

The relationships between acoustic mate attraction signaling,
age, aggressiveness, and sperm quality in spring field crickets

by

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Abstract

Indirect influences upon reproductive success, such as signaling effort and aggressiveness, are often studied independently, and we therefore know relatively little about their interplay. Understanding how behaviours interact can help us predict organismal response to total sexual selection. The aim of my dissertation was to investigate acoustic signaling behaviour, aggression, and sperm quality of the spring field cricket, *Gryllus veletis*.

In my second chapter, I investigated lifetime patterns of acoustic signaling. I found that the fine-scale properties of acoustic signals changed as males aged, suggesting that females may use signal properties to inform mating decisions. Older males signaled with less effort, likely reflecting the degradation of signaling muscles.

The relationship between signaling and aggression has rarely been investigated, but both behaviours influence mating success. In my third chapter, I investigated the relationship between signaling and aggression. Aggressive behaviour was not influenced by signaling behaviour, suggesting that these behaviours are independent of one another in crickets, perhaps serving different functions in female attraction and male-male competition, respectively.

In my fourth chapter I examined whether the presence and sex of an audience influenced contest and post-contest behaviour. Males were more aggressive in the presence of a female audience, and produced more victory display behaviour in the presence of a male audience. My results suggest that male crickets dynamically adjust their aggressive behaviour to gain a reproductive benefit *via* female eavesdropping and

may also deter future aggression from potential rivals *via* the advertisement of the victory.

In my fifth chapter I investigated the relationship between the quality and quantity of mate attraction signals and sperm traits. I found no relationship between pre- and postcopulatory traits, providing no support for either the sperm competition or phenotype-linked fertility hypothesis.

Future studies should investigate the fine-scale components of acoustic signals produced immediately prior to, during, and immediately following aggressive behaviour and courtship to test whether these signals indicate male fighting ability, aggressive intent, or sperm quality in crickets.

Complex systems require investigation of the network of behaviours that exist in nature, and that is what I have strived to do in my dissertation.

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Co-authorship Statement

I am the principal author for all research herein. All of the data chapters in this dissertation (Chapters 2, 3, 4, and 5) are co-authored with my supervisor, Susan M. Bertram. Chapter 2 has been published in *Behaviour* and Chapters 3-5 are currently under review. My co-author on these chapters supported my research, shared in the research design, and provided helpful feedback on the writing of the manuscripts.

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Chapter 1: General introduction

Why does variation in sexually-selected traits exist? If a trait is heritable and subject to either directional or stabilizing selection, we would expect genetic variation in that trait to decrease over time; behavioural ecologists have long struggled with the paradox of widespread genetic variation in sexually-selected traits (Pomiankowski and Møller 1995). Two ideas put forward to explain maintenance in genetic variation are pleiotropy and genic capture. Pleiotropy, in which each trait is affected by many genes and each gene affects many traits, is widespread and important to our understanding of the mechanism of maintenance of genetic variation (Keightley and Hill 1990; Barton and Keightley 2002). Traits generally do not evolve independently (Kimura and Ohta 1969; Barton and Turelli 1989), and as selection acts on alleles that affect the overall fitness of the organism, it is necessary to consider all traits that influence fitness together. Before we examine the genetics underlying trait relationships it is important to understand how selection influences phenotypes by exploring interactions between traits.

Given that signaling is costly to produce and maintain, males that maximize the conspicuousness of their sexual signals may exhibit higher genetic quality than less showy males (Andersson 1994). Rowe and Houle (1996) introduced the genic capture hypothesis as an alternative explanation for the maintenance of genetic variation in fitness conferring traits. The genic capture hypothesis suggests that sexual signals exhibit condition-dependent expression. It assumes that signals are dependent on overall condition because they are costly to produce, that energy is limiting, (Zahavi 1977; Kotiaho 2000; Gil and Gahr 2002; Hunt et al. 2004), and that condition is dependent on

genes at many loci. Given these assumptions, genetic variation is thought to persist through mutation-selection balance. Genic capture suggests that sexual signals should exhibit condition dependent expression (Rowe and Houle 1996; Kotiaho et al. 2001; Tomkins et al. 2004). Genic capture also indicates that the signaling system should be closed to cheaters because males in poor condition should not be able to maximize the conspicuousness of their signals, while males in good condition should be better able to bear the costs of signaling better than males in poor condition (e.g. Hunt et al. 2004). Understanding how signaling traits correlate is important to assess whether genetic quality or trade-offs prevail in signaling allocation. Given the costs of signaling, positive correlations between signaling effort and signal attractiveness would support the hypothesis that sexually selected traits are honest signals.

