congener *Teleogryllus oceanicus* on the island of Kauai have been strongly selected against by an acoustically orienting parasitoid fly to the point that they have been lost entirely (Zuk et al., 2006). In between these two extremes are a range of diverse call types and structures, yet the neuromuscular and neuropharmacological factors underlying variation in cricket call structure are seldom considered. Consequently, despite the attention paid to the fitness effects of cricket calls, we lack an understanding of the functional mechanisms affecting cricket call structure.

We used a synthetic OA receptor antagonist, epinastine, to dampen muscular activity in male *Acheta domesticus* house crickets. Because the stridulatory apparatus enabling sound production is driven by muscular contractions, variation in the neuromuscular activity is likely to drive variation in call structure both among and within call types. Altering muscular function should change overall calling frequency because of the effect muscular contractions have on the file and scraper mechanism involved with male sound production. House crickets exhibit three distinct calls (advertisement calls, courtship calls, and aggressive calls) which vary in frequency structure (Stevenson, 2005). The courtship song specifically comprises three types of sound pulses: two alternating low-frequency, low-intensity pulses of ~5kHz (i.e. “chirps”), and occasional higher-frequency (15- to 20-kHz) "ticks", which replace the low-frequency pulses (Nelson and Nolen, 1997). We tested the hypothesis that blocking OA receptors affects call structure by recording the calls of courting males supplemented with epinastine and comparing the resulting call structures to those of courting control males. Specifically, we predict that males treated with epinastine will exhibit simpler calls that are easier to produce with a compromised signalling apparatus. Furthermore, we also predict that the calls of epinastine-treated crickets will comprise more ticks than chirps because ticks, although higher in frequency than chirps, are produced at lower amplitudes than the more complex chirps, and consequently are likely to be energetically cheaper and thus easier to produce.

Methods and Materials

The *A. domesticus* crickets we used for this experiment were bred in our lab at the University of New Orleans from 1000 count cricket stocks originating from Fluker's Cricket Farm, Baton Rouge, Louisiana. We maintained our cricket supply in separate containers of similar densities of crickets with mesh nets in the lid for ventilation, similar to previous studies (e.g Condon & Lailvaux, 2016). We provided Purina dry cat feed for food, and water via tube every three days. We isolated males at the 7th instar to ensure virginity and increase the motivation of males to court the paired female. We measured the width at the base of the pronotum under a Leica dissection scope as a proxy for body size to test whether size was a factor in call production.

Behavioral trials

We induced courtship behaviour and calls in males by pairing them with females in experimental trials. Before pairing with females, males were placed for two hours inside a plastic container with either plain pureed sweetcorn for control crickets, or sweetcorn supplemented with epinastine at a concentration of 15mg/ml for the epinastine treated crickets (as in Bubak et al., 2013). At the end of those two hours, we recorded male courtship calls during pairings with females to determine differences in call structure of carrier frequencies of males that were supplemented with epinastine. To allow cricket pairs to engage in mating encounters, we placed pairs comprising one male and one female in 17x11.5x6 cm containers with clear lids. We placed a damp paper towel covering the base in each container to provide favorable mating conditions (as in Hall et al., 2013). Males and females were only permitted to engage in mating attempts with their assigned partner once the trial began. Pairs were given an average of approximately 2.5 hours to initiate mating.

Courtship call recording and analysis

We recorded male courtship calls during the trials with a linear microphone (TASCAM DR-40 Linear PCM Recorder) as uncompressed audio files. Our sample size was 92 male crickets. We observed 47 males in the control group and 45 males in the epinastine treatment group. Overall, although we obtained 47 recorded calls from individual crickets in the control group, we found that supplementation with epinastine tended to eliminate calling entirely in many of the treatment crickets. However, we recorded calls from 15 individual crickets in the epinastine-treated cricket group. We clipped each of our audio samples into the clearest 8-second interval and visually expressed our audio data as waveforms and power spectral density charts which we used to identify the maximum frequency density within the sound (Figure 1; Figure 2). Using Audacity 2.3.3 (audacityteam.org) and Raven Lite 2.0.1 (Cornell Lab of Ornithology) software we analyzed the call structures of individual male crickets in each experimental group by applying a high-pass bandpass filter of 3200 Hz to remove lower frequency instrumental noise within the laboratory setting. Frequencies higher than the 3200 Hz cut-off were processed post-filtering. Once we filtered the files, we used the seewave package in R (Sueur et al., 2008) to create a Power Spectral Density plot from which we determined the frequencies of the signals present throughout the recorded call and, ultimately, the peak frequency of dominant signals throughout each call (Figure 1). We determined call structure by analyzing ranges of carrier frequencies crickets produced in the high range consisting of ticks (10kHz+) and low range consisting of chirps (4kHz-5.5kHz) for each of the treatment types (control or epinastine). We were thus able to determine if the stridulatory courtship calls produced by males are within the detection range of the female auditory neurons after manipulating male locomotor function. To test quantitatively whether epinastine might dampen propensity to call at all, we also documented whether each of the tested males produced calls.

Statistical Analysis

We conducted all analyses using R version 4.0.4 (R Development Core Team, http://www.R-project.org). To test whether epinastine treatment affected the propensity to produce a courtship call, we used a

logistic regression model (i.e. a generalized linear model with binomial errors) with calling or not calling as a binary dependent variable and treatment; pronotum width; and the interaction between treatment and pronotum width as factors. Of the crickets that produced calls, we performed an ANCOVA to test whether supplementing the crickets with epinastine affected the average number of syllables the crickets produced during courtship while controlling for size by including pronotum width as a covariate. To characterize how the differences between treatment types affected the dominant carrier signal within the subset of crickets that produced courtship calls, we again fit a logistic model to the data, with carrier frequency coded as a binomial variable (i.e. dominantly in the ticks (10kHz+) or chirps (4kHz-5.5kHz) range) and treatment; pronotum width; and the interaction between treatment and pronotum width as factors.

For all GLMs we used log-likelihood ratio reduction tests to determine the significance of individual terms in the model and arrive at the minimum adequate model [that is, the simplest model that explains the most amount of variation;(Crawley, 2012; Quinn and Keough, 2002)] in each case. We used the ggplot2 package (Gómez-Rubio, 2017) in R (R Core Team 2021, version 4.0.4) for data visualizations.

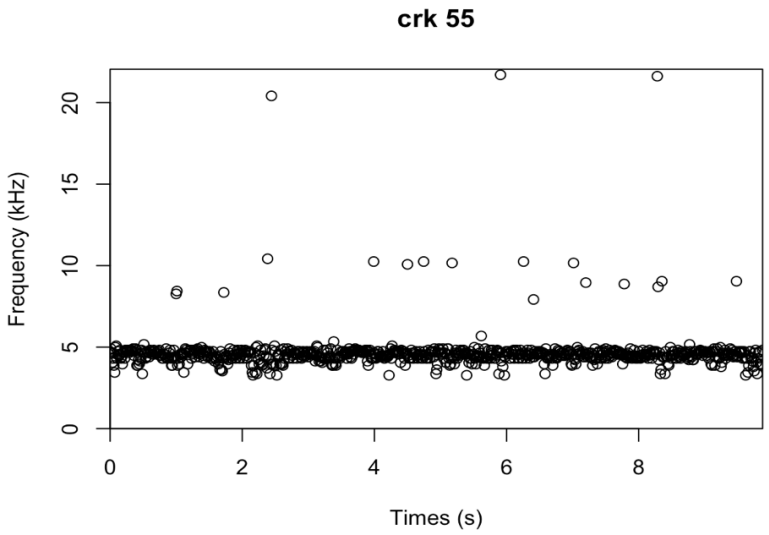
Results

We observed 47 males in the control group and 45 males in the epinastine treatment group, of which we recorded courtship calls from all 47 control crickets, but only 15 males treated with epinastine. Our minimum adequate model for propensity to call retained an effect of treatment, such that males supplemented with epinastine were significantly less likely than control males to call at all (Table 1). Of the filtered dominant carrier frequencies we measured, the minimum carrier frequency of the control group was 3.467 kHz and the maximum was 11.822 kHz, where 2 of the 47 control crickets sampled expressed their carrier frequency in the higher range. Within the epinastine treated group, the minimum

carrier frequency recorded was 3.790 kHz and the maximum was 12.489 kHz, where 8 of the 15 males expressed their carrier frequencies in ranges consistent with tick production rather than chirp production. The frequency ranges of the chirps we recorded here fell within the range of previously observed chirp frequencies (Nelson & Nolen, 1997; Stout et al., 1988).

Our logistic regression results show that while size (as measured by pronotum width) is not a significant factor in changes of carrier frequency ranges of calls, treatment type does affect dominant carrier frequency expressed by males (Table 2). Specifically, crickets treated with epinastine were more likely to dominantly produce ticks at higher frequencies as carrier signals as opposed to producing chirps during their courtship song. Crickets treated with epinastine were also less likely to produce calls at all during trials (Figure 3). Control males were more likely to produce chirps in the 4-5kHz range frequency as opposed to producing ticks in the 10kHz+ frequency range as their carrier signal (Figure 4). Finally, the calls of epinastine-treated and control crickets differed significantly in complexity, with those of treatment males comprising fewer syllables on average than those of control males (Figure 2; Figure 5). Despite this difference in complexity, overall calling patterns did not differ, as the calls were expressed with repeated down sweep patterns, where the frequency decreases towards the end of every syllable produced, regardless of treatment, chirp period, or syllable period repetition (Figure 6).

A



B

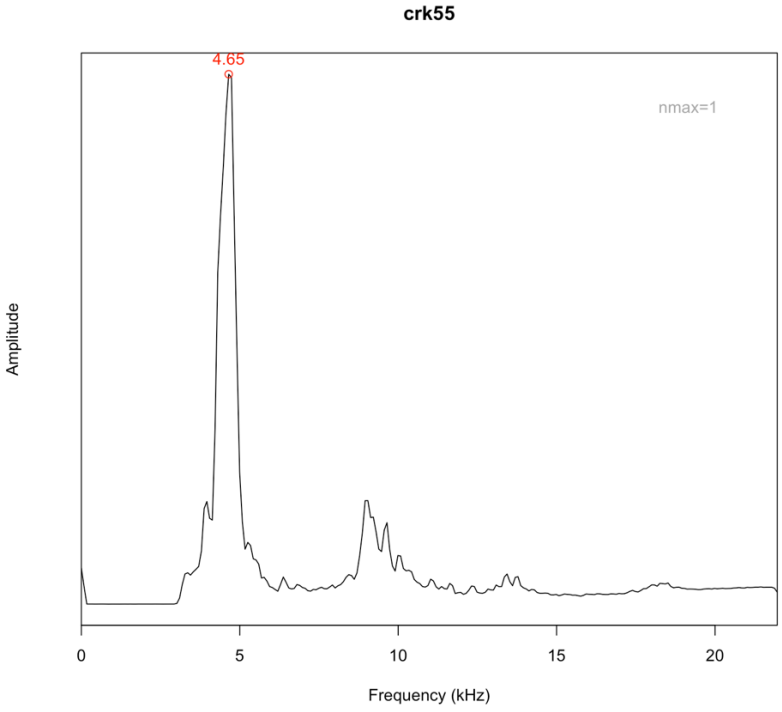


Figure 1 : Individual frequencies shown in a cricket (crk55) recorded throughout the call duration. On the left (A) each of the frequencies from the cricket's produced call are plotted on by frequency over time. On the right (B) the labelled peak is the frequency that is dominantly expressed throughout the call duration of the power spectral density chart. We use the dominant frequency for range classification where amplitude is the distance of a wave moved from an equilibrium position.

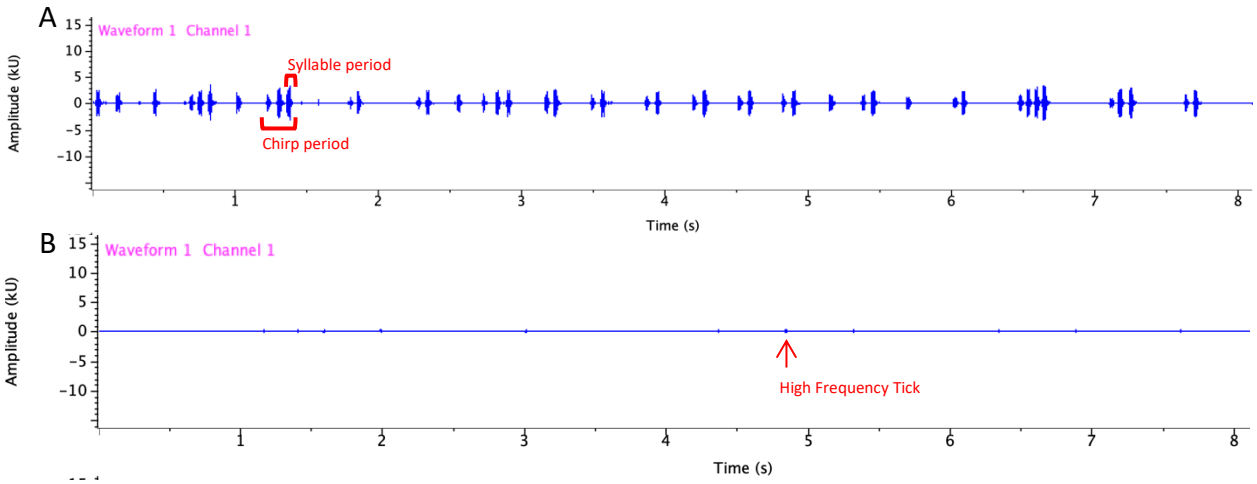


Figure 2 : Chirp period and syllable period of a cricket (crk87) within the control group (A). For each cricket we obtained the count data for number of syllables in a chirp period. Chirp period and syllable period of a cricket (crk135) within the epinastine treatment group (B).

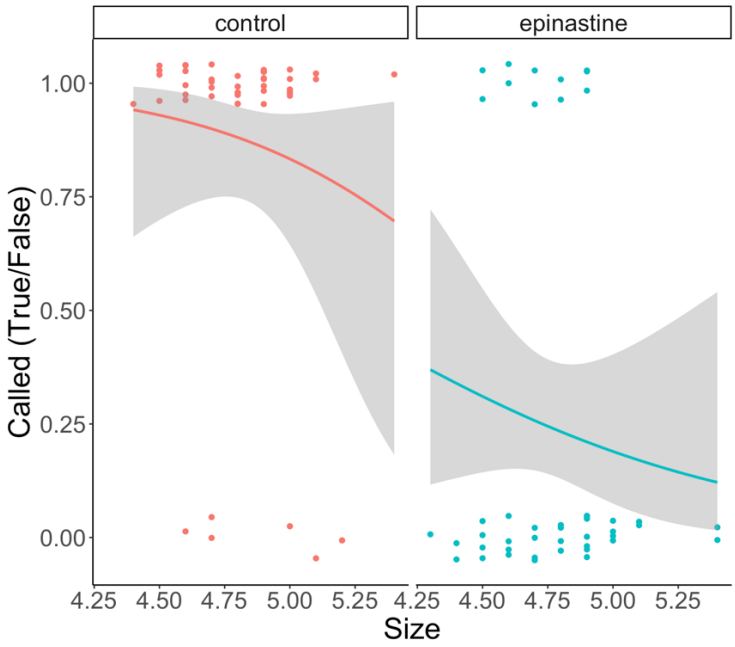


Figure 3 : Treatment type affects whether crickets of each group were able to produce any call during courtship call trials. Males treated with epinastine were less likely to produce calls at all. Size is not a significant factor in the calling model, but is shown here as the x-axis for clarity.

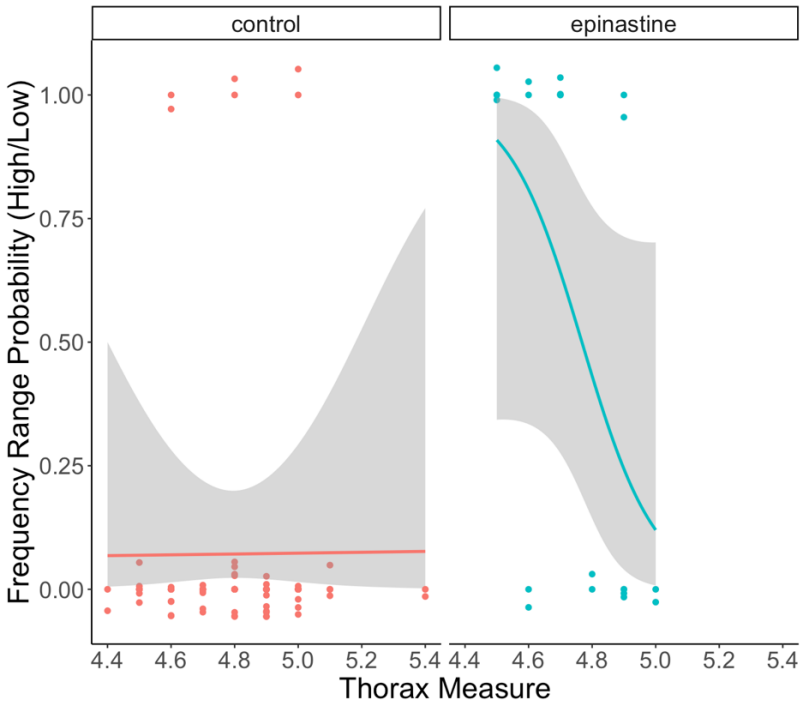


Figure 4 : Treatment type alters dominant carrier frequencies produced by male cricket. The relationship between control and epinastine treatments and the probability of dominant carrier frequencies being expressed as chirps (~4-5kHz) at lower frequencies or ticks (10kHz) at higher frequencies during courtship interactions. Circular symbols depict sounds in ranges expressed by individual males. Size is not a significant factor in the frequency model, but is shown here as the x-axis for clarity.

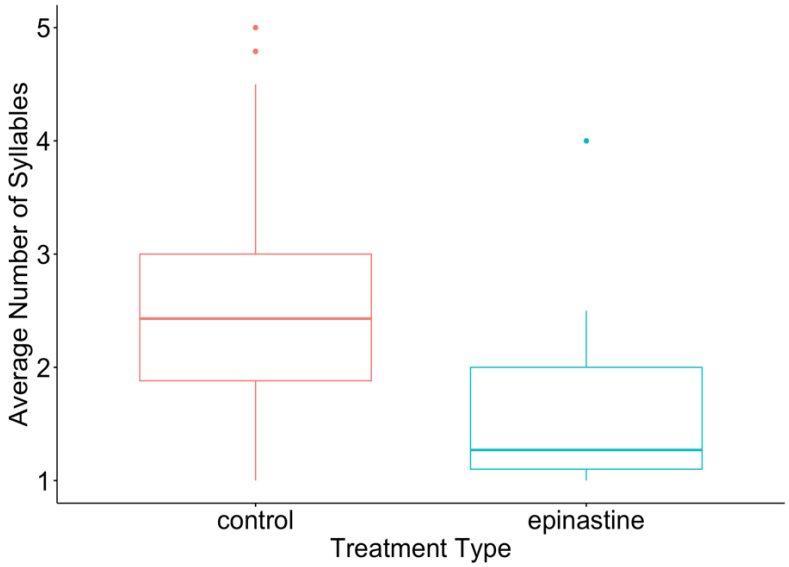


Figure 5: Average number of syllables within each chirp period of the courtship call duration produced by crickets of each group.