Typically researchers examine one aspect of signaling behaviour when studying mate choice, but what if there are trade-offs between, for example, the quantity of signaling and the quality of the signal? Insects and anurans are known to exhibit trade-offs between components of signaling. Signaling animals generally show trade-offs between components related to conspicuousness, such as trade-offs between signaling rate and signal duration (Gerhardt and Huber 2002). Trade-offs may result from physiological constraints (such as the metabolic cost of signaling; Prestwich 1994), or from natural selection pressures (such as predators and parasitoids; Zuk and Kolluru 1998).

It is important to examine many components of signaling behaviour because there may be trade-offs between various components of signaling and because we may not know which aspects are most relevant to mate choice in an animal's natural environment.

For example, anurans have often been used as model organisms in studies of sexual selection in the laboratory and field, and numerous studies have shown that females exhibit preferences for calls with high call rates and long call durations (reviewed in Gerhardt and Huber 2002). However, these experiments were performed in the laboratory using a simple two-choice phonotaxis design; under natural conditions, there is little evidence that such preferences are expressed (Friedl 2006). Studies that have instead measured chorus attendance (nights spent at the breeding pond) have revealed a correlation between mating success and chorus attendance (e.g. Friedl and Klump 2005), and it is now generally accepted that chorus attendance is the best indicator of male mating success in the field. This finding highlights the importance of studying many aspects of signaling behaviour and conducting experiments in realistic, natural environments.

The aim of my dissertation was to investigate the acoustic signaling behaviour, agonistic behaviour, and sperm quality of the spring field cricket, *Gryllus veletis*. Indirect measures of reproductive success, such as signaling effort and aggressiveness, are most often studied independently and we therefore know relatively little about the interplay between these factors. Understanding the dynamics of selection on reproductive success requires us to examine both competition between males and mate choice by females. Given that male crickets use acoustic signals for both male-male competition and mate attraction, they are an ideal candidate to investigate male signaling, fighting, and mating behaviour and female mating decisions.

Female preference for older males has been documented in many taxa, but the evidence that males signal their age is inconclusive. Brooks and Kemp (2001) review the

evidence for male traits to signal age, and thereby quality, and conclude that females that mate with old males might receive indirect fitness benefits under many circumstances because old males demonstrate their superior genetic quality for viability (Brooks & Kemp, 2001). Three recent studies explore how the fine-scale structure of cricket signals change over time, and found that several acoustic parameters changed as males aged (Jacot et al. 2007; Judge 2011; Verburgt et al. 2011). These studies reveal that the fine-structure of male calls may reliably change as male age, potentially allowing females to distinguish between certain age classes when choosing a mate. In my second chapter, I investigated lifetime patterns of signaling in a longitudinal study of male spring field crickets. My study asked whether calls change as males age, and what is the relationship between calling effort and longevity? I found that signals changed with age; the signals of older males were quieter, with more silent periods within and between chirps, and produced less often than those of younger males. Together, my findings provide the most thorough exploration of lifetime signaling patterns in crickets to date.

Non-lethal patterns of settling disputes, such as agonistic displays and ritualized fighting, have likely evolved because such behaviours benefit the individuals engaged in the disputes. Aggression is generally regarded as a behavioural strategy for asserting claim to a limited resource, such as a territory or a mate, to increase reproductive success (reviewed in Cunningham and Birkhead 1998). For agonistic displays to be reliable, only those individuals who can back them up with physical displays should use them. Enquist (1985) suggested that honest signaling can occur in competitive situations due to the potential cost of fighting against a strong individual, and Számadó (2008) extended this

model by suggesting that proximity to the opponent maintains the reliability of agonistic displays.

Field crickets are excellent models for the study of agonistic behaviour; males frequently compete for territorial burrows, females, and other resources (Hack 1997). Fights between male crickets are often composed of discrete agonistic displays (Alexander 1961). A classification scheme of behaviours, generally increasing in energetic cost and aggression, has been developed for crickets (first described by Alexander 1961; later modified by Hofmann and Schildberger 2001), and most cricket fights progress through this stereotypic pattern of behaviours. Winning fights increases male mating success *via* male-male competition and possibly through female choice favouring dominant males (Simmons 1986a). Females of many animal species select dominant males as mates (fish: Doutrelant and McGregor 2000; birds: Mennill et al. 2002; e.g. crustaceans: Aquiloni and Gherardi 2010), and thus fighting ability can also directly influence reproductive success.

In crickets, both signaling and fighting are important aspects in determining which males will be successful in mating. Male crickets signal acoustically to attract females and repel rival males (Alexander 1961). They rub their raised forewings together; each closing stroke produces a pulse of sound, and pulses are concatenated into chirps (Figure 1; Pfau and Koch 1994). Females use the acoustic properties of the male's long-distance signal to distinguish between potential mates. Female crickets tend to prefer males who invest the most effort into acoustic signaling. For example, female *Teleogryllus commodus* prefer males that signal most often (Hunt et al. 2004). In several cricket species, males that signal most through the night tend to mate most often (Cade

and Cade 1992; Rodríguez-Muñoz et al. 2010). Signaling requires a territory, and males frequently compete for territorial signaling sites and access to females using discrete agonistic displays (fights; Alexander 1961; Hack 1997; Hofmann and Schildberger 2001; Jang et al. 2008; Bertram et al. 2011).

The relationship between signaling and aggression has rarely been investigated, even though both types of behaviour are important in determining which males will be successful in mating. However, there is a growing interest in studying signals that predict aggressive behaviour or fighting ability (Searcy and Beecher 2009). My third chapter had three goals: I investigated the relationship between signaling and aggression, the influence of body condition on aggression, and the effect of fighting experience on subsequent signaling behaviour in field crickets. Aggressive behaviour was not related to signaling behaviour. Further, there was no evidence for a winner or loser effect on signaling.

In nature, communication often occurs in the context of a communication network, with several individuals within range of one another. Audiences are individuals that are present during, but do not directly take part in, signaling interactions between others (Matos and Schlupp 2005). An audience effect is a change in the signaling behaviour during an interaction between individuals caused by the mere presence of an audience (Matos and Schlupp 2005). Studies of animal behaviour have recently begun to incorporate the influence of the social environment, providing new opportunities for the study of signaling strategies and signal evolution. While there is some evidence in vertebrate taxa that audiences gather information from social interactions and change the behaviour of signallers, little is known about whether audiences influence behaviour in

invertebrates. An audience may introduce extra costs or benefits to signalers (Zahavi 1979); for example, eavesdropping fish are more likely to initiate aggressive interactions with a loser than a winner (Oliveira et al. 1998), imposing an immediate cost to the loser, and female fish and birds may copy the mate choice of other females, resulting in mating benefits to the chosen male (Galef Jr and White 1998; Godin et al. 2005). In my fourth chapter, I examined whether the presence and sex of an audience influenced contest and post-contest behaviour in male field crickets. I provide the first investigation of audience effects on contest and post-contest behaviour in an invertebrate.

My fifth chapter focused on the relationship between male signaling and sperm quality, to determine whether males honestly indicate their sperm quality to females (as we would expect from the phenotype-linked fertility hypothesis), or whether there is a trade-off between signaling effort and sperm quality (as predicted by sperm competition hypothesis). Studies of sexual selection tend to focus on events prior to copulation, and consequently we know most about how the selective forces leading up to mating act on female choice and male-male competition. However, females in many species mate multiply, which can lead to sexual selection continuing beyond copulation *via* sperm competition and cryptic female choice (Parker 1970; Eberhard 1996; Simmons 2001). Postcopulatory sexual selection can counteract mating success (e.g. Preston et al. 2001) or can amplify mating success (e.g. Thomas and Simmons 2009) which may lead to inaccurate estimates of total sexual selection (Hunt et al. 2009).

The sperm competition hypothesis and the phenotype-linked fertility hypothesis predict opposite association patterns between precopulatory and postcopulatory sexually selected traits. Sperm competition predicts a negative association between traits involved

in mate acquisition and sperm quality (Parker 1998). Conversely, phenotype-linked fertility predicts that mate attraction traits and sperm quality will covary positively (Sheldon 1994). Studies have found evidence consistent with both hypotheses, highlighting the difficulty in predicting the relationship between investment in mate attraction and sperm quality. I investigated the relationship between long-distance mate attraction signals and sperm traits in the spring field cricket to test the predictions of the sperm competition and phenotype-linked fertility hypotheses. Females use the acoustic properties of the male's acoustic signal to distinguish between potential mates, and tend to prefer males who invest the most effort into acoustic signaling (e.g. Cade and Cade 1992; Hunt et al. 2004; Rodríguez-Muñoz et al. 2010). Crickets provide a powerful system to study pre- and postcopulatory traits because females mate multiply, female receive only ejaculates from their mates, and we know how ejaculate quality influences fertilization success in several species (e.g. Sakaluk and Eggert 1996; Schaus and Sakaluk 2001). My study is the first to examine the relationship between long-distance acoustic signals and sperm quality in a natural cricket population, as well as the first to examine whether wild-captured males exhibit different sperm traits than laboratory-reared males.