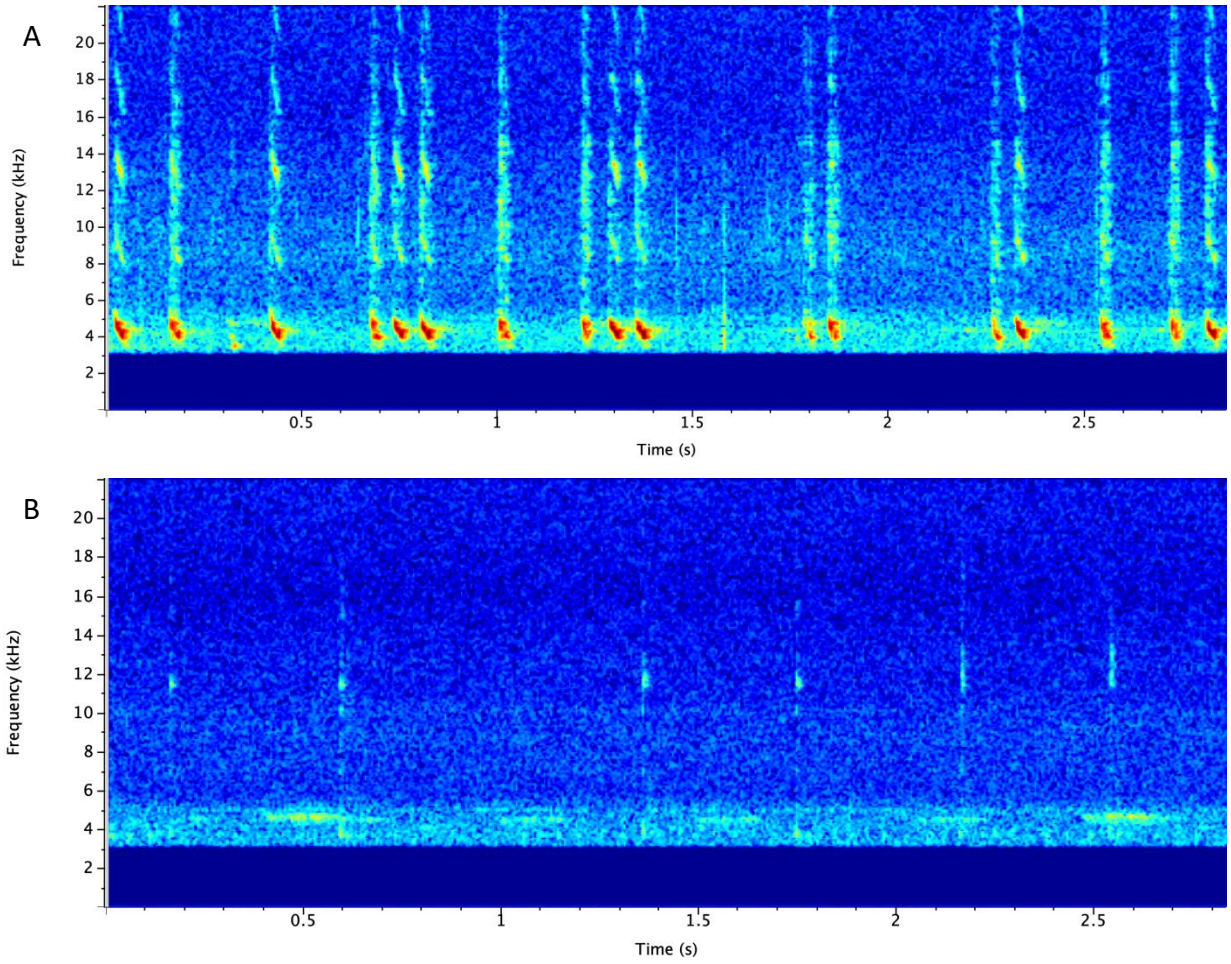


Figure 6: Spectrogram a cricket (crk87) within the control group (A). In the first three seconds of the calling song there are repeated down sweep patterns (~4-5kHz) at lower frequencies. (B) The below spectrogram (crk119) shows frequencies produced by crickets treated with epinastine. We use the same time interval and magnification in both images. u Frequencies below 3.2kHz were filtered out for environmental laboratory noise.

Discussion

Sexual displays may vary in conspicuousness and in component structure, yet despite considerable inter- and intraspecific variation in dynamic signalling, the proximate mechanisms contributing to that variation are poorly understood. We tested the hypothesis that the biogenic amine octopamine (OA), which acts as a neurotransmitter, neurohormone, and neuromodulator of muscular action, affects the expression of courtship calls in the house cricket *A. domesticus* crickets by altering muscle function. Specifically, we tested the predictions that blocking OA receptors via epinastine supplementation would

cause male crickets to produce less complex courtship calls, and that those calls would also be higher in frequency, comprising predominantly "ticks", compared to untreated controls.

Acheta domesticus courtship calls exhibit a general down sweep pattern (Figure 6A) consisting of a chirp period comprising two to three syllables per period (Henley et al., 1992; Stout et al., 1988) and a tick that is produced at a variable rate throughout the duration of the courtship song (Nelson and Nolen, 1997). Our first prediction was supported, as we found that the courtship calls produced by males treated with epinastine were simpler than those of control males, independent of size effects, comprising overall fewer syllables on average per call period in epinastine-treated crickets compared to untreated controls (Table 3; Figure 5). Our second prediction regarding the overall call frequency was also supported, with the calls of epinastine-treated males exhibiting more high-frequency chirps as opposed to the low-frequency ticks which dominate the calls of control males, again independent of the size of the calling cricket. (Table 2). Thus, the calls of control and treated males differ markedly in key components of call structure, even while other aspects of the courtship call are clearly observable in both experimental groups.

This difference in call complexity could be attributed to at least two non-mutually exclusive effects of our pharmacological intervention that our current data do not allow us to distinguish between. In the cricket *Telogryllus oceanicus*, increasing octopamine levels increased twitch amplitude contraction rate and relaxation rate of metathoracic longitudinal muscles (O'Gara & Drewes, 1990) which are associated with rapid wing movements during both stridulation and flight. While we did not directly measure these muscular contractions, the clear differences in average syllable number and frequency observed between treatment crickets and untreated controls indicate the strong possibility that it is these same muscles powering stridulation that are targeted by the OA antagonist in *A. domesticus*, and that are responsible for the altered call structure. However, these likely effects of an OA antagonist on muscle function do not preclude a simultaneous effect of call motivation on call structure

and expression. Specifically, although our experimental results suggest that epinastine administration altered the action of these stridulatory muscles as opposed to eliminating it entirely, there were nonetheless instances in the experiment where epinastine treated males did not produce any calls; did not move; or both remained stationary and did not call. Indeed, males treated with epinastine in our study were significantly less likely than control males to produce a courtship call at all under the same experimental conditions (Table 1; Figure 3). Males treated with epinastine in our study also did not successfully mate with females.

Previous studies using vertebrates have implicated brain function in the propensity to express locomotor behaviour (Baldo et al., 2003; Cardinal et al., 2002; Rhodes et al., 2003), and experimental studies using mice have shown that selection for voluntary wheel running results in mice who are incentivised, and thus more motivated, to exhibit wheel-running via the dopamine based reward system (Rhodes et al., 2005). The observed reluctance of epinastine treated crickets in our dataset to engage in courtship mirrors the reduced likelihood of engaging in aggression exhibited by individuals treated with OA antagonists in other insect species (Hoyer et al., 2008; Stevenson and Rillich, 2012). Furthermore, *A. domesticus* males are subject to the "loser effect" (Hack, 1997), an OA-mediated phenomenon in insects whereby males who have recently lost a fight experience a decrease in circulating OA and are more likely to lose subsequent fights compared to males who have not recently lost (Stevenson and Rillich, 2012). Condon and Lailvaux (2016) found that such "loser" *A. domesticus* males also are also less likely to exert their maximum bite forces when measured, and a recent study by Bubak et al. (in press) found that this “motivation” to bite is in fact eliminated in *A. domesticus* by dietary supplementation with epinastine, and restored by supplementation with OA. Collectively these findings both implicate OA in mediating courtship song structure and production, and suggest an important role for OA in affecting not just the absolute ability to perform integrated neuromuscular tasks, such as biting, calling, and courting, but their motivational capacity to perform such tasks to their

maximum functional capacity. Although our data here do not speak to "motivation" in the incentivized reward sense in which the term is used in vertebrate species, the notion that OA is involved in such a motivation system in crickets, perhaps in conjuction with dopamine (Bromberg-Martin et al., 2010) and/or serotonin (Bubak et al., 2016), is a testable one.

Our findings here regarding the impact of epinastine treatment on the production and complexity contradict those of several previous studies. For example, Zhou et al. (2008) found that mutant fruit flies lacking OA nonetheless express normal courtship, and blocking OA receptors with epinastine did not affect courtship call production in *Gryllus bimaculatus* crickets (Rillich et al., 2019). The reasons for these contrasting results here are unclear; it could be that our "bulk" method of epinastine supplementation, although not novel and commonly used in other invertebrates (Bubak et al., 2013) resulted in a greater OA receptor blocking efficacy than the injection approach of Rillich et al. (2019). However, Solari et al., (2018) also found that OA application modulates the activity of motoneurons that affect calling behavior in *Lymantria dispar* gypsy moths, particularly enhancing the activity of the terminal abdominal ganglia that control calling behavior, which suggests that blocking OA activity might affect aspects of calls in this species as well. Given the complexity of courtship interactions, as well as the known stage-specific action of OA during different phases of aggressive escalation (Brown et al., 2007) it might be that certain components of courtship are affected to a greater extent than others, perhaps in a taxon-specific fashion, although our current data offer no insight into this possibility. From our results, males produced calls within female courtship call hearing sensitivity ranges though there were differences between experimental groups in their call expression at those frequency ranges.

Octopamine is present in invertebrates in high concentrations and has significant functionality in the peripheral nervous system and central nervous system along with other tissues (Farooqui, 2007). Consequently, changes in circulating OA could, and likely do, affect multiple systems, phenotypes, and,

potentially, behaviors simultaneously, yet may do so in different ways or to different extents (Husak and Lailvaux, 2022). For example, a study conducted on octopaminergic neuromodulation within *Drosophila* resulted in differential effects to both song and flight such that increased OA levels stabilized flight rather than song motor patterns, even though *Drosophila* flies use their wings both for flight and for courtship (O'Sullivan et al., 2018). Our results here constitute further evidence for the conserved nature of OA function in insects, but also point towards the complexity of that function, implicating it as a proximate mechanism affecting call production and structure in addition to the known effects on aggression and locomotion. The multivariate functional nature of OA, as well as the multiple ecological and social effectors of OA dynamics, could provide insight into the context-specific nature of courtship activity in these insects, and ultimately into the various factors affecting call expression. Indeed, given our finding that fundamental aspects of courtship calls are altered by the neuropharmacological milieu in these animals, similar experiments considering OA dynamics might provide further insight into the neuromuscular control of both other song types within *A. domesticus*, and the same song types in other cricket species.

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Table1: Calls Produced					
Term	Estimate	Std. Error	Statistic	Conf.low	Conf.high
1 (Intercept)	6.83	0.437	4.40	3.13	17.9
2 Type Epinastine	0.0434	0.558	-5.47	0.00146	0.133

Table 1: Results of logistic regression analysis testing the propensity of epinastine-treated males to produce courtship calls compared with untreated controls. The above model is the minimum adequate model which retained only an effect of treatment, and did not retain either an effect of cricket size or an interaction between size and treatment. The baseline level for the treatment factor is "Control".

Table 2: Effect on Sound Production					
Term	Estimate	Std. Error	Statistic	Conf.low	Conf.high
1 (Intercept)	0.0769	0.599	-4.28	0.0186	0.212
2 Type Epinastine	15.6	0.852	3.23	3.16	96.1

Table 2: Results of logistic regression analysis examining the effect of treatment type on male-produced frequencies. This table represents the minimum adequate model which retained only an effect of treatment and did not retain either an effect of cricket size or an interaction between size and treatment. The baseline level for the treatment factor is "Control".

Table 3: Average Syllable ANCOVA						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
TypeEpinastine	1	7.50	7.505	7.584	0.008	**
Size	1	2.66	2.664	2.692	0.107	
Residuals	50	49.48	0.990			
—						
Signif. Codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1						

Table 3: ANCOVA of the average number of syllables produced by each cricket throughout their produced number of calls. Size is not a significant factor.

CHAPTER III: Antennae removal affects calling effort and lifespan but not metabolic rate in adult male *Teleogryllus commodus* crickets

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Abstract

Males of many animal species exhibit costly signals that are subject to sexual selection via female choice. In crickets, the energetic cost of producing such signals in particular drives trade-offs with other important life-history traits such as longevity, such that increased calling effort often results in decreased male lifespan. But despite the fitness benefits of enhanced calling, the factors affecting variation in calling effort in adult males are poorly understood. The removal of male antennae affects courtship call structure in house crickets, and is linked to variation in both mating behavior and whole-organism performance. We tested the hypothesis that antennae removal will also affect calling effort in the black field cricket, *Teleogryllus commodus*, a model system for understanding life-history trade-offs. To understand the consequences for both energetic expenditure and longevity, we also measured metabolic rate and lifespan in addition to calling effort, and calculated metabolic scope. Our data show that antennae removal modifies the relationship between calling effort and longevity, such that antennaectomized males who call more than those with intact antennae do not live as long, yet energetic expenditure is not affected. These data demonstrate that key life-history trade-offs can potentially be decoupled from energetic expenditure, and offer insight into the factors affecting signal expression in adult male insects.

Introduction

The expression of dynamic phenotypic traits incurs a variety of costs, ranging from the energetic costs of development, maintenance, and use, to survival costs potentially associated with trait expression in specific ecological contexts (Clark 2012; Husak and Lailvaux 2017; Reznick et al. 1990; Searcy and Nowicki 2005). Sexually selected signaling traits are often considered to be especially costly, and the energetic costs of expressing such traits frequently drive trade-offs with other aspects of the integrated organismal phenotype (Hunt et al. 2004; Kilpimaa et al. 2004; Lailvaux and Irschick 2006). But despite the ubiquity and importance of these costs constraining sexually selected trait expression, the proximate, mechanistic factors underlying variation in signal expression are seldom identified and consequently poorly understood.

Male signals and displays in many animal species comprise or otherwise incorporate dynamic, muscle-powered movements such as jumping or flying. Because these movements can incur significant energetic costs (Clark 2012; Husak and Lailvaux 2017), males may be forced to trade-off signal expression against other aspects of the integrated organismal phenotype if those costs are large enough. These trade-offs may manifest as reduced expression of one or more other important traits that depend on a similar pool of acquired resources for their expression, or as compromised longevity in cases where signal expression is sustained at high levels throughout adulthood. In field crickets, for example, male calling effort (i.e. how often males express their advertisement call) is under directional sexual selection via female choice, such that males who exhibit higher calling effort tend to enjoy higher fitness; however, given appropriate resources, such males will also call so frequently that they suffer reduced longevity (Hunt et al. 2004; Maklakov et al. 2009). Because of these crucial costs and benefits, researchers have manipulated calling effort in several ways to understand both its causes and consequences. In addition to dietary manipulation, calling effort can also be affected by the juvenile

demographic context (Kasumovic et al. 2012; Kasumovic et al. 2011), presence of rivals (Callander et al. 2013), mating opportunities (Houslay et al. 2017) or by mating status, with virgin males exhibiting higher calling effort compared to mated males (Zajitschek et al. 2012). However, the role of muscular activity in affecting overall calling effort has seldom been explored, despite the energetic costs of calling purportedly being driven by the repeated muscular contraction of the stridulation apparatus.

The insect biogenic amine octopamine (OA), acts as a neurohormone, neurotransmitter, and neuromodulator regulating several physiological and behavioral processes including courtship, cognition, and locomotion. In particular, OA directly affects muscle function, and flight muscle power can be amplified or reduced by treatment with OA or an OA antagonist respectively in the locust *Schistocerca americana gregaria* (Malamud et al. 1988). Octopamine also affects both the twitch amplitude contraction rate and the relaxation rate of the metathoracic longitudinal muscles in the cricket that power rapid wing movements during flight and stridulation (O'Gara and Drewes 1990). A recent study in *Acheta domesticus* house crickets showed that courtship call structure is altered by the administration of an OA antagonist, epinastine, such that epinastine treated males exhibited both simpler and higher frequency calls than controls (Adeola et al. 2022). Additionally, Bubak et al. (2022) found that removing the antennae of *A. domesticus* males decreases motivation to bite, and further that this removal effect can be abolished by supplementation with OA, and replicated with the administration of epinastine. These results suggest that antennae removal might inhibit overall calling effort as well via the same OA-mediated effects on the calling apparatus that alter *A. domesticus* call structure. If so, then both muscular activity and the energetic costs of call production that are attributable to muscular contraction could be dampened by antennaectomy, which might allow us to quantify the contribution of the muscular activity underlying calling effort to the calling-longevity trade-off.

To test the hypothesis that antennae removal affects both lifespan and calling effort, we antennaectomized *Teleogryllus commodus* males upon eclosion to adulthood and measured calling effort over their entire lifetimes. *Telogryllus commodus* are ideal for this experiment because of the large literature on calling effort in this species. We predicted that antennae removal would decrease calling effort via OA mediated effects on the calling apparatus, and consequently that antennaectomized males would enjoy longer lifespans than controls. In addition, we also measured both resting and active metabolic rates to test the secondary hypothesis that antennaectomy would affect energetic expenditure in treatment males. Specifically, we predicted that antennae removal would reduce energetic expenditure compared to control males, and that this would also be reflected in a reduced metabolic scope in treatment crickets.

Methods and Materials

Crickets used in this experiment were 5th generation descendants of approximately 200 males and females collected at Smith's Lake, NSW, Australia (32°22'S, 152°30'E). Nymphs were collected before the penultimate juvenile instar and isolated into individual plastic containers (5x5x3cm) with an egg carton for refuge, and supplied with *ad libitum* Friskies Go-Cat senior cat food and water. Upon eclosion, crickets were allocated randomly to either a control or treatment group. Treatment (i.e. antennaectomized) crickets had their antennae removed with scissors following Bubak et al. (2022). All experimental methods complied with the national and institutional ethical guidelines where this work was conducted.