Most studies examine the mechanisms of sexual selection in isolation, but this approach can result in a misleading understanding of the total strength and form of sexual selection on male traits (Hunt et al. 2009). In studying several indirect measures of reproductive success, such as signaling effort, age, condition, aggressiveness, and mate choice, I hope to further our understanding of the interplay between these factors and generate a more integrated concept of an animal's behaviour and fitness.

Figure

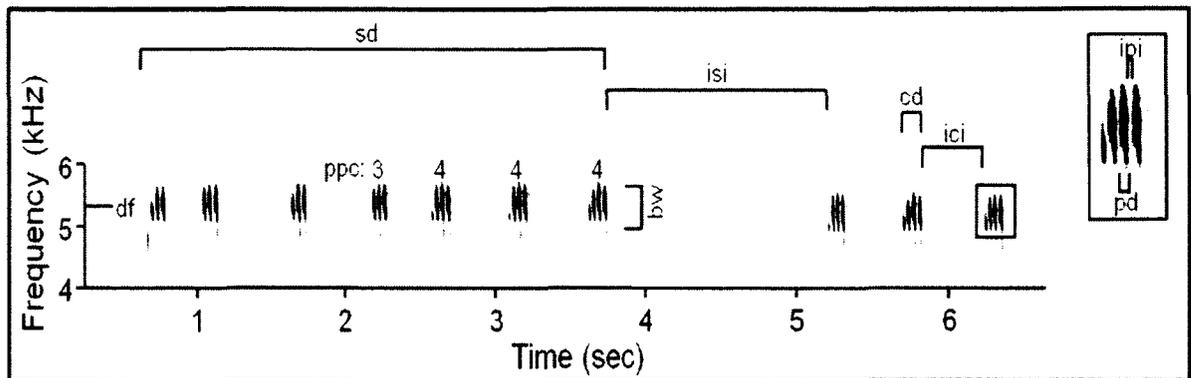


Figure 1.1. Spectrogram of long-distance mate attraction song of *Gryllus veletis*. One chirp is enlarged to show fine-scale pulse measurements.

sd: song duration; isi: intersong interval; cd: chirp duration; ici: interchirp interval; ppc: pulses per chirp; pd: pulse duration; ipi: interpulse interval; df: dominant frequency; bw: bandwidth.

Chapter 2: The calling songs of male spring field crickets (*Gryllus veletis*) change as males age

Abstract

Sexual traits are typically thought to convey information about a male's quality or condition. Female preference for older males has been documented in many taxa, but the evidence that males signal their age is inconclusive. We investigated lifetime patterns of acoustic mate attraction signaling in a longitudinal study of the spring field cricket, *Gryllus veletis*. We recorded males continuously throughout their lives, such that every pulse of sound produced by every male was analyzed. Our study answers two main questions: (1) Do calls change as males age? Our results reveal that the calls of male spring field crickets change with age; the calls of older males were quieter, with more silent periods within and between chirps, and produced less often than those of younger males. As males aged most of the changes in call structure reflect decreased calling effort. (2) What is the relationship between calling effort and longevity? Lifetime calling effort was positively related to longevity, such that males that called the most over their life also lived longer than males that called less. Together, our findings provide the most thorough exploration of lifetime signaling patterns in crickets to date.

Introduction

Much research in behavioural ecology is dedicated to investigating what information males communicate to females through their sexual displays. Fisher (1958) proposed that female preference for male displays might increase her chance of choosing a high-quality male with whom to mate. Recently, questions about whether males signal their age and whether females prefer older males have been debated in the literature.

Brooks & Kemp (2001) review the evidence for male traits to signal age, and thereby quality, to the extent that male quality and longevity are correlated. They conclude that females that mate with old males might receive indirect fitness benefits under many circumstances, simply due to the fact that old males demonstrate their superior genetic quality for viability (Brooks and Kemp 2001). However, there are also potential costs associated with mating with older males. For example, older males likely have a greater number of deleterious viability mutations due to the accumulation of germ-line mutations over their lifetime (Hansen and Price 1995; Crow 2000). The existence and strength of female preference for older males will likely be species-specific and depend on a variety of factors, including the balance of benefits (e.g. genetic quality) and costs (e.g. deleterious mutations).

Crickets (Orthoptera: Gryllidae) produce acoustic long-distance mate attraction calls that have been the subject of many studies on sexual traits with respect to female preference, male quality, and male age. Due to limitations of recording technology, most studies only analyze a snap-shot of calling effort and largely ignore the fine-scale structure of calls. Three recent thorough studies are, however, worthy of mention as they explore how the fine-scale structure of calls change over time. Judge (2011) conducted a longitudinal study of male *Gryllus pennsylvanicus* and found that several acoustic parameters changed as males aged; older males produced calls with more pulses per chirp and shorter pulse durations. Verburgt et al. (2011) similarly found that nearly all song traits measured changed as male *G. bimaculatus* aged, indicating a general slowing down of wing movements in older males. Jacot et al. (2007) found that pulses per chirp increased and carrier frequency decreased as male *G. campestris* aged. These studies

reveal that the fine-structure of male calls may reliably change as male age, potentially allowing females to distinguish between certain age classes when choosing a mate.

To assess whether the calls of male spring field crickets, *G. veletis*, change as males age, we conducted a longitudinal study in which we investigated the lifetime patterns of acoustic mate attraction signaling. Whereas previous studies have taken short sound samples at different male ages (e.g. Jacot et al. 2007; Judge 2011; Verburgt et al. 2011), we recorded males continuously throughout their lives, such that every pulse of sound produced by every male is included in our analyses. We provide the most thorough exploration of lifetime calling patterns in crickets to date.

The core assumption of handicap signaling theory is that sexual signaling is costly (Zahavi 1975), which leads to the prediction that signaling, quality, and longevity will be positively correlated because high-quality males can bear the costs of signaling better than low-quality males (Hunt et al. 2004). Researchers often assume that sexual trait expression and longevity will be positively correlated when the trait is an honest advertisement of male quality (Höglund and Sheldon 1998), and that a positive relationship is most likely to occur when sexual traits are condition dependent and energy is limiting. Alternatively there may be a direct trade-off between sexual and natural selection (Selander 1965) whereby investing in sexual traits is balanced by reduced longevity.

In support of the prediction that signaling and longevity will be positively correlated, a meta-analysis of studies of sexually selected traits and survivorship found that males with greater expression of sexual traits had greater longevity (Jennions et al.

2001). For example, Kotiaho (2000) found a weak positive relationship between signaling rate and longevity in male wolf spiders (*Hygrolycosa rubrofasciata*). Similarly, Judge et al. (2008) found that male fall field crickets, *G. pennsylvanicus*, that were in good condition lived longer and called more than males in poor condition. On the contrary, several other studies have reported a negative relationship between signaling and longevity. For example, Hunt et al. (2004) found a condition-dependent relationship between longevity and calling effort in the black field cricket, *Teleogryllus commodus*. Males in good condition called more early in life, and had higher lifetime calling effort, but died sooner than males in poor condition. Gustafsson et al. (1995) argue that we should expect trade-offs between life-history traits and secondary sexual characteristics because the latter are condition dependent and costly to display, thus making them part of the trade-off between current and future reproductive effort.

We examined the relationship between calling effort and longevity in *G. veletis* to assess whether males that invest heavily in calling effort pay a longevity cost or whether high-effort callers live longer than low-effort callers. We also investigated whether longevity was related to the onset of calling, and whether longevity was related to male body size or condition. We explored the relationship between calling effort and longevity with a higher degree of precision in the measure of calling effort than previous studies (Hunt et al. 2004; Judge et al. 2008) since we recorded males continuously throughout their lives.

Together, our study answered two main questions: (1) do calls change as males age; and (2) what is the relationship between calling effort and longevity?

Methods

Experimental animals

We captured adult male and female *G. veletis* in Ottawa, Ontario, Canada (45°19' N, 75°40' W) in May and June 2008. Males used in this experiment were the first generation of lab-reared offspring from the field-captured individuals. Late-instar nymph males were removed from the colony and checked daily for adult eclosion. Adult males were individually housed in circular, clear plastic 540 mL containers. Crickets were provided with water and food (Harlan Teklad Laboratory Rodent Diet #8604: 24% crude protein, 4% crude fat, 4.5% crude fibre) *ad libitum* and were held in a temperature-controlled room (30 ± 2°C).