Calling effort

To determine age-specific calling effort, upon maturity we placed males overnight every three days for 12h within a custom-built electronic call monitoring device (Hunt et al. 2004; Kasumovic et al. 2012;

Lailvaux et al. 2010) until death. A DaqBook 120 IO Tech datalogger connected to a personal computer monitored 64 microphones embedded in the lids of separate 5x5x3 cm plastic containers holding individual males and surrounded by plastic, acoustic isolating foam. The DaqBook is programmed to check for a signal from each microphone 10 times/second, where that signal is recorded as a 1 if it is at least 10dB higher than the level of background noise, and as a 0 if not. We calculated the average daily calling rate for each male.

Metabolic rate and metabolic scope

We measured active and resting metabolic rates of all male crickets using a Fibox3 oxygen system by placing them individually within a 9.6 cm^3 sealed respirometer with a 3x8 mm magnetic stir bar (as in Kasumovic and Seebacher 2013). By placing the respirometer over a magnetic stirrer at the lowest setting, the crickets were induced to move continuously for 5-10 min. After stopping the stir bar, we monitored oxygen consumption for approximately 30 minutes until a steady state was established to establish the resting metabolic rate. We calculated metabolic scope as the difference between maximum active and resting metabolic rates.

Statistical Analysis

To explain variation in calling effort, we first used a Tukey transformation to normalize calling effort data. We then conducted a general linear model with Tukey transformed calling $effort^{0.42}$ as the dependent variable, and thorax size, treatment, active and resting metabolic rates, and the interaction between size and treatment as factors. To test for an effect of treatment on lifespan, we fit a general linear model with lifespan as a dependent variable and thorax size, treatment, calling effort, resting and active metabolic rates, and an interaction between treatment and calling effort as factors. To test for an effect of antennaectomy treatment on metabolic rate, we fit a generalized linear mixed model with

metabolic rate as a dependent variable; thorax size, treatment, metabolic rate type, and the interaction between metabolic rate type and treatment as fixed effects; and individual as a random effect (because the same individuals were measured for both standard and resting metabolic rates). To test for an effect of antennae removal on metabolic scope, we fit a final general linear model with metabolic scope as the dependent variable, and size, treatment, and the interaction between size and treatment as factors.

In all cases, we used log-likeilhood ratio reduction tests to find the minimum adequate model (i.e. the simplest model that explain the most amount of variation in metabolic rate) (Crawley 1993). This is particularly important for the mixed models where the interpretation of p-values can be misleading due to shrinkage caused by inclusion of random factors. Once we arrived at the final model in each case, we refit them using REML.

Results

The minimum adequate model for calling effort retained size, antennae removal, and resting metabolic rate as significant factors (Table 1; Fig 1a, b). The final model for overall lifespan retained antennae removal, calling effort, and the interaction between antennae removal and calling effort as significant factors, such that males with antennae removed exhibited markedly increased calling effort, but also reduced lifespan compared to control males (Table 2; Figure 2). Metabolic rate was significantly affected by both size and metabolic rate type, such that larger crickets exhibited higher metabolic rates and active metabolic rates were higher than resting ones; however, we found no evidence for any effect of antennae removal on energetic expenditure. The minimum adequate model for metabolic scope retained only an effect of size, such that larger crickets exhibited higher metabolic scope. Consequently, antennaectomy had no effect on metabolic scope either.

Discussion

Investment in secondary sexual signals can drive trade-offs with other aspects of the integrated phenotype over individual lifetimes, and with lifespan itself. Calling effort in male crickets is subject to female choice, but the specific contribution of power- based muscular activity to both overall calling effort and the energetic costs of calling are seldom estimated. We removed antennae in adult male *T. commodus* crickets as a manipulation of muscular activity and tested for effects on both calling effort and longevity.

Our hypothesis that antennae removal affects calling effort was supported, albeit in some unexpected ways. Although we predicted that antennae removal would reduce calling effort, we found that this was only the case for crickets with the highest resting metabolic rates after accounting for effects of body size; for all other males, antennaectomized animals called more than their control counterparts (Table 1; Fig 1a). This result comes about because of the significant interaction between resting metabolic rate and antennae removal, such that control crickets exhibit a generally positive relationship between resting metabolic rate and calling effort, whereas antennaectomized males exhibit a negative relationship (Fig 1b). These results clearly suggest that removing antennae modifies the general relationship between energetic expenditure and calling effort, and are generally consistent with the links among antennae removal, OA, and call structure in *A. domesticus*, as well as the known connections between the antennae neural circuitry and OA in insects in general (Adeola et al. 2022; Bubak et al. 2022; Cayre et al. 1999; Heisenberg 1998; Schendzielorz et al. 2015). However, it is also important to note that the antennae are important components of insect sensory systems, and that by removing the antennae, we deprived treatment males of an important sensory modality. The antennae of *T. commodus* serve an important chemosensory recognition function (Rence and Loher 1977), and calling effort in this species is known to be sensitive to both demographic and social context (Callander et al. 2013; Kasumovic et al. 2012; Kasumovic et al. 2011). It may be that, lacking a key means of

perceiving that context, antennaectomized crickets instead decrease overall calling effort by default, depending on their energetic status at the time. Similarly, Sakura and Aonuma (2013) found that antennaectomized *Gryllus bimaculatus* males were reluctant to initiate combat, likely due to that reduced sensory input. The fact the antennae manipulation almost certainly has implications for insect social behavior is an important shortcoming of the antennaectomy procedure, and limits our ability to assign causality of our findings solely to changes in the neuropharmacological milieu. Experiments that manipulated OA directly, perhaps by the use of OA antagonists as in Bubak et al. (2022) and others, would be extremely valuable in this regard.

Although calling effort depended on both resting metabolic rate and on the presence or absence of antennae, we also predicted that antennaectomy would reduce overall metabolic expenditure compared to controls. Instead, we found that metabolic rate was unaffected by antennae removal. Active males exhibited higher active metabolic rates than resting males, as expected, and those active rates were also higher in larger males (Table 3; Figure 3). However, the final model did not retain an effect of antennaectomy treatment (Table 3), which means that the presence or absence of male antennae did not influence the rate at which crickets expended energy. Metabolic scope was similarly unaffected by antennae removal in our dataset, being a function only of cricket size (Table 4; Fig 4).

Energetic costs associated with movement and whole-organism performance account for significant amounts of most organisms' daily energy budgets (Garland 1983). In mammals, these costs appear to trade-off against longevity and reproduction, such that short-lived species with fast life-histories (and therefore elevated reproductive output) spend relatively less energy on daily locomotion compared to long-lived mammals who reproduce more slowly (Lailvaux and Husak 2017). We predicted that antennaectomized males should enjoy longer lifespans compared to control males with intact antennae, given the inhibitory effects of antennae removal on performance in other cricket species (Bubak et al. 2022; Adeola et al. 2022). Instead, our results show an interaction between antennae

removal and calling effort, with control males living longer but calling much less frequently than males with antennae removed (Table 3; Figure 2). Taken together with the lack of an effect of calling effort on metabolism, these results suggest that the dynamic, muscular-powered movements associated with insect call production are not significant drivers of lifetime energetic expenditure in this species, even though calling effort itself affects lifespan. This result is curious given both the known instantaneous energetic costs of calling in other cricket species (e.g. Hoback and Wagner 1997), as well as the documented trade-off between calling effort and key life-history traits, including lifespan, in *T. commodus* (Hunt et al. 2004). The relationships among metabolic rate, calling effort, and longevity in particular therefore appear to be complex. Okada et al. (2011) similarly found no relationship between resting metabolic rate and calling effort in *Gryllodes sigillatus*, contrary to our results here, but did find a trade-off between resting metabolism and longevity. That trade-off was not apparent in our dataset, and indeed we found a generally positive relationship between calling and lifespan in our experiment that was altered by antennae removal, but not to the extent that it resulted in any trade-offs (Table 2; Figure 2). Instead, our results show that antennae removal diminishes, but does not eliminate, that positive relationship between calling effort and lifespan that we see in our control animals. Collectively, our results suggest that antennae removal increases calling effort and decreases lifespan, but does not do so by increasing energetic expenditure. Instead, our results indicate that the efficacy of antennae removal to affect calling effort depends on the metabolic status of the organism in question.

The energetic costs of displays have received a great deal of attention, but relatively few studies have manipulated the functional basis of dynamic displays to assess the relationships among energetic expenditure, display expression, and longevity. We adopt such a functional approach here, and show that the links between calling effort and longevity can be modified independent of energetic expenditure in *T. commodus* by applying a procedure that is known to compromise both dynamic function and the call apparatus in other cricket species. Our results suggest that the trade-off between

energetic expenditure and sexual signal expression is not inevitable, and points towards the neuropharmacological milieu as a potentially important source of variation in sexual signal expression (Adeola et al. 2022), just as it has been implicated previously in aspects of aggression and male combat (reviewed in Bubak et al. 2014).

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d.f.	Coefficient	S.E.
Intercept	-41.9	45.71
Size	2.069	1.031
Antennae (yes)	-41.97	19.19
MR type (resting)	547.73	6539.4
Antennae (yes):MR type (resting)	15262.7	9984.75

Table 1: Best-fit minimum adequate model describing variation in calling effort$^{0.42}$ in male *T. commodus*. The coefficients describe the estimated change in calling effort between the baseline categories and the categories named in the table. The baseline category for Antennae is "Yes" (i.e. males with intact antennae), and for MR type is "active".

d.f.	Coefficient	S.E.
Intercept	27.08	5.46
Antennae (yes)	-3.45	8.36
Calling effort	0.0012	0.0005
Antennae (yes):Calling effort	0.0054	0.0023

Table 2: Best-fit minimum adequate model describing variation in overall lifespan in male *T. commodus*. The coefficients describe the estimated change in calling effort between the baseline categories and the categories named in the table. The baseline category for Antennae is "Yes" (i.e. males with intact antennae).

d.f.	Coefficient	S.E.
Intercept	0.011	0.01
Size	0.00043	0.0003
MR type (resting)	-0.03	0.0016

Table 3: Best-fit minimum adequate model describing variation in metabolic rate in male *T. commodus*. The coefficients describe the estimated change in metabolic rate between the baseline categories and the categories named in the table. The baseline category for MR type is “resting”.

d.f.	Coefficient	S.E.
Intercept	-0.012	0.019
Size	0.0009	0.0005

Table 4: Best-fit minimum adequate model describing variation in metabolic scope in male *T. commodus*. Size was retained as the only significant factor affecting metabolic scope.

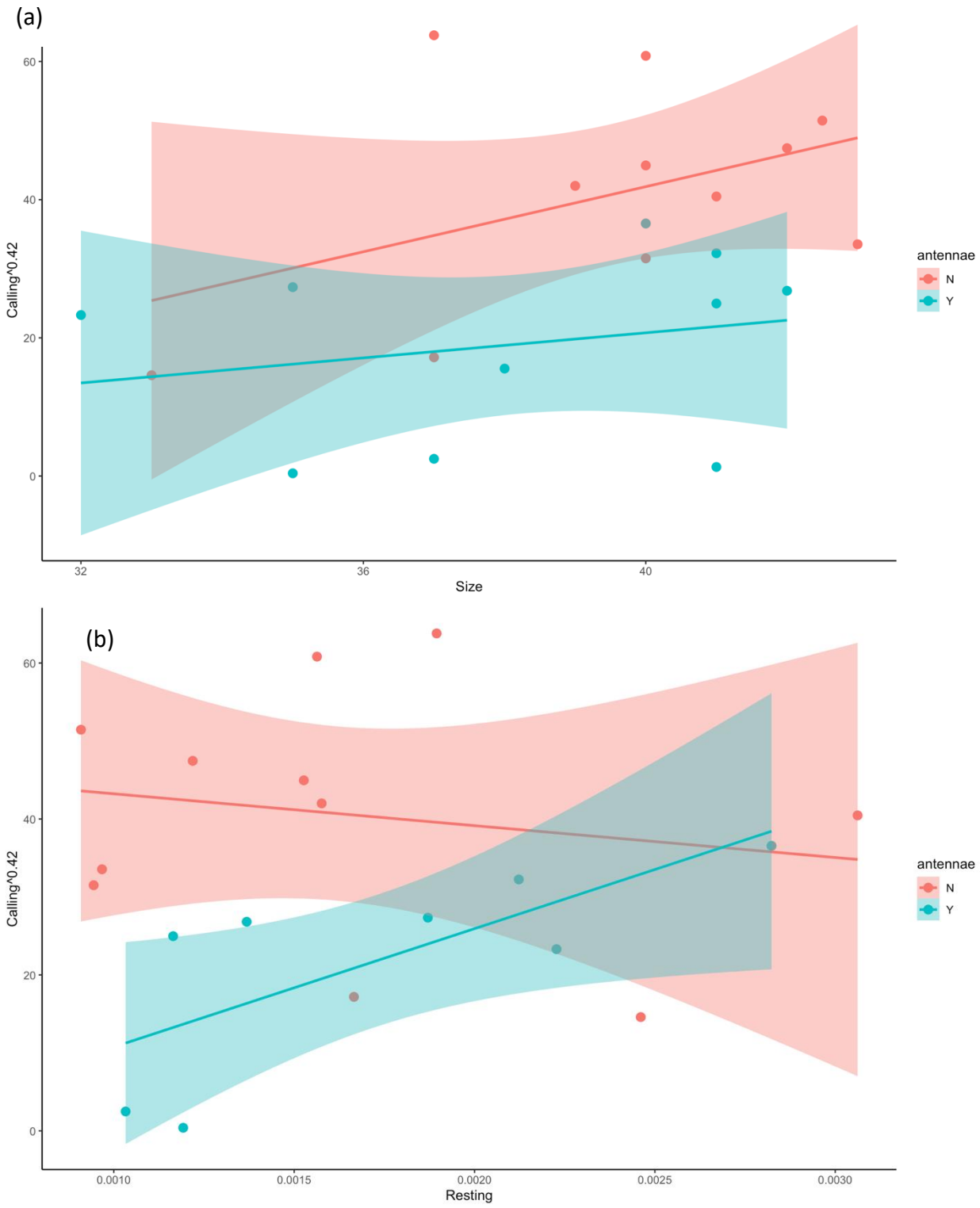


Figure 1: Power-transformed lifetime calling effort as a function of antennae removal and (a) body size, and (b) resting metabolic rate respectively in male *T. commodus* crickets.

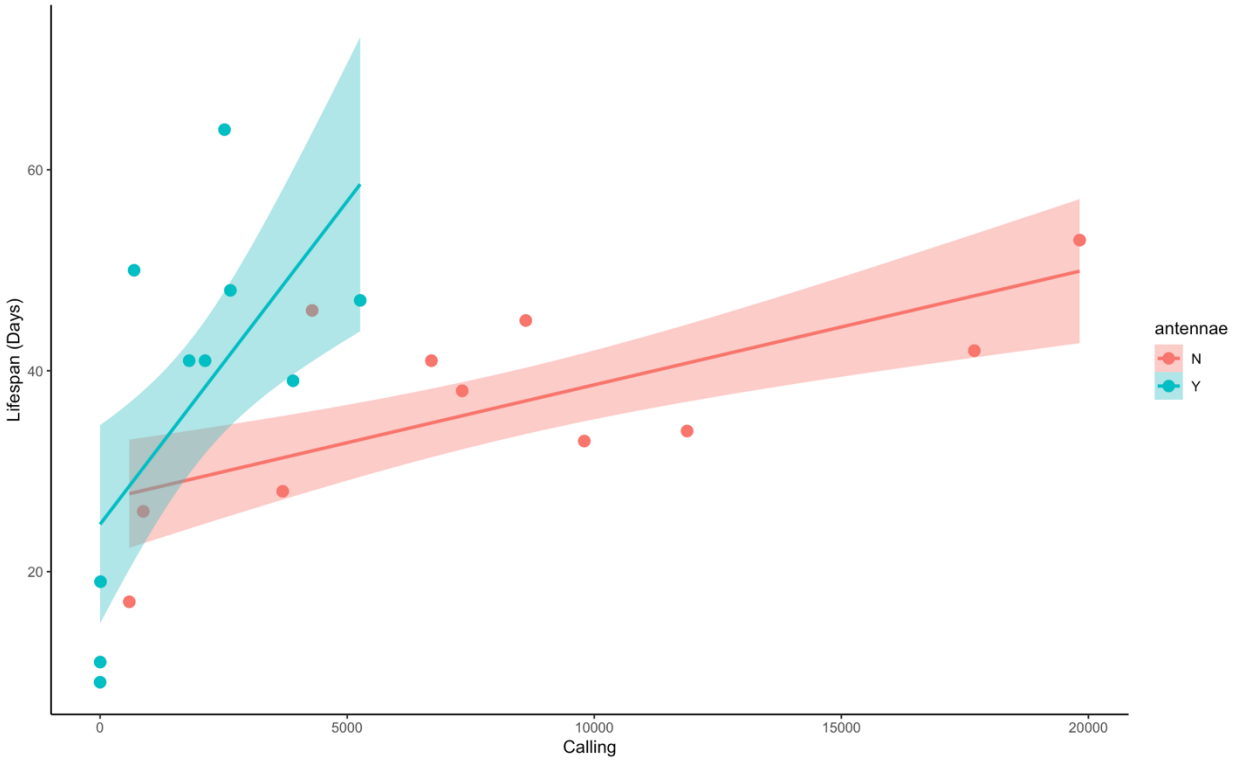


Figure 2: Lifespan in days as a function of lifetime calling effort and antennae treatment in male *T. commodus* crickets.

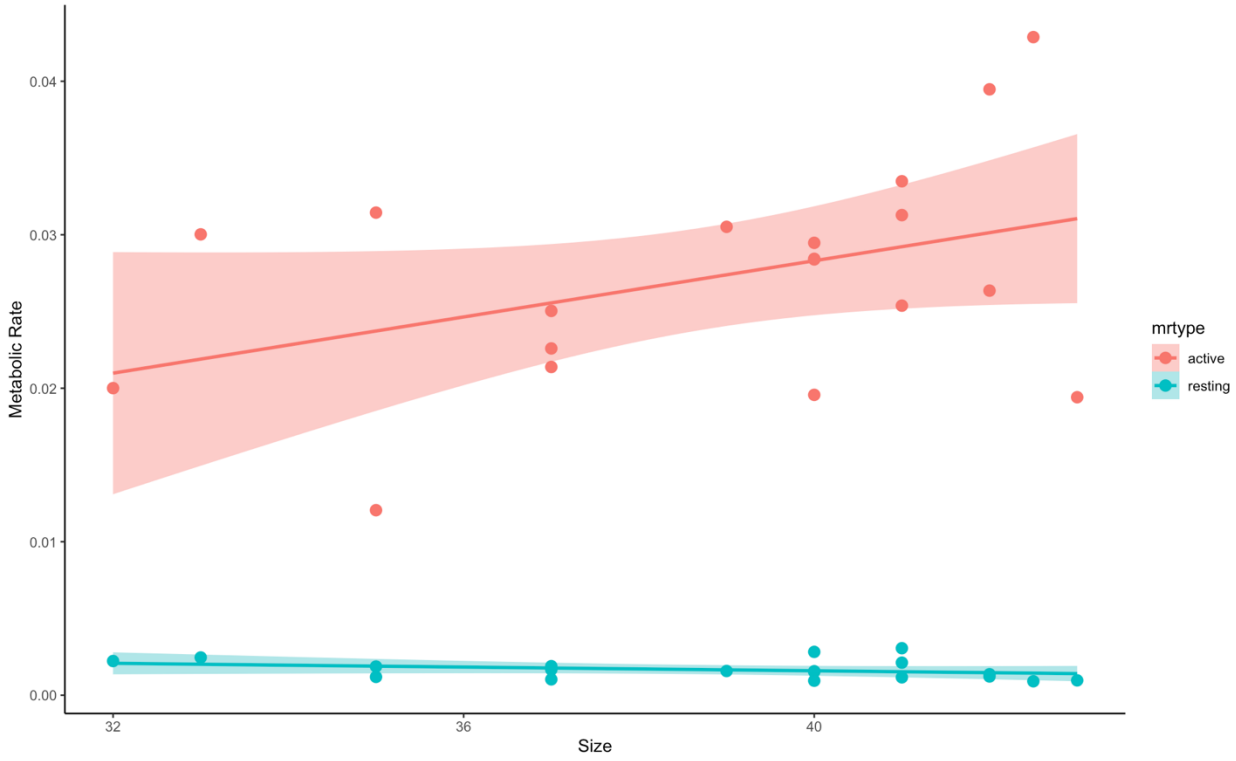


Figure 3: Metabolic rate is significantly affected by both body size and by metabolic rate type, such that active metabolic rate increases as does size.

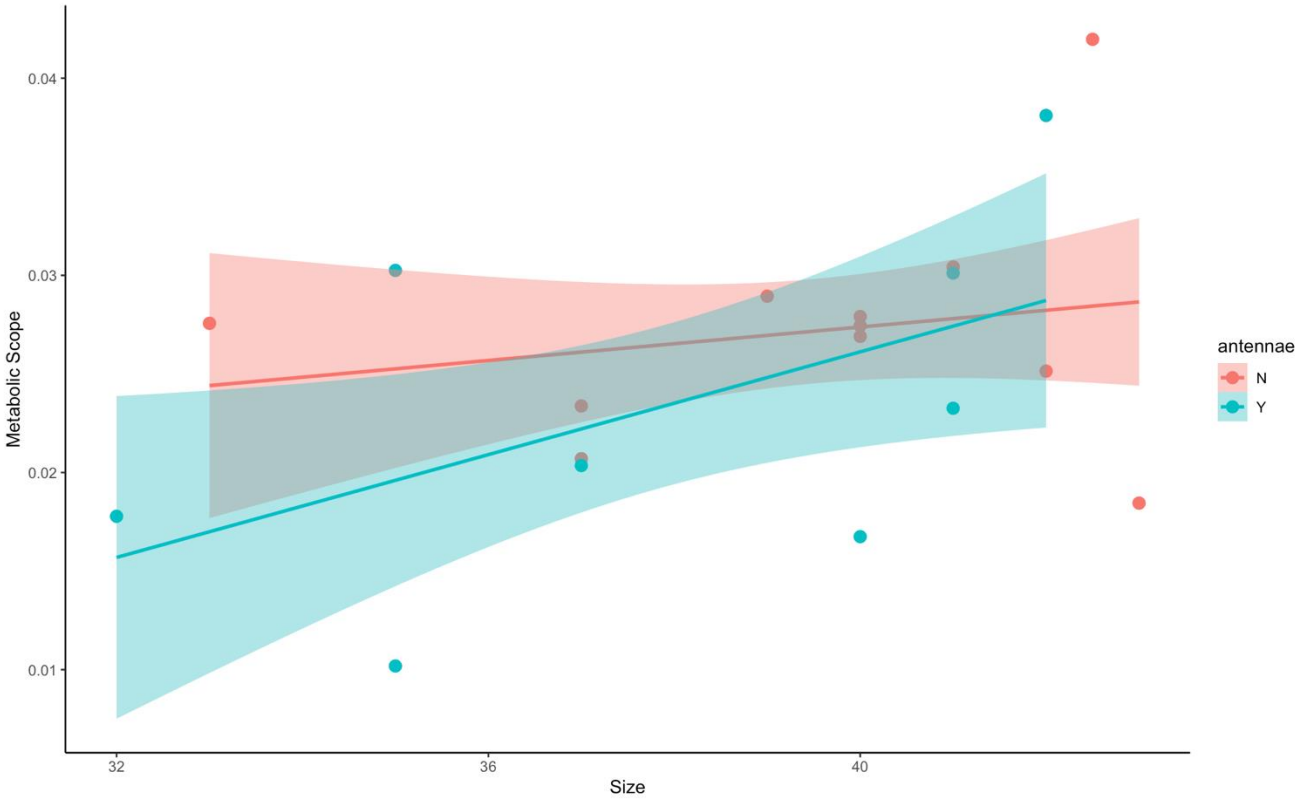


Figure 4: Metabolic scope is significantly affected only by body size in our dataset; however, we show the body size patterns for each level of our antennae treatment as well here for completeness.

Conclusion:

Despite the large literature on sexual selection and sexual conflict, the proximate mechanisms underlying important phenomena such as secondary sexual signal production and sexual coercion remain understudied. As a result, we lack a proper understanding of *how* organisms are able to conduct these crucial fitness-determining tasks, as well as the consequences of expressing these mechanisms for the integrated organismal phenotype. The experiments described in this dissertation therefore represent an important step forward in our understanding of the functional ecology of both sexual selection and intralocus sexual conflict, and also add to our growing body of knowledge regarding the behavioral and potential fitness effects of biogenic amines such as octopamine.

I investigated effects of both direct and indirect manipulation of the key invertebrate biogenic amine associated with aggression and locomotion octopamine, on mating behavior, call structure, and bite force. My data clearly show that animal functional capacities are relevant to sexual conflict, just as they are to sexual selection, and suggest that selection might act on such capacities in both males and females of cricket species. Because crickets use muscular based movements to produce their calls through stridulation I was able to explore the effects dampening octopamine would have due to the locomotor function association. Through blocking octopamine receptors with a synthetic antagonist, I was able to show that reducing active octopamine changes courtship call structure in male *A. domesticus* crickets based on the dominant frequency expressed throughout the duration of their calls. I used the same manipulation to demonstrate that epinastine eliminated the motivation of males to mate. This suggests that octopamine is necessary for one or more stages of the courtship interaction in this species, and points to some intriguing interspecific variation in the role of neuropharmacology in insect courtship behavior. Furthermore, these data also show a key role for bite force in both effecting and resisting male coercion during reproductive interactions in this same species. Few studies thus far

have tested explicitly for any effects of functional capacities in interlocus sexual conflict, and this experiment represents an important reference point for future studies of this phenomenon.

The development, maintenance, and use of physical and neuromuscular mechanisms are energetically costly and frequently drive trade-offs with other life history traits. The results of my *T. commodus* cricket antennaectomy and calling effort experiment revealed antennae removal increases calling effort and decreases lifespan, but does not do so by increasing energetic expenditure. Instead, the results indicate that the efficacy of antennae removal to affect calling effort depends on the metabolic status of the organism in question. This finding suggests that the mechanisms driving important life history trade-offs are more nuanced than is often appreciated, and also implicates octopamine indirectly in the many compromises in trait expression that collectively shape the multivariate organismal phenotype.

Appendix:

Octopamine mediates mating interactions in the house cricket (Acheta domesticus)

```
library(dplyr)
library(tidyverse)
library(fields)
library(plotly)
library(broom)

crk_mate <- read.csv("~/Desktop/Cricket experiments/cricketdatathis.csv")
glimpse(crk_mate)
attach(crk_mate)
```

Generalized Linear models

For our collected variables we run a series of generalized linear models to visualize results

```
model_init <- glm(init ~ massdiff + bitediff + glanddiff, data = crk_mate)

model_latency2 <- glm(latency2 ~ bitediff * glanddiff, data = crk_mate)
summary(model_latency2)
```

```
##
## Call:
## glm(formula = latency2 ~ bitediff * glanddiff, data = crk_mate)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -4079.9  -1091.2      1.1    917.8   5265.6
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         9942.19     298.25  33.336  < 2e-16 ***
## bitediff             809.40    2282.90   0.355  0.72391
## glanddiff             86.78     175.50   0.494  0.62242
## bitediff:glanddiff -4475.89    1505.50  -2.973  0.00395 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 3221266)
##
##     Null deviance: 292161034  on 79  degrees of freedom
## Residual deviance: 244816180  on 76  degrees of freedom
##   (79 observations deleted due to missingness)
## AIC: 1431.7
##
## Number of Fisher Scoring iterations: 2
```

```
model_matetime2 <-glm(matetime2 ~ massdiff + bitediff * glanddiff, data = crk_mate)
summary(model_matetime2)
```

```
##
## Call:
## glm(formula = matetime2 ~ massdiff + bitediff * glanddiff, data = crk_mate)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -167.88   -29.40   -11.58    49.06   144.67
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)          28.74      30.50   0.942  0.35439
## massdiff           -732.29     210.75  -3.475  0.00174 **
## bitediff            136.48     173.34   0.787  0.43794
## glanddiff           -16.98      12.86  -1.320  0.19794
## bitediff:glanddiff   23.20     113.76   0.204  0.83991
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 6152.159)
##
##     Null deviance: 270560  on 31  degrees of freedom
## Residual deviance: 166108  on 27  degrees of freedom
##   (127 observations deleted due to missingness)
## AIC: 376.56
##
## Number of Fisher Scoring iterations: 2
```

Logistic Regression Models

Confidence intervals of bite force difference on accessory gland difference on cricket mating outcomes.

```
logit_mated <- glm(mated ~ bitediff + glanddiff, data = crk_mate, family = binomial(link = "logit"))
summary(logit_mated)
```

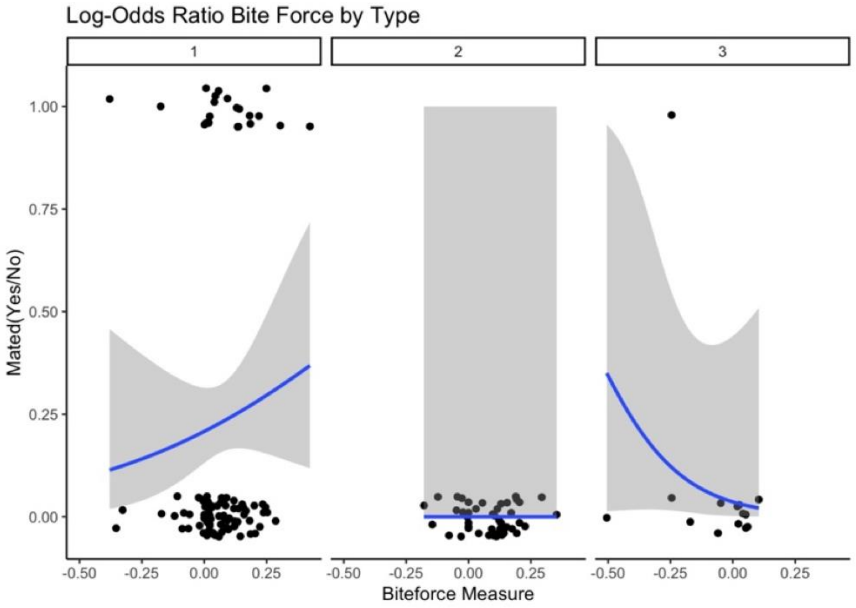
```
##
## Call:
## glm(formula = mated ~ bitediff + glanddiff, family = binomial(link = "logit"),
##     data = crk_mate)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.0449  -0.6531  -0.4980  -0.3666   2.4974
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -2.3047     0.4752   -4.85 1.24e-06 ***
## bitediff      5.5047     2.7384    2.01   0.0444 *
## glanddiff    -0.4474     0.1998   -2.24   0.0251 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 87.646  on 90  degrees of freedom
## Residual deviance: 79.815  on 88  degrees of freedom
##   (68 observations deleted due to missingness)
## AIC: 85.815
##
## Number of Fisher Scoring iterations: 4
```

```
tidy(logit_mated, exponentiate = TRUE, conf.int = TRUE)
```

```
## # A tibble: 3 x 7
##   term        estimate std.error statistic    p.value conf.low conf.high
##   <chr>          <dbl>     <dbl>     <dbl>      <dbl>    <dbl>     <dbl>
## 1 (Intercept)   0.0998     0.475     -4.85 0.00000124   0.0353     0.233
## 2 bitediff    246.         2.74       2.01 0.0444       1.32   73710.
## 3 glanddiff     0.639      0.200     -2.24 0.0251       0.423      0.937
```

```
plotsubsets <- ggplot(data = crk_mate, aes(y = mated, x = bitediff, group = type)) +
  geom_jitter(width = 0, height = 0.05) +
  xlab("Biteforce Measure") +
  ylab("Mated(Yes/No)") +
  ggtitle("Log-Odds Ratio Bite Force by Type")

plotsubsets + geom_smooth(method = 'glm',
        method.args = list(binomial(link = 'logit')),
        se = TRUE) + facet_wrap(~type) + theme_classic()
```

```
## `geom_smooth()` using formula 'y ~ x'
```

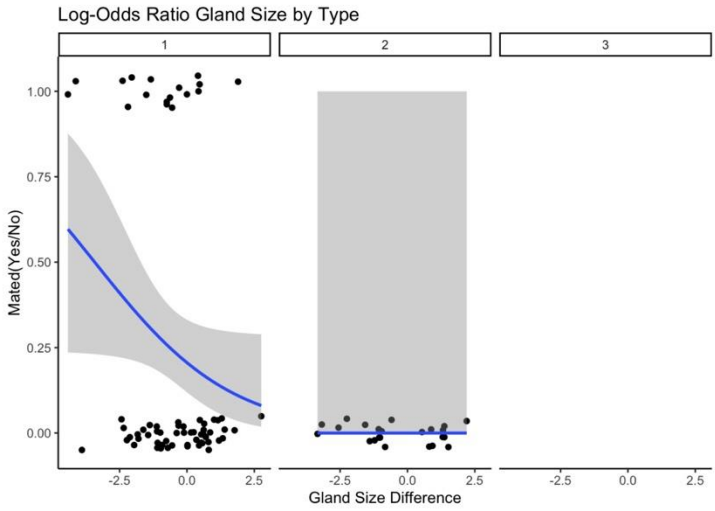



```
plotsubsets0 <- ggplot(data = crk_mate, aes(y = mated, x = glanddiff, group = type)) +
  geom_jitter(width = 0, height = 0.05) +
  xlab("Gland Size Difference") +
  ylab("Mated(Yes/No)") +
  ggtitle("Log-Odds Ratio Gland Size by Type")

plotsubsets0 + geom_smooth(method = 'glm',
    method.args = list(binomial(link = 'logit')),
    se = TRUE) + facet_wrap(~type) + theme_classic()
```

```
## `geom_smooth()` using formula 'y ~ x'
```

```
## Warning: Removed 68 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 68 rows containing missing values (geom_point).
```



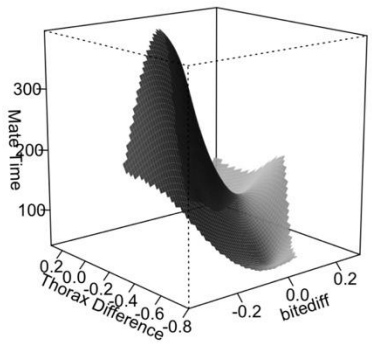
Surface renderings

```
##fix them use the scale parameter
trials.fitness <- Tps(cbind(bitediff,thoraxdiff),matetime)
summary(trials.fitness)
```

```
## CALL:
## Tps(x = cbind(bitediff, thoraxdiff), Y = matetime)
##
##  Number of Observations:               25
##  Number of unique points:              25
##  Number of parameters in the null space 3
##  Parameters for fixed spatial drift    3
##  Effective degrees of freedom:         11
##  Residual degrees of freedom:          14
##  MLE tau                               80.02
##  GCV tau                               88.44
##  MLE sigma                             3491000
##  Scale passed for covariance (sigma)   <NA>
##  Scale passed for nugget (tau^2)       <NA>
##  Smoothing parameter lambda            0.001834
##
## Residual Summary:
##     min   1st Q  median   3rd Q     max
## -142.60  -51.69   11.29   33.95  211.30
##
## Covariance Model: Rad.cov
##   Names of non-default covariance arguments:
##        p
##
## DETAILS ON SMOOTHING PARAMETER:
##  Method used:   GCV    Cost:  1
##    lambda       trA       GCV   GCV.one GCV.model    tauHat
## 1.834e-03 1.100e+01 1.396e+04 1.396e+04        NA 8.844e+01
##
##  Summary of all estimates found for lambda
##              lambda    trA   GCV tauHat -lnLike Prof converge
## GCV        0.001834 10.996 13963  88.44        136.4        10
## GCV.model        NA     NA    NA     NA           NA        NA
## GCV.one    0.001834 10.996 13963  88.44           NA        10
## RMSE             NA     NA    NA     NA           NA        NA
## pure error       NA     NA    NA     NA           NA        NA
## REML       0.048355  4.289 15463 113.18        136.0         1
```

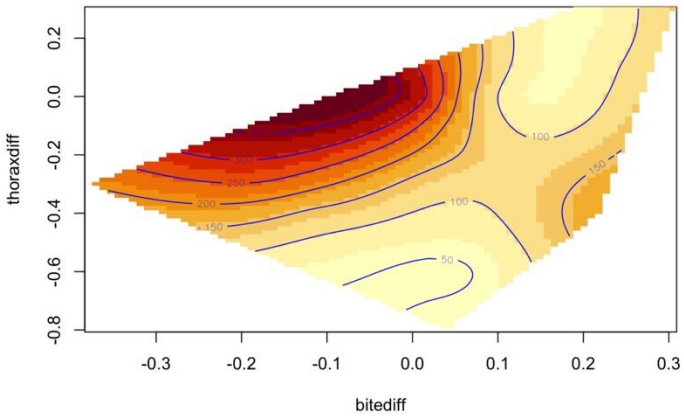
```
data.output <- predictSurface(trials.fitness)

persp(data.output,col="lightgrey",border=NA,shade=0.75,theta=320,phi=15,d=5,axes=T,ticktype="detailed",xlab="bite
diff",ylab="Thorax Difference", zlab="Mate Time", main="Mating time Differences based on biteforce and thorax wid
th")
```

Mating time Differences based on biteforce and thorax width




```
image(data.output, xlab="bitediff",ylab="thoraxdiff", main="Mating time Differences vs biteforce and thorax widt
h")
contour(data.output, col = "blue", add=T)
```