Acoustic mate attraction displays

On the first day of adulthood, males were placed into individual containers in an electronic acoustic recording system. The recording system consisted of 32 individually recording microphones (electret condenser type KECG2742PBL-A; Kingstate Electronics Corporation, Tamshui, Taipei, Taiwan), each with a single LED light that provided males with a 12-h:12-h L:D cycle, positioned 6.6 cm above the top of the male's container. Each male was separated from its neighbours by an acoustically isolated enclosure (a 7 cm thick Styrofoam box that was internally lined with 3.5 cm thick acoustic foam) that contained the microphone and the LED light. This design minimized the likelihood of individuals detecting their neighbours' signals. Further, although the calls could be heard faintly through the acoustic foam, the minimum amplitude threshold of the recording system ensured that neighbouring microphones did not mistakenly record non-focal

males. Microphones were calibrated relative to a known signal level so that input values from different channels could be compared.

To quantify whether cricket calls change as males age, we continuously recorded long-distance acoustic mate attraction signals until day 35 of adulthood using real-time electronic analysis (N = 64 males). To quantify the relationship between signaling and longevity, a subset of the males was left in the recording system beyond 35 days until they died a natural death (N = 35 males). Recording was paused for 15 min each day while we replenished food and water supplies. The microphones were continuously monitored and analyzed using CricketSong software (Cambridge Electronic Design Ltd., Cambridge, UK). Songs were recorded at 31.25 Hz. We used an amplitude threshold of 55 db to determine pulse onset. However, this threshold was dynamically adjusted by CricketSong to account for males that called at higher than average amplitudes. For these individuals, the threshold was raised to a level proportional to the amplitude of the pulse and decayed back to the original value within 1 - 8000 ms (the system is self-scaling, and thus the exact rate of decay is proportional to the size of the pulse). For very quiet individuals, the minimum threshold was manually set below the species-specific value to ensure that all of their pulses were scored correctly. We established species-specific thresholds to classify pulse, interpulse, chirp, interchirp, song, and intersong periods (Table 2.1). The system recorded 30 seconds of waveform data for each individual, which we then manually scanned to confirm that the program thresholds were correctly scoring the pulses and chirps correctly. We processed the resulting audio files using a series of scripts in Spike2 6.10 (Cambridge Electronic Design Ltd.).

Crickets produce sound by rubbing their forewings together. The fine-scale structure of cricket calls is determined by wing properties and the rate and pattern of wing movement. Each closing stroke produces a pulse of sound, and pulses are concatenated together to produce chirps. A series of chirps is concatenated together to produce a bout of song. We define a song as a series of chirps in which the male does not pause for longer than one minute; once a male pauses for thirty seconds or longer, he is considered to have begun a new song bout (following Bertram and Warren 2005; Bertram and Bowen 2006). Because our recording system continuously monitored males' acoustic behaviour, our measure of daily time spent calling quantifies the total amount of time each male spent calling during a 24-hour period.

To quantify whether daily acoustic signaling parameters were affected by male age, we determined each male's mean values for time spent calling, calling effort, song duration, pulse rate, number of pulses per chirp, chirp duration, interchirp duration, pulse duration, interpulse duration, amplitude, and carrier frequency (Figure 2.1) for each full day of recording (measuring all sound pulses produced). Hourly calling parameter averages were weighted by the number of pulses produced in the hour. In this way, hours with many pulses were given heavier weight than hours when relatively few pulses were produced. Pulse rate (#pulses/sec) was calculated at a later date using the equation: (#pulses per chirp/chirp duration)*1000.

Body size

Males were weighed on day 7 of adulthood using a Denver Instruments balance (Pinnacle Series model PI-314; precision = ± 0.1 mg). We measured head width,

pronotum length, and pronotum width for each cricket at the end of the experiment (either 35 days old or following natural death; see below for details). Measurements were taken using a Zeiss Axio Observer inverted dissecting microscope and highly magnified photographs (AxioVision v4.8, Carl Zeiss; magnification: ~8.5x, resolution: ~1.60 μ m).

Statistical methods

We conducted statistical analyses in JMP v9.0.0 (SAS Institute Inc., Cary, NC, USA) and SPSS Statistics v17.0 (IBM, Armonk, NY, USA). Data on four signaling parameters (time spent calling, song duration, pulse rate, interchirp duration) were Box-Cox transformed to meet the assumption of normality necessary for parametric statistical analysis. We calculated the mean \pm standard error (SE) for all 11 calling parameters averaged across each day for each male. All values reported are mean \pm SE.