Mating time Differences vs biteforce and thorax width




```
trials.fitness <- Tps(cbind(bitediff,thoraxdiff),matetime)
summary(trials.fitness)
```

```
## CALL:
## Tps(x = cbind(bitediff, thoraxdiff), Y = matetime)
##
##  Number of Observations:                25
##  Number of unique points:               25
##  Number of parameters in the null space 3
##  Parameters for fixed spatial drift     3
##  Effective degrees of freedom:          11
##  Residual degrees of freedom:           14
##  MLE tau                                80.02
##  GCV tau                                88.44
##  MLE sigma                              3491000
##  Scale passed for covariance (sigma)    <NA>
##  Scale passed for nugget (tau^2)        <NA>
##  Smoothing parameter lambda             0.001834
##
## Residual Summary:
##     min   1st Q  median   3rd Q     max
## -142.60  -51.69   11.29   33.95  211.30
##
## Covariance Model: Rad.cov
##   Names of non-default covariance arguments:
##        p
##
## DETAILS ON SMOOTHING PARAMETER:
##  Method used:   GCV    Cost:  1
##    lambda       trA       GCV   GCV.one GCV.model    tauHat
## 1.834e-03 1.100e+01 1.396e+04 1.396e+04        NA 8.844e+01
##
##  Summary of all estimates found for lambda
##              lambda    trA   GCV tauHat -lnLike Prof converge
## GCV        0.001834 10.996 13963  88.44        136.4       10
## GCV.model        NA     NA    NA     NA           NA       NA
## GCV.one    0.001834 10.996 13963  88.44           NA       10
## RMSE             NA     NA    NA     NA           NA       NA
## pure error       NA     NA    NA     NA           NA       NA
## REML       0.048355  4.289 15463 113.18        136.0        1
```

```
##second pairing
trials.fitness11 <- Tps(cbind(bitediff,thoraxdiff),matetime2)
```

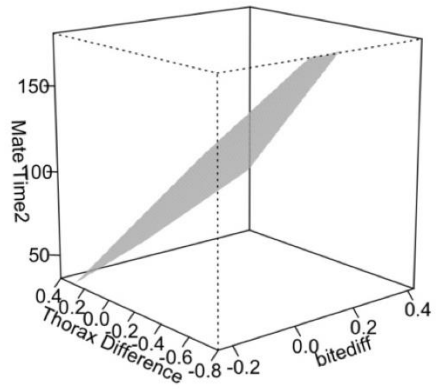
```
## Warning:
## Grid searches over lambda (nugget and sill variances) with  minima at the endpoints:
##   (GCV) Generalized Cross-Validation
##    minimum at  right endpoint  lambda  =  144.1948 (eff. df= 3.000991 )
```

```
summary(trials.fitness11)
```

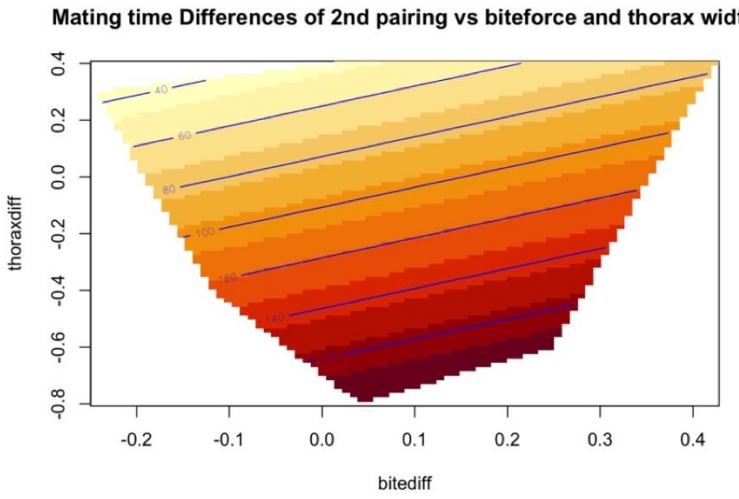
```
## CALL:
## Tps(x = cbind(bitediff, thoraxdiff), Y = matetime2)
##
##  Number of Observations:                37
##  Number of unique points:               36
##  Number of parameters in the null space 3
##  Parameters for fixed spatial drift     3
##  Effective degrees of freedom:          3
##  Residual degrees of freedom:           34
##  MLE tau                                80.18
##  GCV tau                                83.77
##  Pure error tau                         26.87
##  MLE sigma                              44.59
##  Scale passed for covariance (sigma)    <NA>
##  Scale passed for nugget (tau^2)        <NA>
##  Smoothing parameter lambda             144.2
##
## Residual Summary:
##      min    1st Q   median    3rd Q      max
## -132.000  -64.870    2.575   64.730  160.700
##
## Covariance Model: Rad.cov
##   Names of non-default covariance arguments:
##        p
##
## DETAILS ON SMOOTHING PARAMETER:
##  Method used:   GCV    Cost:  1
##    lambda       trA       GCV   GCV.one GCV.model    tauHat
##   144.195     3.001  8723.418  7637.218  8586.127    83.772
##
##  Summary of all estimates found for lambda
##                lambda    trA   GCV tauHat -lnLike Prof converge
## GCV        1.442e+02  3.001  8723  83.77        193.4       NA
## GCV.model  3.113e-06 34.200  5148  20.00           NA       NA
## GCV.one    5.312e-06 33.577  4954  21.41           NA       14
## RMSE              NA     NA    NA     NA           NA       NA
## pure error 1.310e-05 32.245 73293  26.87        206.7       NA
## REML       1.442e+02  3.001  8723  83.77        193.4       NA
```

```
data.output11 <- predictSurface(trials.fitness11)

persp(data.output11,col="lightgrey",border=NA,shade=0.75,theta=320,phi=15,d=5,axes=T,ticktype="detailed",xlab="bi
tediff",ylab="Thorax Difference", zlab="Mate Time2", main="Mating time Differences of 2nd pairing based on bitefo
rce and thorax width")
```

Mating time Differences of 2nd pairing based on biteforce and thorax wi



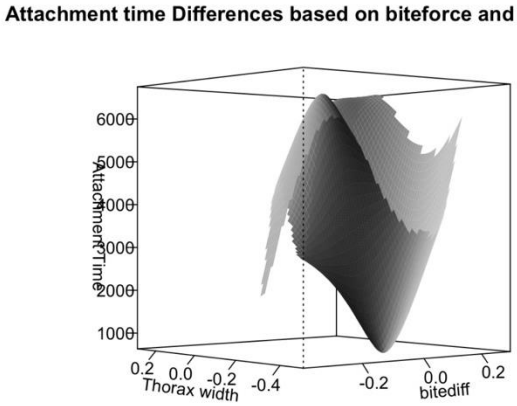
```
image(data.output11, xlab="bitediff",ylab="thoraxdiff", main="Mating time Differences of 2nd pairing vs biteforce
and thorax width")
contour(data.output11, col = "blue", add=T)
```



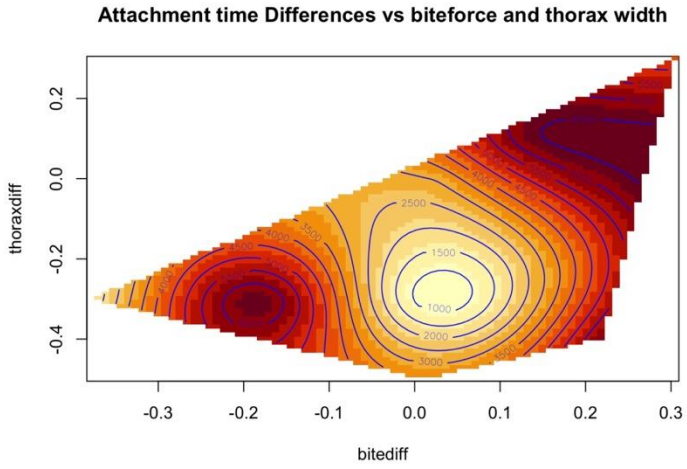

```
#first pairing
trials.fitness0 <- Tps(cbind(bitediff,thoraxdiff),attachtime)
summary(trials.fitness0)
```

```
## CALL:
## Tps(x = cbind(bitediff, thoraxdiff), Y = attachtime)
##
##  Number of Observations:               11
##  Number of unique points:              11
##  Number of parameters in the null space 3
##  Parameters for fixed spatial drift     3
##  Effective degrees of freedom:          8.4
##  Residual degrees of freedom:           2.6
##  MLE tau                                919.4
##  GCV tau                                1109
##  MLE sigma                              1.655e+09
##  Scale passed for covariance (sigma)    <NA>
##  Scale passed for nugget (tau^2)        <NA>
##  Smoothing parameter lambda             0.0005106
##
## Residual Summary:
##        min      1st Q     median      3rd Q        max
## -1126.0000  -185.7000    -0.9822   314.7000   753.4000
##
## Covariance Model: Rad.cov
##   Names of non-default covariance arguments:
##        p
##
## DETAILS ON SMOOTHING PARAMETER:
##  Method used:   GCV    Cost:  1
##    lambda       trA       GCV   GCV.one GCV.model    tauHat
## 5.106e-04 8.447e+00 5.295e+06 5.295e+06        NA 1.109e+03
##
##  Summary of all estimates found for lambda
##               lambda   trA     GCV tauHat -lnLike Prof converge
## GCV        5.106e-04 8.447 5295370   1109        74.38       19
## GCV.model         NA    NA      NA     NA           NA       NA
## GCV.one    5.106e-04 8.447 5295370   1109           NA       19
## RMSE              NA    NA      NA     NA           NA       NA
## pure error        NA    NA      NA     NA           NA       NA
## REML       4.856e+01 3.001 8778486   2527        74.03       NA
```

```
data.output0 <- predictSurface(trials.fitness0)

persp(data.output0,col="lightgrey",border=NA,shade=0.75,theta=320,phi=360,d=5,axes=T,ticktype="detailed",xlab="bi
tediff",ylab="Thorax width", zlab="Attachment Time", main="Attachment time Differences based on biteforce and tho
rax width")
```




```
image(data.output0, xlab="bitediff",ylab="thoraxdiff", main="Attachment time Differences vs biteforce and thorax
 width")
contour(data.output0,col = "blue", add=T)
```

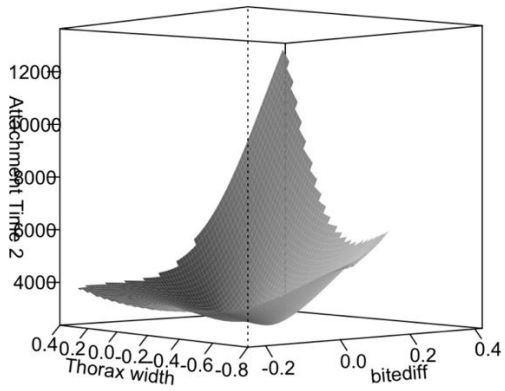



```
#2nd pairing
trials.fitness00 <- Tps(cbind(bitediff,thoraxdiff),attachtime2)
summary(trials.fitness00)
```

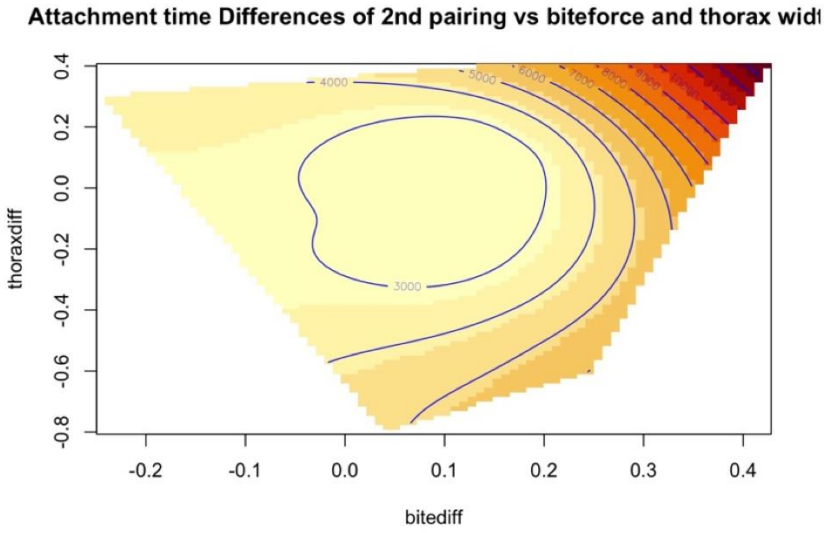
```
## CALL:
## Tps(x = cbind(bitediff, thoraxdiff), Y = attachtime2)
##
##  Number of Observations:                15
##  Number of unique points:               15
##  Number of parameters in the null space 3
##  Parameters for fixed spatial drift     3
##  Effective degrees of freedom:          6.5
##  Residual degrees of freedom:           8.5
##  MLE tau                                2084
##  GCV tau                                2254
##  MLE sigma                              717100000
##  Scale passed for covariance (sigma)    <NA>
##  Scale passed for nugget (tau^2)        <NA>
##  Smoothing parameter lambda             0.006059
##
## Residual Summary:
##     min   1st Q  median   3rd Q     max
## -2778.0 -1363.0   240.3  1279.0  2432.0
##
## Covariance Model: Rad.cov
##   Names of non-default covariance arguments:
##        p
##
## DETAILS ON SMOOTHING PARAMETER:
##  Method used:   GCV    Cost:  1
##    lambda       trA       GCV   GCV.one GCV.model    tauHat
## 6.059e-03 6.460e+00 8.925e+06 8.925e+06        NA 2.254e+03
##
##  Summary of all estimates found for lambda
##               lambda   trA     GCV tauHat -lnLike Prof converge
## GCV         0.006059 6.460 8925412   2254        112.9        17
## GCV.model         NA    NA      NA     NA           NA        NA
## GCV.one     0.006059 6.460 8925412   2254           NA        17
## RMSE              NA    NA      NA     NA           NA        NA
## pure error        NA    NA      NA     NA           NA        NA
## REML        0.004118 7.062 9010481   2184        112.8         2
```

```
data.output00 <- predictSurface(trials.fitness00)

persp(data.output00,col="lightgrey",border=NA,shade=0.75,theta=320,phi=360,d=5,axes=T,ticktype="detailed",xlab="b
itediff",ylab="Thorax width", zlab="Attachment Time 2", main="Attachment time Differences of 2nd pairing based on
biteforce and thorax width")
```

ttachment time Differences of 2nd pairing based on biteforce and thorax v



```
image(data.output00, xlab="bitediff",ylab="thoraxdiff", main="Attachment time Differences of 2nd pairing vs bitef
orce and thorax width")
contour(data.output00,col = "blue", add=T)
```

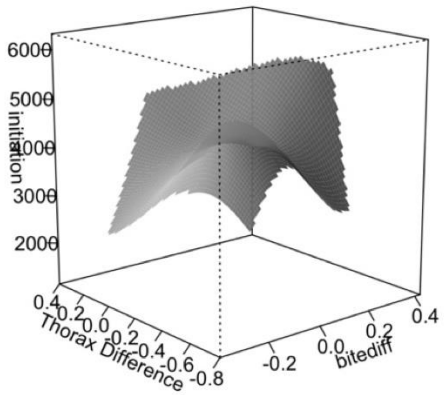



```
##initiation times compared with biteforce and thorax parameters
trials.fitnessa <- Tps(cbind(bitediff,thoraxdiff),init)
summary(trials.fitnessa)
```

```
## CALL:
## Tps(x = cbind(bitediff, thoraxdiff), Y = init)
##
##  Number of Observations:               27
##  Number of unique points:              27
##  Number of parameters in the null space 3
##  Parameters for fixed spatial drift    3
##  Effective degrees of freedom:         6.6
##  Residual degrees of freedom:          20.4
##  MLE tau                               2114
##  GCV tau                               2257
##  MLE sigma                             3.32e+08
##  Scale passed for covariance (sigma)   <NA>
##  Scale passed for nugget (tau^2)       <NA>
##  Smoothing parameter lambda            0.01347
##
## Residual Summary:
##     min   1st Q  median   3rd Q     max
## -4175.0 -1397.0   633.3  1524.0  2790.0
##
## Covariance Model: Rad.cov
##   Names of non-default covariance arguments:
##        p
##
## DETAILS ON SMOOTHING PARAMETER:
##  Method used:   GCV    Cost:  1
##    lambda       trA       GCV   GCV.one GCV.model    tauHat
## 1.347e-02 6.602e+00 6.742e+06 6.742e+06        NA 2.257e+03
##
##  Summary of all estimates found for lambda
##              lambda   trA     GCV tauHat -lnLike Prof converge
## GCV         0.01347 6.602 6741811   2257        221.7        17
## GCV.model        NA    NA      NA     NA           NA        NA
## GCV.one     0.01347 6.602 6741811   2257           NA        17
## RMSE             NA    NA      NA     NA           NA        NA
## pure error       NA    NA      NA     NA           NA        NA
## REML        0.02025 5.831 6761418   2302        221.7         3
```

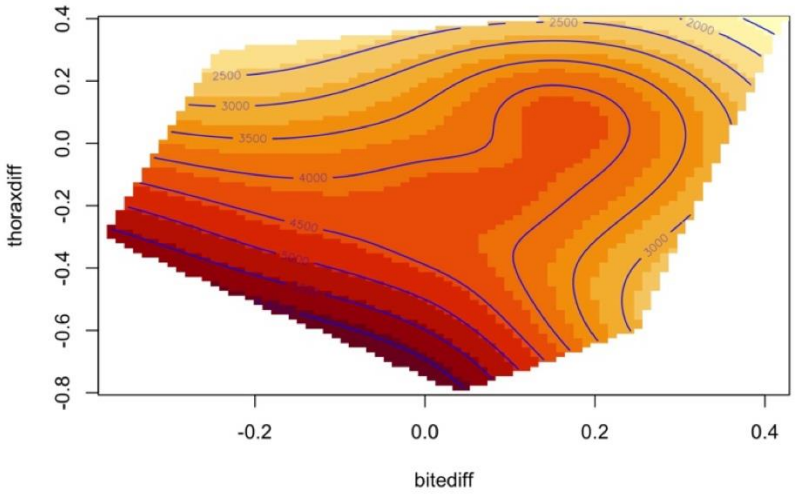
```
data.outputa <- predictSurface(trials.fitnessa)

persp(data.outputa,col="lightgrey",border=NA,shade=0.75,theta=320,phi=15,d=5,axes=T,ticktype="detailed",xlab="bit
ediff",ylab="Thorax Difference", zlab="initiation", main="Initiation time of mating based on biteforce and thorax
width")
```

Initiation time of mating based on biteforce and thorax width




```
image(data.outputa, xlab="bitediff",ylab="thoraxdiff", main="Initiation time of mating vs biteforce and thorax wi
dth")
contour(data.outputa, col = "blue", add=T)
```

Initiation time of mating vs biteforce and thorax width



Extracting Peak Frequencies From Calls

```
{r setup, include=FALSE} knitr::opts_chunk$set(echo = TRUE)
```

Cricket Frequency Data

I obtained the mean relative amplitude of the frequency distribution from each male cricket courtship call.

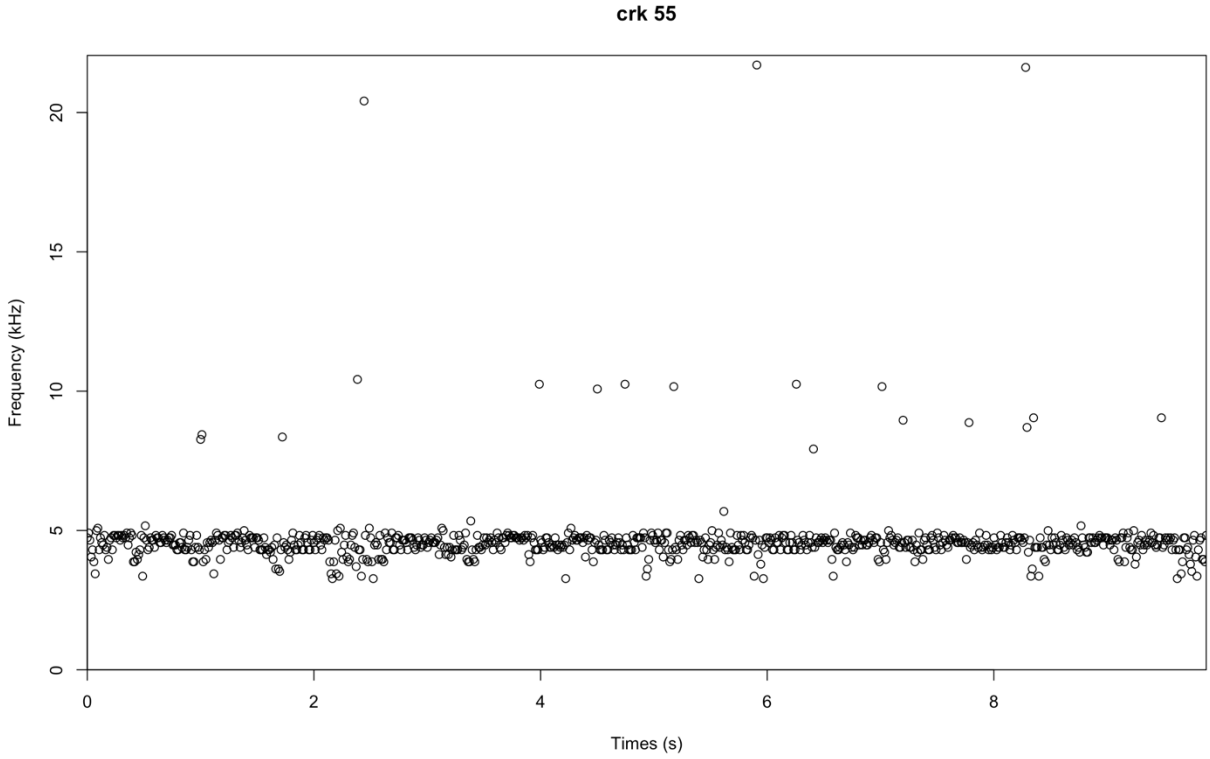
```
library(seewave)
setwd("~/Desktop/Cricket experiments/bpfaudio")

cricket_calls <- read_csv("~/Desktop/Chapter 2/chirps_ticks0.csv")
syllable <- read.csv("~/Desktop/Chapter 2/chirps_ticks_called.csv")
call_verification <- read.csv("cricketcallveri1.csv")
```

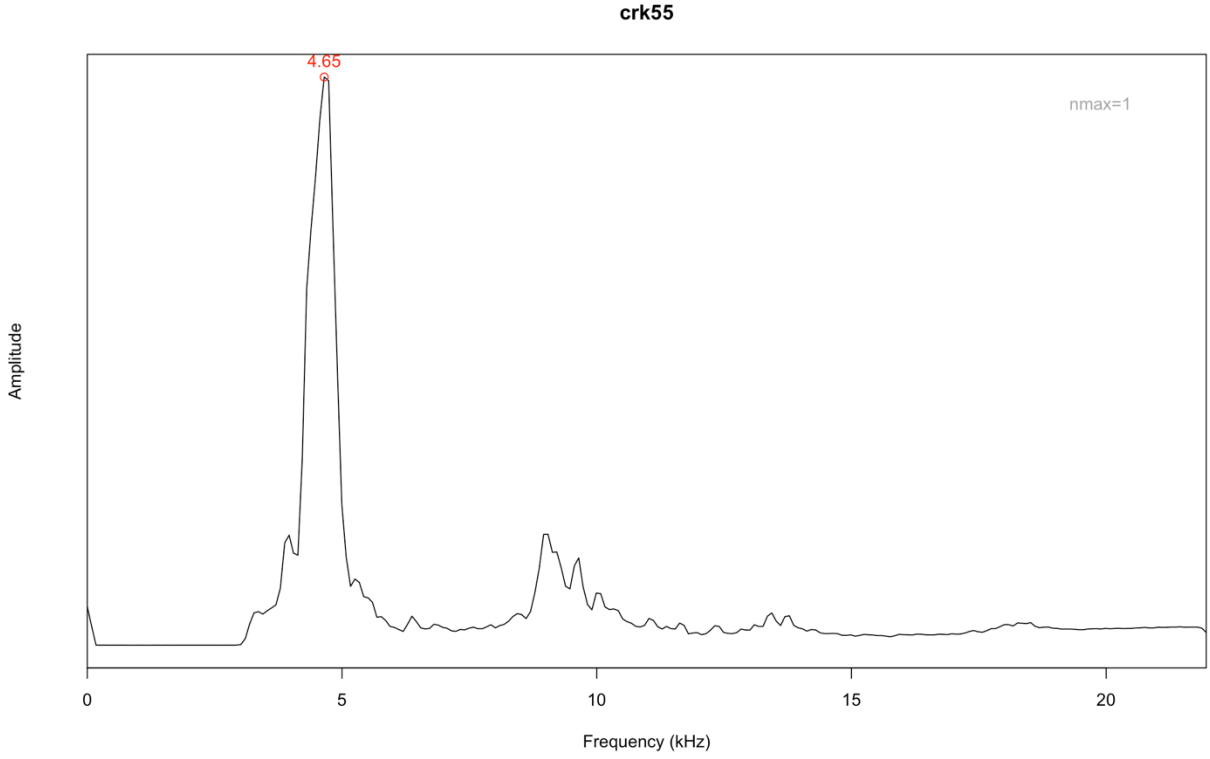
Dataset

Cricket Calls

The `cricket_calls` dataset provides a data of the dominant frequency of calls produced by male crickets during courtship calls. I render the frequency vs amplitude graphs and find the peak frequency of the audio file. Since our audio is prefiltered our lower frequency limit is where we we filtered our audio at 3200Hz our frequency value starts at 3200 Hz.

```
readWave("filtercrk55cut.wav")
C<- readWave("filtercrk55cut.wav")
f <- 44100
temp_slice <- c(from = 0, to = 8)
dfreq(C,f,tlim =NULL, main = "crk 55")

temp_lim <- c(from = 3.2, to = 22)
meanspec(C,f, main = "crk55", flim = temp_lim)

CS<-meanspec(C,f,main = "crk55",flim = temp_lim)
CSdB<-meanspec(C,f, main = "crk55", dB = "C",flim = temp_lim)
CSdB1<-meanspec(C,f, main = "crk55", dB = "C")

fpeaks(CS, nmax = 1, main = "crk55", title = FALSE)
```


crk 55
Frequency (kHz)
0
5
10
15
20
0
2
4
6
8
Times (s)


crk55
4.65
nmax=1
Amplitude
0
5
10
15
20
Frequency (kHz)

Octopamine affects courtship call structure in male Acheta domesticus crickets

Cricket Call Data

Invertebrates exhibit a variety of visual and auditory signals and displays that function within the contexts of **female choice**. Male cricket courtship calls are examined in this dataset where males of different trials had octopamine levels altered.

Dataset

Cricket Calls

The `cricket_calls` dataset provides a data of the dominant frequency of calls produced by male crickets during courtship calls.

```
glimpse(cricket_calls)
```

```
## Rows: 53
## Columns: 15
## $ `Sample name:`   <chr> "1crk54cut", "1crk61cut", "1crk65cut", "1crk72cut", …
## $ Type             <chr> "control", "control", "control", "control", "control…
## $ Type0            <dbl> 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0…
## $ Range            <chr> "Low", "Low", "Low", "Low", "High", "Low", "Low", "L…
## $ Range0           <dbl> 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0…
## $ `Length (s)`     <dbl> 8.173, 7.985, 12.931, 7.962, 8.026, 8.026, 6.986, 8.…
## $ chirps           <dbl> 11, 0, 2, 2, 7, 7, 10, 6, 9, 0, 0, 0, 0, 1, 10, 10, …
## $ ticks            <dbl> 0, 9, 0, 9, 7, 7, 0, 0, 0, 7, 6, 10, 8, 0, 0, 0, 0, …
## $ avg.syllables    <dbl> 2.000, 2.110, 2.500, 1.360, 4.790, 4.790, 3.000, 3.6…
## $ avg.chirp.period <dbl> 0.0960, 0.0000, 0.1368, 0.0976, 0.0455, 0.0455, 0.15…
## $ max.freq.den     <dbl> 4393, 4820, 4264, 4587, 11822, 4694, 4737, 4737, 445…
## $ CP1              <dbl> 0.0670, 0.0000, 0.1971, 0.1376, 0.2886, 0.2886, 0.10…
## $ CPL              <dbl> 0.2043, 0.0000, 0.1424, 0.0567, 0.2070, 0.2070, 0.15…
## $ biteforce        <dbl> 0.0594, 0.0408, 0.1152, 0.1524, 0.2044, 0.2044, 0.08…
## $ size             <dbl> 4.9, 4.7, 4.9, 5.0, 5.0, 5.0, 4.8, 5.0, 4.9, 4.4, 4.…
```

The `syllable` dataset contains data of treatment types affect on the number of syllables each cricket produces

```
glimpse(syllable)
```

```
## Rows: 53
## Columns: 16
## $ Sample.name.     <chr> "1crk54cut", "1crk61cut", "1crk65cut", "1crk72cut", …
## $ Type             <chr> "control", "control", "control", "control", "control…
## $ Type0            <int> 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0…
## $ Range            <chr> "Low", "Low", "Low", "Low", "High", "Low", "Low", "L…
## $ Range0           <int> 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0…
## $ Length..s.       <dbl> 8.173, 7.985, 12.931, 7.962, 8.026, 8.026, 6.986, 8.…
## $ chirps           <int> 11, 0, 2, 2, 7, 7, 10, 6, 9, 0, 0, 0, 0, 1, 10, 10, …
## $ ticks            <int> 0, 9, 0, 9, 7, 7, 0, 0, 0, 7, 6, 10, 8, 0, 0, 0, 0, …
## $ avg.syllables    <dbl> 2.000, 2.110, 2.500, 1.360, 4.790, 4.790, 3.000, 3.6…
## $ avg.chirp.period <dbl> 0.0960, 0.0000, 0.1368, 0.0976, 0.0455, 0.0455, 0.15…
## $ max.freq.den     <int> 4393, 4820, 4264, 4587, 11822, 4694, 4737, 4737, 445…
## $ CP1              <dbl> 0.0670, 0.0000, 0.1971, 0.1376, 0.2886, 0.2886, 0.10…
## $ CPL              <dbl> 0.2043, 0.0000, 0.1424, 0.0567, 0.2070, 0.2070, 0.15…
## $ biteforce        <dbl> 0.0594, 0.0408, 0.1152, 0.1524, 0.2044, 0.2044, 0.08…
## $ size             <dbl> 4.9, 4.7, 4.9, 5.0, 5.0, 5.0, 4.8, 5.0, 4.9, 4.4, 4.…
## $ called           <int> 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0…
```

The `call_verification` dataset contains call data on male cricket sound production. Male crickets either produced a call or dis not produce a call.

```
glimpse(call_verification)
```

```
## Rows: 92
## Columns: 6
## $ X.          <int> 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68…
## $ generation <chr> "1a", "1a", "1a", "1a", "1a", "1a", "1a", "1a", "1a", "1a"…
## $ type       <chr> "control", "control", "control", "control", "control", "co…
## $ mmass      <dbl> 0.3142, 0.3369, 0.2862, 0.2948, 0.3339, 0.3258, 0.2836, 0.…
## $ mthorax    <dbl> 4.9, 5.2, 4.7, 4.8, 5.1, 5.0, 4.9, 4.7, 4.4, 4.5, 5.1, 4.9…
## $ called     <int> 1, 0, 0, 1, 0, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 0…
```

Effect of Treatment on Sound Production

The `cricket_calls` dataset has been processed through long format logistic regression. Results of logistic regression analysis examining the effect of treatment type on male-produced frequencies. This table represents the minimum adequate model which retained only an effect of treatment and did not retain either an effect of cricket size or an interaction between size and treatment.

```
library(tidyverse)
```

```
## ── Attaching packages ─────────────────────────────────────── tidyverse 1.3.0 ──
```

```
## ✓ ggplot2 3.3.5     ✓ purrr   0.3.4
## ✓ tibble  3.0.6     ✓ stringr 1.4.0
## ✓ tidyr   1.1.2     ✓ forcats 0.5.1
```

```
## ── Conflicts ────────────────────────────────────────── tidyverse_conflicts() ──
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(MASS)
```

```
##
## Attaching package: 'MASS'
```

```
## The following object is masked from 'package:dplyr':
##
##     select
```

```
library(broom)
Range_logit_U <- glm(Range0 ~ Type, data = cricket_calls, family = binomial(link = "logit"))
summary(Range_logit_U)
```

```
##
## Call:
## glm(formula = Range0 ~ Type, family = binomial(link = "logit"),
##     data = cricket_calls)
##
## Deviance Residuals:
##    Min      1Q  Median      3Q     Max
## -1.256  -0.385  -0.385  -0.385   2.297
##
## Coefficients:
##                Estimate Std. Error z value Pr(>|z|)
## (Intercept)     -2.5649     0.5991  -4.281 1.86e-05 ***
## Typeepinastine   2.7473     0.8518   3.225  0.00126 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 48.292  on 52  degrees of freedom
## Residual deviance: 36.773  on 51  degrees of freedom
## AIC: 40.773
##
## Number of Fisher Scoring iterations: 5
```

```
tidy(Range_logit_U, exponentiate = TRUE, conf.int = TRUE)
```

```
## # A tibble: 2 x 7
##   term           estimate std.error statistic   p.value conf.low conf.high
##   <chr>             <dbl>     <dbl>     <dbl>     <dbl>    <dbl>     <dbl>
## 1 (Intercept)      0.0769     0.599     -4.28 0.0000186   0.0186     0.212
## 2 Typeepinastine  15.6        0.852      3.23 0.00126     3.16      96.1
```

ANOVA of the average number of syllables produced by each cricket throughout their produced number of calls using the `syllable` dataset.

```
syllable.anova <- aov(syllable$avg.syllables ~ syllable$Type0)
summary(syllable.anova)
```

```
##                Df Sum Sq Mean Sq F value  Pr(>F)
## syllable$Type0  1   7.50   7.505    7.34 0.00916 **
## Residuals      51  52.15   1.022
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Results of logistic regression analysis testing the propensity of epinastine-treated males to produce courtship calls compared with untreated controls using the `call_verification` dataset. The model is the minimum adequate model which retained only an effect of treatment, and did not retain either an effect of cricket size or an interaction between size and treatment.

```
glimpse(call_verification)
```

```
## Rows: 92
## Columns: 6
## $ X.         <int> 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68…
## $ generation <chr> "1a", "1a", "1a", "1a", "1a", "1a", "1a", "1a", "1a", "1a"…
## $ type       <chr> "control", "control", "control", "control", "control", "co…
## $ mmass      <dbl> 0.3142, 0.3369, 0.2862, 0.2948, 0.3339, 0.3258, 0.2836, 0.…
## $ mthorax    <dbl> 4.9, 5.2, 4.7, 4.8, 5.1, 5.0, 4.9, 4.7, 4.4, 4.5, 5.1, 4.9…
## $ called     <int> 1, 0, 0, 1, 0, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 0…
```

```
Range_logit_CALLED1 <- glm(called ~ type, data = call_verification, family = binomial(link = "logit"))
summary(Range_logit_CALLED1)
```

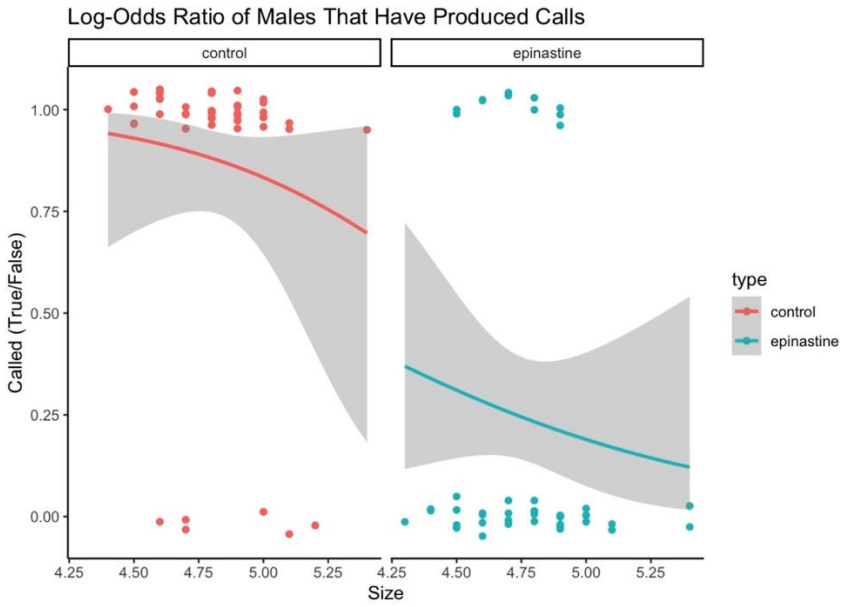
```
##
## Call:
## glm(formula = called ~ type, family = binomial(link = "logit"),
##     data = call_verification)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -2.0290  -0.7487   0.5226   0.5226   1.6785
##
## Coefficients:
##                Estimate Std. Error z value Pr(>|z|)
## (Intercept)      1.9218     0.4371   4.397 1.10e-05 ***
## typeepinastine  -3.0503     0.5580  -5.466 4.59e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 125.969  on 91  degrees of freedom
## Residual deviance:  85.953  on 90  degrees of freedom
## AIC: 89.953
##
## Number of Fisher Scoring iterations: 4
```

```
tidy(Range_logit_CALLED1, exponentiate = TRUE, conf.int = TRUE)
```

```
## # A tibble: 2 x 7
##   term           estimate std.error statistic      p.value conf.low conf.high
##   <chr>             <dbl>     <dbl>     <dbl>        <dbl>    <dbl>     <dbl>
## 1 (Intercept)      6.83       0.437      4.40 0.0000110      3.13      17.9
## 2 typeepinastine   0.0473     0.558     -5.47 0.0000000459   0.0146     0.133
```

```
plotsubsetsVERI <- ggplot(data = call_verification, aes(y = called, x = mthorax, group = type, color = type)) +
  geom_jitter(width = 0, height = 0.05) +
  xlab("Size") +
  ylab("Called (True/False)") +
  ggtitle("Log-Odds Ratio of Males That Have Produced Calls")

plotsubsetsVERI + geom_smooth(method = 'glm',
                              method.args = list(binomial(link = 'logit')),
                              se = TRUE) + facet_wrap(~type) + theme_classic()
```

```
## `geom_smooth()` using formula 'y ~ x'
```

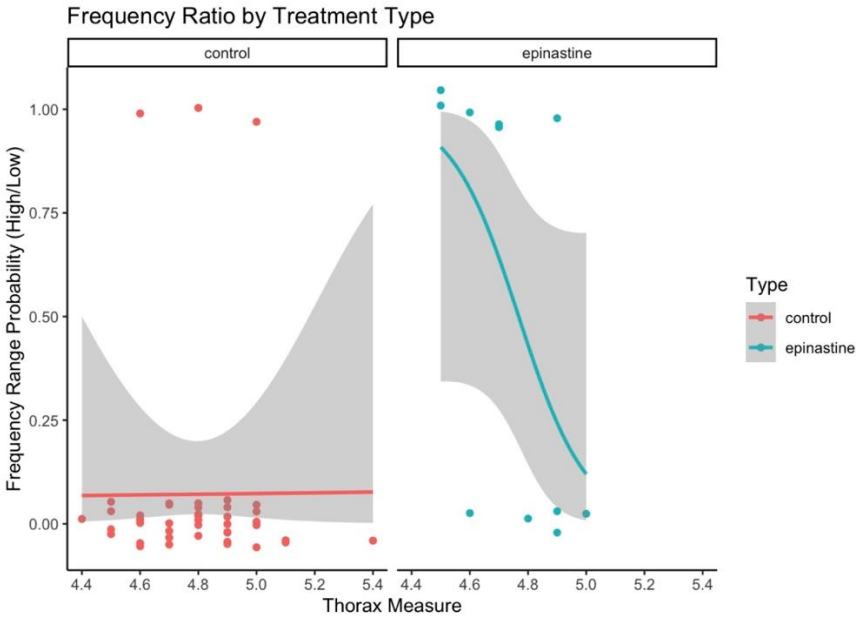


Treatment Effect Summary

The `cricket_calls` dataset can be visualized using a log-odds plot of frequencies determined by high and low ranges of dominant peak frequencies of each cricket.