Calling effort was quantified as the first principal component from a Principal Component Analysis (PCA) of time spent calling and amplitude; PC1 explained 78% of the variation in calling effort (loadings: time spent calling = 0.88, amplitude = 0.88). Body condition was determined as the first principal component from a PCA of body mass and three morphological measurements (head width, pronotum length, pronotum width; Judge 2011); PC1 explained 76% of the variation in body condition (loadings: head width = 0.93, weight = 0.90, pronotum width = 0.85, pronotum length = 0.81). We also performed analyses involving condition with the more commonly-used residuals of mass on body size. Our results did not differ when analyzed with residuals of mass, and given the problems associated with use of residuals as a response variable (Freckleton 2002), we present results obtained from PCA scores only. For all of our PCA, we

conducted Kaiser-Meyer-Olkin measures of sampling adequacy and Bartlett's test of sphericity to ensure that the data met the criteria for factor analysis.

To address the question of whether calls change as males age, we analyzed the calling data with generalized linear mixed models (GLMM). We included male age (repeated measure), male identity (random effect), body condition, body size (pronotum length; Judge et al. 2008), and the interactions age*condition and age*size in the models. We examined relationships with our full data set (N = 64 males; those removed from the experiment at day 35 and those that remained in the experiment until natural death), and then with a subset of our data that represents the expected natural lifespan in the field (28 days). We were interested in comparing our analyses using our full data set of calling metrics to a similar set of analyses performed on a subset of our data representing the natural lifetime of a cricket in the wild (Zuk 1987). Extending the lifespan of organisms in the laboratory is beneficial because relationships between age and life history traits may be revealed that would not have been detected within the natural lifespan (Hoikkala et al. 2008). Therefore we examined the relationships between age and calling parameters on both a natural and extended lifespan.

To assess whether calls could be statistically classified to age classes, we performed a discriminant function analysis (DFA). We took daily mean values for all calling parameters over the expected natural lifespan (28 days), and classified them by week (4 levels). Due to multicollinearity of calling parameters, we performed a PCA that generated two new variables with eigenvalues > 1 for use in the DFA (Tabachnick and Fidell 2007). PC1 explained 79.4% of the variation in calling parameters, and was heavily loaded by all parameters (all loadings > |0.67|; highest loadings: calling effort =

0.99, amplitude = 0.99, pulse rate = 0.98). PC2 explained 12.3% of the variation, and was loaded by pulse duration (0.65), carrier frequency (-0.54), and chirp duration (0.51). We used a fraction of our data to generate a training function, and we then used a cross-validated approach to classify the remainder of our data using this function.

To address the question of what is the relationship between calling effort and longevity, we performed regressions of lifetime and mean daily calling effort with longevity. We also performed pairwise regressions to examine the relationships between calling effort and male age and onset of calling. We used regression to examine the relationship between longevity and male body size and condition.

Results

In total, we recorded 64 males for an average of 45.5 ± 2.46 days (range: 16-111 days). The average onset of calling was day 5.7 ± 0.34 of adulthood (range: 2-13 days old, N = 49 males); two males never called. Of the subset of males that were recorded until death, average lifespan was 60.0 ± 3.65 days (range: 28-111 days, N = 35 males).

Question 1: Do calls change as males age?

Our GLMM analyses revealed that male age explained significant amounts of variation for all calling parameters (range: 7 - 92%; Table 2.2). Age explained the most variation for time spent calling (92%), song duration (42%), chirp duration (41%), and interchirp duration (47%). Identity explained variation in all calling parameters but time spent calling (range: 11 - 60%), and was highest for carrier frequency (60%) and chirp duration (49%). Size was not a significant factor in any of the models, and condition was only significant in explaining 7% of the variation in song duration. The interactions

between condition and age, and size and age, were significant in some models but never explained more than 0.2% of the variation for any calling parameter (Table 2.2).

Given that age explains a significant amount of variation for all calling parameters, we plotted the relationships between acoustic parameters and age to help understand how calls changed as males aged. We found that both the quantity of calling and the acoustic structure of calls changed as males aged (Table 2.2; Figure 2.1). Song duration and pulse rate decreased, while the number of pulses per chirp, chirp duration, and interchirp duration increased (Figure 2.2). Pulse duration increased with age, and interpulse duration peaked late in life. Both call amplitude and carrier frequency decreased with age (Figure 2.2). In general, older males called with reduced effort compared to young males. Older males had less time spent calling, shorter song durations, quieter songs, longer interchirp durations, and longer interpulse durations. However, some of the changes reflect higher effort in older males: older males produced more pulses per chirp, and consequently longer chirp durations, as well as longer pulse durations. The small peaks in some parameters at old ages, such as time spent calling (Figure 2.2a), likely reflect noise due to small sample sizes at those times (e.g. only two males were sampled beyond day 80), and likely do not represent meaningful changes in call parameters over time.