```
library(ggplot2)
library(tidyverse)
plotsubsets <- ggplot(data = cricket_calls, aes(y = Range0, x = size, group = Type, color = Type)) +
  geom_jitter(width = 0, height = 0.06) +
  xlab("Thorax Measure") +
  ylab("Frequency Range Probability (High/Low)") +
  ggtitle("Frequency Ratio by Treatment Type")

plotsubsets + geom_smooth(method = 'glm',
                              method.args = list(binomial(link = 'logit')),
                              se = TRUE) + facet_wrap(~Type) + theme_classic()
```

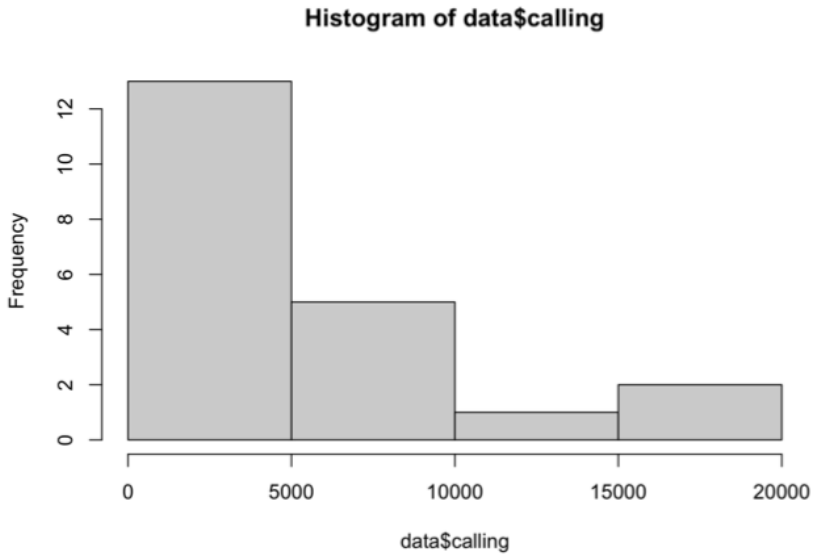
```
## `geom_smooth()` using formula 'y ~ x'
```


Frequency Ratio by Treatment Type
control
epinastine
Frequency Range Probability (High/Low)
1.00
0.75
0.50
0.25
0.00
4.4
4.6
4.8
5.0
5.2
5.4
Thorax Measure
Type
control
epinastine

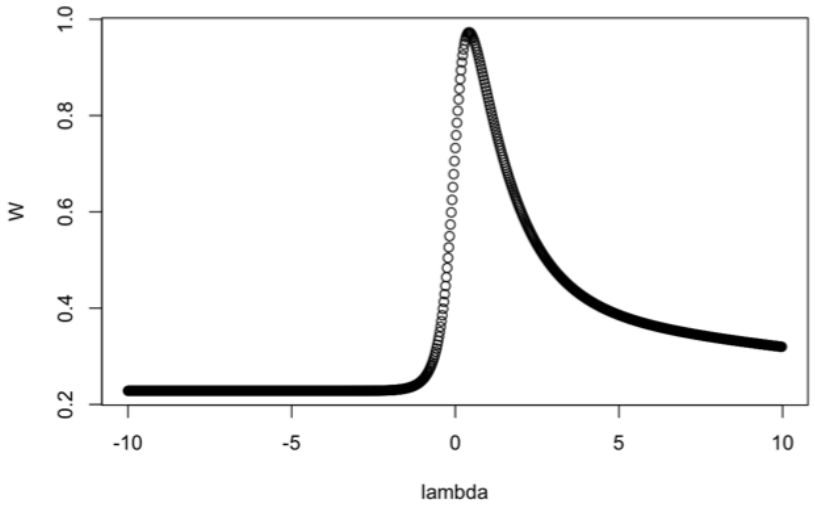
Teleogryllus commodus calling and metabolic rates

```
library(tidyverse)
library(ggplot2)
library(rcompanion)
library(nlme)
library(MASS)

setwd("~/Desktop/Cricket experiments/antennae")
data <- read.csv("antennae.csv",header=T)
```

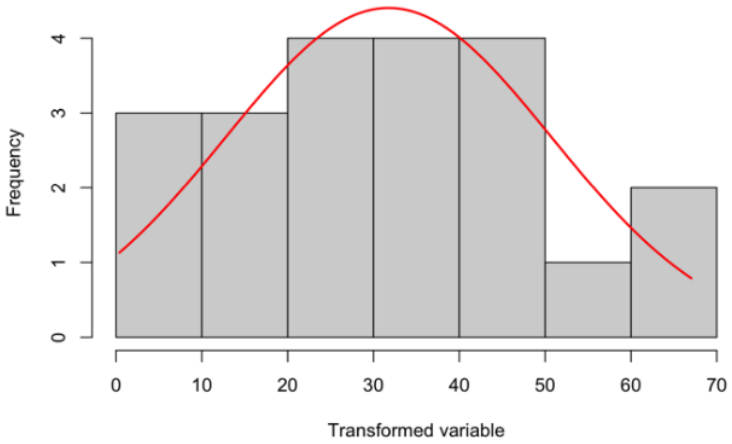
```
##Find a transformation that normalizes calling, since it's always skew
hist(data$calling)
```

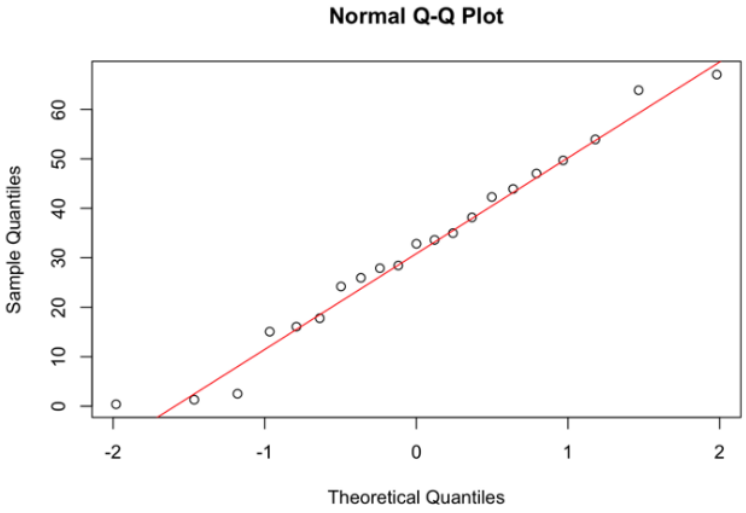



```
transformTukey(data$calling)
```



```
##
##     lambda     W Shapiro.p.value
## 418  0.425 0.973          0.7973
##
## if (lambda >  0){TRANS = x ^ lambda}
## if (lambda == 0){TRANS = log(x)}
## if (lambda <  0){TRANS = -1 * x ^ lambda}
```






```
##  [1] 67.0337331 47.0416496 49.6857326 15.0652632 17.7799712 63.8696066
##  [7] 42.2856123 32.8281827 53.9232384 43.9131200 34.9816406 24.1945900
## [13] 38.1548324 27.8890076 16.0701112  2.5173201  0.3930492 33.6067737
## [19] 28.4397793  1.3162892 25.9470544
```

```
data<- data %>%
  mutate(metabolicscope = active - resting)
data
```

```
##    cricket      calling antennae lifespan     resting     active   mass size
## 1      M10 1.982262e+04        N       53 0.001894901 0.02259331 0.5225 37.0
## 2      M13 8.614956e+03        N       45 0.001526776 0.02843250 0.6007 40.0
## 3      M14 9.797909e+03        N       33 0.001218776 0.02636008 0.6349 42.0
## 4      M15 5.911667e+02        N       17 0.002460852 0.03002754 0.3841 33.0
## 5      M17 8.730000e+02        N       26 0.001665709 0.02504060 0.4751 37.0
## 6      M19 1.769095e+04        N       42 0.001563298 0.02947388 0.6391 40.0
## 7       M2 6.704024e+03        N       41 0.003062060 0.03348793 0.5589 41.0
## 8      M21 3.695214e+03        N       28 0.000944125 0.02839424 0.6321 40.0
## 9       M3 1.187865e+04        N       34 0.000908878 0.04287946 0.7584 42.5
## 10      M5 7.327026e+03        N       38 0.001576230 0.03051989 0.5630 39.0
## 11      M7 4.291065e+03        N       46 0.000967298 0.01941519 0.7625 43.0
## 12      M1 1.802220e+03        Y       41 0.002227291 0.02000741 0.2968 32.0
## 13     M11 5.263723e+03        Y       47 0.002823378 0.01956982 0.5531 40.0
## 14     M12 2.517787e+03        Y       64 0.001368358 0.03947716 0.6339 42.0
## 15     M16 6.881633e+02        Y       50          NA          NA 0.5187 38.0
## 16     M18 8.777778e+00        Y       19 0.001032727 0.02138641 0.4844 37.0
## 17     M20 1.111111e-01        Y        9 0.001192309 0.03144332 0.3418 35.0
## 18      M4 3.904744e+03        Y       39 0.002122691 0.02538192 0.6436 41.0
## 19      M6 2.636349e+03        Y       48 0.001870665 0.01205615 0.3888 35.0
## 20      M8 1.909091e+00        Y       11          NA          NA 0.5761 41.0
## 21      M9 2.124545e+03        Y       41 0.001164425 0.03128197 0.6404 41.0
##    metabolicscope
## 1      0.02069841
## 2      0.02690573
## 3      0.02514131
## 4      0.02756669
## 5      0.02337489
## 6      0.02791058
## 7      0.03042587
## 8      0.02745011
## 9      0.04197058
## 10     0.02894366
## 11     0.01844790
## 12     0.01778012
## 13     0.01674644
## 14     0.03810880
## 15             NA
## 16     0.02035368
## 17     0.03025101
## 18     0.02325923
## 19     0.01018549
## 20             NA
## 21     0.03011754
```

```
##Transpose resting and active metabolic rates into a new variable "mr" with "mrtype" as
##factor; pass to new object "tidymr"
tidymr <- data %>%
  dplyr::select(cricket, calling, antennae, lifespan,resting,active,mass,size,metabolicscope)%>%
  gather("resting","active",key="mrtype",value="mr")
tidymr
```

```
##    cricket      calling antennae lifespan   mass size metabolicscope  mrtype
## 1      M10 1.982262e+04        N       53 0.5225 37.0     0.02069841 resting
## 2      M13 8.614956e+03        N       45 0.6007 40.0     0.02690573 resting
## 3      M14 9.797909e+03        N       33 0.6349 42.0     0.02514131 resting
## 4      M15 5.911667e+02        N       17 0.3841 33.0     0.02756669 resting
## 5      M17 8.730000e+02        N       26 0.4751 37.0     0.02337489 resting
## 6      M19 1.769095e+04        N       42 0.6391 40.0     0.02791058 resting
## 7       M2 6.704024e+03        N       41 0.5589 41.0     0.03042587 resting
## 8      M21 3.695214e+03        N       28 0.6321 40.0     0.02745011 resting
## 9       M3 1.187865e+04        N       34 0.7584 42.5     0.04197058 resting
## 10      M5 7.327026e+03        N       38 0.5630 39.0     0.02894366 resting
## 11      M7 4.291065e+03        N       46 0.7625 43.0     0.01844790 resting
## 12      M1 1.802220e+03        Y       41 0.2968 32.0     0.01778012 resting
## 13     M11 5.263723e+03        Y       47 0.5531 40.0     0.01674644 resting
## 14     M12 2.517787e+03        Y       64 0.6339 42.0     0.03810880 resting
## 15     M16 6.881633e+02        Y       50 0.5187 38.0             NA resting
## 16     M18 8.777778e+00        Y       19 0.4844 37.0     0.02035368 resting
## 17     M20 1.111111e-01        Y        9 0.3418 35.0     0.03025101 resting
## 18      M4 3.904744e+03        Y       39 0.6436 41.0     0.02325923 resting
## 19      M6 2.636349e+03        Y       48 0.3888 35.0     0.01018549 resting
## 20      M8 1.909091e+00        Y       11 0.5761 41.0             NA resting
## 21      M9 2.124545e+03        Y       41 0.6404 41.0     0.03011754 resting
## 22     M10 1.982262e+04        N       53 0.5225 37.0     0.02069841  active
## 23     M13 8.614956e+03        N       45 0.6007 40.0     0.02690573  active
## 24     M14 9.797909e+03        N       33 0.6349 42.0     0.02514131  active
## 25     M15 5.911667e+02        N       17 0.3841 33.0     0.02756669  active
## 26     M17 8.730000e+02        N       26 0.4751 37.0     0.02337489  active
## 27     M19 1.769095e+04        N       42 0.6391 40.0     0.02791058  active
## 28      M2 6.704024e+03        N       41 0.5589 41.0     0.03042587  active
## 29     M21 3.695214e+03        N       28 0.6321 40.0     0.02745011  active
## 30      M3 1.187865e+04        N       34 0.7584 42.5     0.04197058  active
## 31      M5 7.327026e+03        N       38 0.5630 39.0     0.02894366  active
## 32      M7 4.291065e+03        N       46 0.7625 43.0     0.01844790  active
## 33      M1 1.802220e+03        Y       41 0.2968 32.0     0.01778012  active
## 34     M11 5.263723e+03        Y       47 0.5531 40.0     0.01674644  active
## 35     M12 2.517787e+03        Y       64 0.6339 42.0     0.03810880  active
## 36     M16 6.881633e+02        Y       50 0.5187 38.0             NA  active
## 37     M18 8.777778e+00        Y       19 0.4844 37.0     0.02035368  active
## 38     M20 1.111111e-01        Y        9 0.3418 35.0     0.03025101  active
## 39      M4 3.904744e+03        Y       39 0.6436 41.0     0.02325923  active
## 40      M6 2.636349e+03        Y       48 0.3888 35.0     0.01018549  active
## 41      M8 1.909091e+00        Y       11 0.5761 41.0             NA  active
## 42      M9 2.124545e+03        Y       41 0.6404 41.0     0.03011754  active
##             mr
## 1  0.001894901
## 2  0.001526776
## 3  0.001218776
## 4  0.002460852
## 5  0.001665709
## 6  0.001563298
## 7  0.003062060
## 8  0.000944125
## 9  0.000908878
## 10 0.001576230
## 11 0.000967298
## 12 0.002227291
## 13 0.002823378
## 14 0.001368358
## 15          NA
## 16 0.001032727
## 17 0.001192309
## 18 0.002122691
## 19 0.001870665
## 20          NA
## 21 0.001164425
## 22 0.022593308
## 23 0.028432504
## 24 0.026360082
## 25 0.030027541
## 26 0.025040600
## 27 0.029473875
## 28 0.033487932
## 29 0.028394235
## 30 0.042879458
```

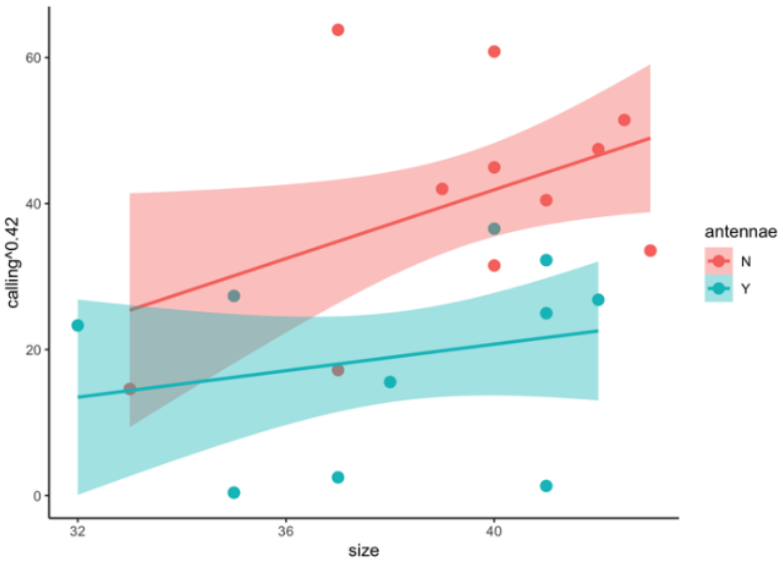
```
## 31 0.030519891
## 32 0.019415193
## 33 0.020007412
## 34 0.019569818
## 35 0.039477160
## 36          NA
## 37 0.021386411
## 38 0.031443316
## 39 0.025381916
## 40 0.012056152
## 41          NA
## 42 0.031281970
```

```
##Model calling effort and plot model with significant factors
callmodel <- lm(calling^0.42~size+antennae+mrtype+antennae:mrtype,data=tidymr)
summary(callmodel)
```

```
##
## Call:
## lm(formula = calling^0.42 ~ size + antennae + mrtype + antennae:mrtype,
##     data = tidymr)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -22.182 -12.652   1.755   8.752  27.014
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)             -2.129e+01  2.895e+01  -0.735  0.46676
## size                     1.570e+00  7.252e-01   2.164  0.03695 *
## antennaeY               -1.957e+01  6.201e+00  -3.156  0.00318 **
## mrtyperesting           -9.287e-15  5.981e+00   0.000  1.00000
## antennaeY:mrtyperesting  1.399e-14  8.668e+00   0.000  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 14.03 on 37 degrees of freedom
## Multiple R-squared:  0.444,  Adjusted R-squared:  0.3839
## F-statistic: 7.386 on 4 and 37 DF,  p-value: 0.0001773
```

```
g <- ggplot(tidymr,aes(x=size,y=calling^0.42,col=antennae))
g+geom_point(size=3)+geom_smooth(aes(fill=antennae),method="lm")+theme_classic()
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

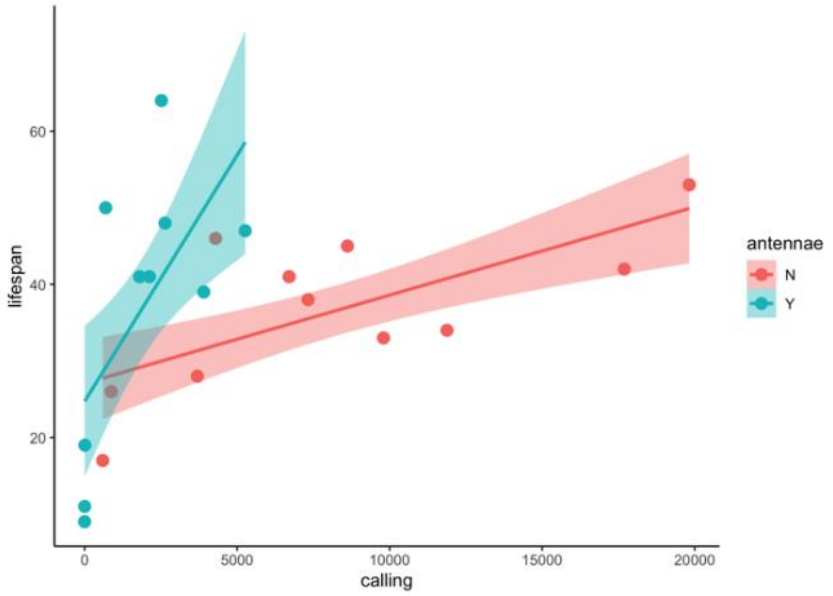


```
##Model lifespan and plot model with significant factors
##(include call effort because we already know that affects longevity)
lifemodel <- lm(lifespan~size+antennae+calling+antennae:calling,data=tidymr)
summary(lifemodel)
```

```
##
## Call:
## lm(formula = lifespan ~ size + antennae + calling + antennae:calling,
##     data = tidymr)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -15.945  -7.494  -1.094   7.732  21.118
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)        4.9383236 23.2077163   0.213  0.83266
## size               0.5730415  0.5916285   0.969  0.33904
## antennaeY         -1.4996556  5.5432873  -0.271  0.78825
## calling            0.0010921  0.0003989   2.738  0.00945 **
## antennaeY:calling  0.0050146  0.0015353   3.266  0.00235 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 11.03 on 37 degrees of freedom
## Multiple R-squared:  0.4388, Adjusted R-squared:  0.3782
## F-statistic: 7.233 on 4 and 37 DF,  p-value: 0.0002081
```

```
##Check out this interaction between antennae treatment and calling effort!
l <- ggplot(tidymr,aes(x=calling,y=lifespan,col=antennae))
l+geom_point(size=3)+geom_smooth(aes(fill=antennae),method="lm")+theme_classic()
```

```
## `geom_smooth()` using formula = 'y ~ x'
```




```
##Metabolic rate is repeated for each cricket (active vs resting). To analyze variation in MR,
##we run a mixed model on metabolic rate with "cricket" as a random factor and simplify
mixedmodel <- lme(mr~size+antennae+mrtype+antennae:mrtype,random=~1|cricket,method="ML",na.action=na.omit, data=t
idymr)
mixedmodel.stp <- stepAIC(mixedmodel,scope = list(upper~size+antennae+mrtype,lower = ~1),trace = T)
```

```
## Start:  AIC=-284.91
## mr ~ size + antennae + mrtype + antennae:mrtype
##
##                   Df     AIC
## - antennae:mrtype  1 -285.43
## <none>               -284.91
## - size             1 -284.67
##
## Step:  AIC=-285.43
## mr ~ size + antennae + mrtype
##
##            Df     AIC
## - antennae  1 -286.92
## <none>        -285.43
## - size      1 -285.28
## - mrtype    1 -208.43
##
## Step:  AIC=-286.92
## mr ~ size + mrtype
##
##          Df     AIC
## <none>      -286.92
## - size    1 -286.09
## - mrtype  1 -210.37
```

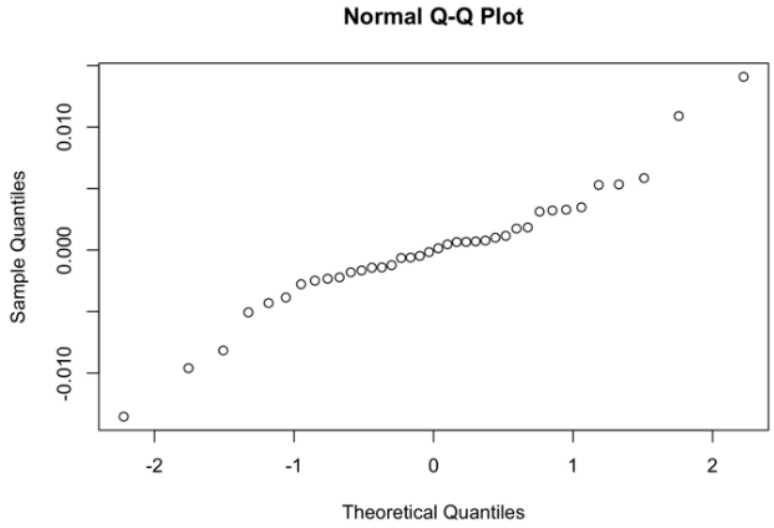
```
mixedmodel.stp$anova
```

```
## Stepwise Model Path
## Analysis of Deviance Table
##
## Initial Model:
## mr ~ size + antennae + mrtype + antennae:mrtype
##
## Final Model:
## mr ~ size + mrtype
##
##
##                Step Df  Deviance Resid. Df Resid. Dev       AIC
## 1                                       31  -298.9072 -284.9072
## 2 - antennae:mrtype  1 1.4730983        32  -297.4341 -285.4341
## 3        - antennae  1 0.5152133        33  -296.9189 -286.9189
```

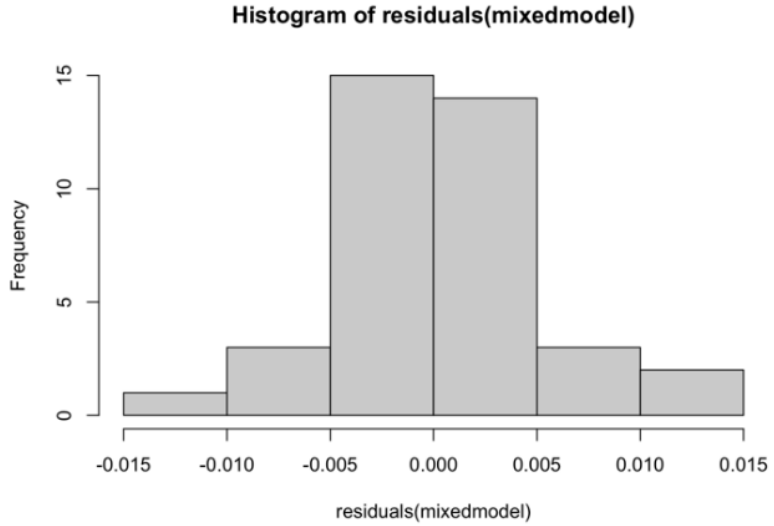
```
##Antennae drops out, only size and mrtype are retained; refit with REML
mixedmodel <- lme(mr~size+mrtype,random=~1|cricket,na.action=na.omit, data=tidymr)
anova.lme(mixedmodel,type="marginal",adjustSigma=F)
```

```
##             numDF denDF   F-value p-value
## (Intercept)     1    18   1.10057  0.3080
## size            1    17   2.70735  0.1182
## mrtype          1    18 241.56160  <.0001
```

```
##mr definitely isn't normal (also can't be easily normalized), so check model fit; it's pretty good
qqnorm(residuals(mixedmodel))
```

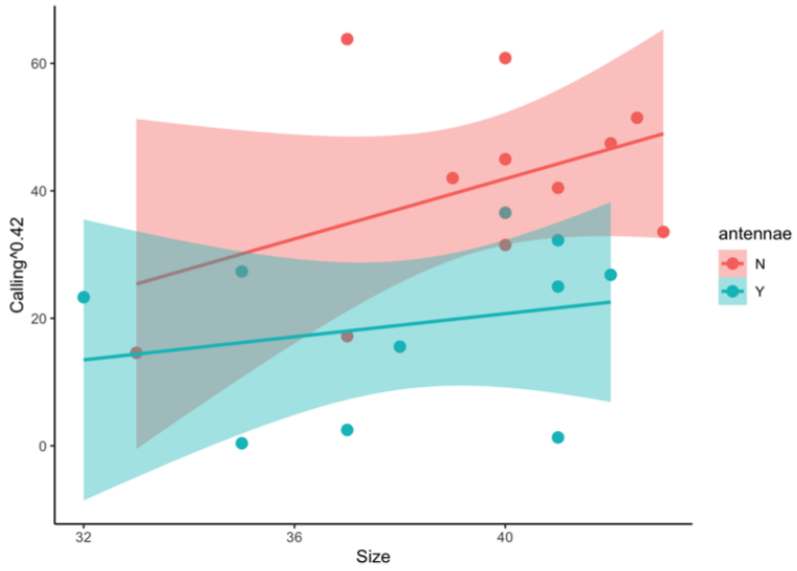



```
hist(residuals(mixedmodel))
```




```
##Plot power transformed calling as a function of size between treatments
s<- ggplot(tidymr,aes(x=size,y=calling^0.42,col=antennae))
s1 <-s+geom_point(size=3)+geom_smooth(aes(fill=antennae),method="lm")+theme_classic()
s1 + xlab("Size") + ylab("Calling^0.42")
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

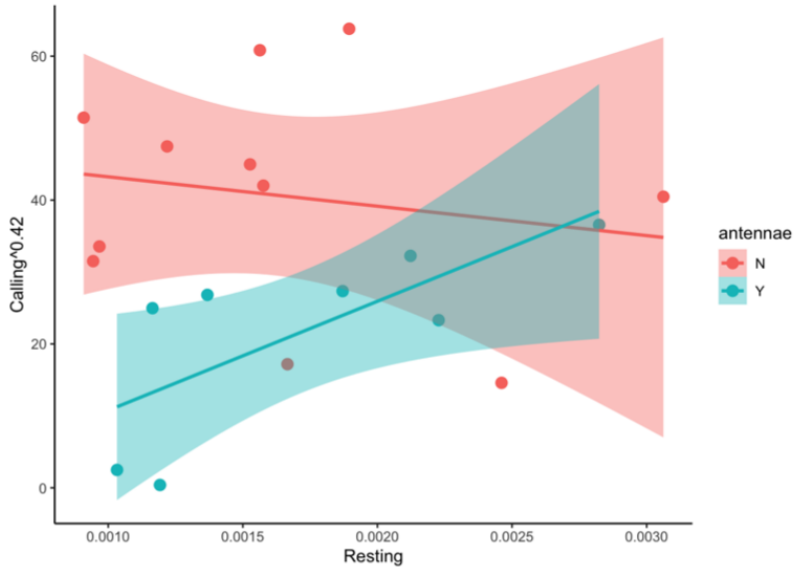



```
##Plot power transformed calling as a function of resting metabolic rate between treatments
r<- ggplot(data,aes(x=resting,y=calling^0.42,col=antennae))
r1 <-r+geom_point(size=3)+geom_smooth(aes(fill=antennae),method="lm")+theme_classic()
r1 + xlab("Resting") + ylab("Calling^0.42")
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

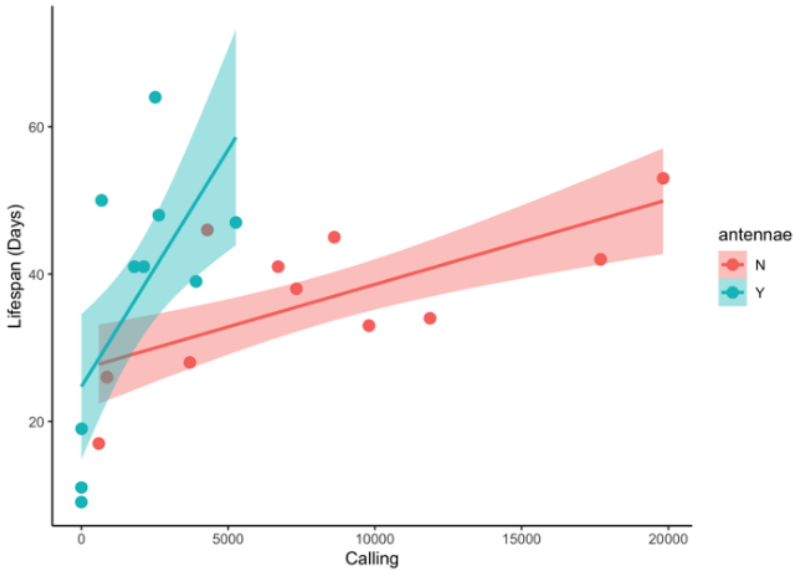
```
## Warning: Removed 2 rows containing non-finite values (`stat_smooth()`).
```

```
## Warning: Removed 2 rows containing missing values (`geom_point()`).
```




```
##Plot lifespan as a function of calling and mrtype between treatments
l<- ggplot(tidymr,aes(x=calling,y=lifespan,col=antennae))
l1 <-l+geom_point(size=3)+geom_smooth(aes(fill=antennae),method="lm")+theme_classic()
l1 + xlab("Calling") + ylab("Lifespan (Days)")
```

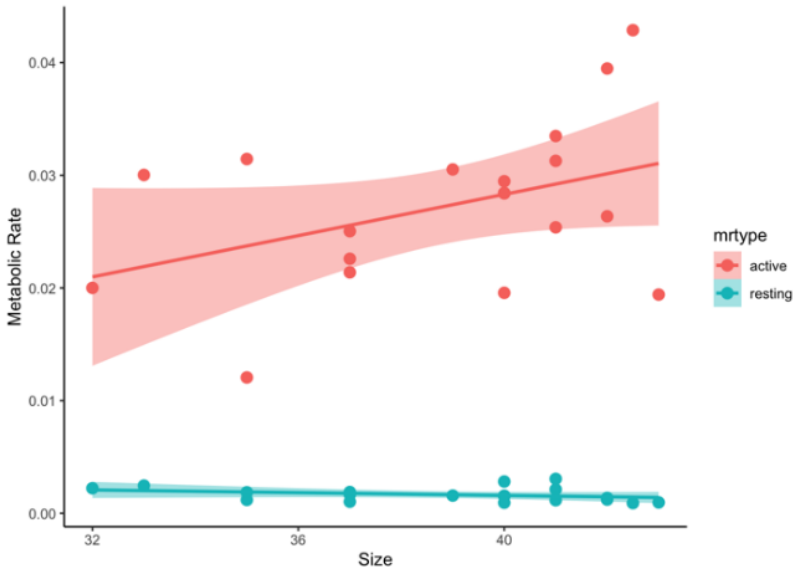
```
## `geom_smooth()` using formula = 'y ~ x'
```




```
##Plot mr as a function of size and mrtype
m <- ggplot(tidymr,aes(x=size,y=mr,col=mrtype))
m1 <-m+geom_point(size=3)+geom_smooth(aes(fill=mrtype),method="lm")+theme_classic()
m1 + xlab("Size") + ylab("Metabolic Rate")
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

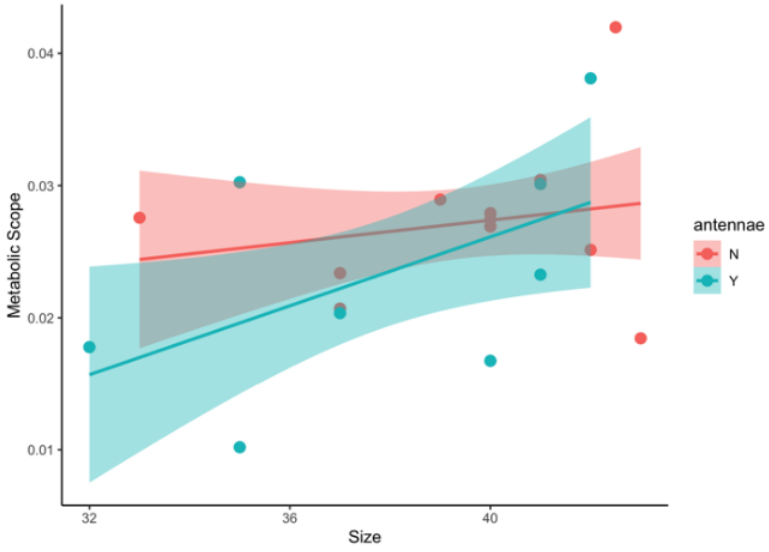
```
## Warning: Removed 4 rows containing non-finite values (`stat_smooth()`).
## Removed 4 rows containing missing values (`geom_point()`).
```




```
##Plot metabolic scope as a function of size between treatments
ms<- ggplot(tidymr,aes(x=size,y=metabolicscope,col=antennae))
ms1 <-ms+geom_point(size=3)+geom_smooth(aes(fill=antennae),method="lm")+theme_classic()
ms1 + xlab("Size") + ylab("Metabolic Scope")
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

```
## Warning: Removed 4 rows containing non-finite values (`stat_smooth()`).
## Removed 4 rows containing missing values (`geom_point()`).
```



Vita

The author grew up in New Orleans, Louisiana. She received her bachelor's degree in biology at the University of New Orleans spring of 2017. She joined the graduate school in the Integrative Biology Department of the University of New Orleans fall of 2017 in the Lailvaux Lab.