The analyses of how calling parameters changed as males aged during their natural lifetime revealed almost identical results to the analyses using our full data set (Table 2.3). The only notable difference was that identity explained a significant amount of variation in time spent calling (26%) when analyzing the natural lifetime but not the extended laboratory lifetime. As in the analyses with the full data set, age explained the

most variation in time spent calling and song duration, whereas individual differences explained more variation for pulse duration, chirp duration, amplitude, and carrier frequency (Table 2.3). When we plotted the relationships, many were similar in slope and direction, but a few changed entirely. For example, in the analyses limited to natural lifespan, daily calling effort peaked late instead of early, amplitude peaked mid-life instead of linearly decreasing, and interchirp duration decreased instead of increased (Figure 2.2f, g).

The DFA correctly classified 85.7% of calling parameter PC scores to the correct age class (week), well above the expected chance level of 25% (binomial test: $p < 0.001$).

For descriptive purposes, mean values for calling parameters at age 10, 30, and 50 days are shown for 15 males that were recorded at all three ages (Table 2.4). Table 2.4 highlights how time spent calling, calling effort, song duration, and pulses per chirp peaked in mid-life and decreased with age, whereas silent intervals (interchirp duration and interpulse duration) increased as males aged. Amplitude and carrier frequency were also lowest when males were 50 days old.

Question 2: What is the relationship between calling effort and longevity?

Longevity was significantly positively related to lifetime calling effort (calling effort: $r^2_{\text{adj}} = 0.20$, $F_{1,31} = 8.90$, $p = 0.006$). However, longevity was not related to mean daily calling effort ($r^2_{\text{adj}} = -0.03$, $F_{1,31} = 0.11$, $p = 0.74$). Together these results suggest short- and long-lived males called with similar effort, but long-lived males achieve greater lifetime calling effort as a result of having lived more days on which to call.

The onset of calling was not related to mean daily or lifetime calling effort (daily calling effort: $r^2_{\text{adj}} = -0.03$, $F_{1,18} = 0.46$, $p = 0.50$; lifetime calling effort: $r^2_{\text{adj}} = 0.02$, $F_{1,18} = 1.30$, $p = 0.27$).

Longevity was not related to male body size or condition ($r^2_{\text{adj}} = -0.03$, $F_{2,25} = 0.58$, $p = 0.57$).

Discussion

Do calls change as males age?

In our study, we found that the acoustic mate attraction call of male *G. veletis* changed as males aged. Age explained a significant amount of variation in all of the calling parameters we measured, and a discriminant function analysis correctly classified call parameters to the correct age class with high success. Many of the changes reflect decreased calling effort; older males increased the amount of silence both within and between their calls. Older males also spent less time calling, produced quieter calls, shorter song durations, longer interchirp durations, slower pulse rates, and longer interpulse durations. Overall, young males tended to produce many loud, fast, short chirps in rapid succession for long periods, while old males tended to produce fewer, quieter, slower, longer chirps for shorter durations with longer pauses between chirps. Our results suggest that females may be able to distinguish between males of different age classes based on their calls, but a direct test of this hypothesis is needed before we can conclude that the variation we detected is biologically significant.

A few hypotheses have been proposed to account for the changes in cricket signaling as males age. Older males are expected to invest relatively more effort in

reproduction as their residual reproductive value decreases as they age (Williams 1966). However, our results do not support this idea, as we found that older males invested less into signaling than younger males. Cricket wings are subject to wear and tear as males age, which likely affects the fine structure of their calls (Jacot et al. 2007; Judge 2011). Further, Verburgt et al. (2011) suggest that since insect flight muscles degrade as males age, and the same muscles are used to produce calls, age-related degradation in flight muscles should lead to predictable changes in call structure. Our results support this hypothesis; older males called with longer interpulse and interchirp durations, increasing the silent portions of their songs, and investing less energy into their calls, as do male *G. bimaculatus* (Verburgt et al. 2011). This may be due to increased muscle recovery time and may be a strategy to conserve energy to perform longer song bouts than would otherwise be possible with degraded calling muscles. More work is needed to test whether wing wear or muscle degradation or both is responsible for the fine structural changes in cricket calls over their lifetime.

How do our results compare to previous studies of time spent calling in *G. veletis*? Cade & Wyatt (1984) reported that *G. veletis* males begin acoustic calling between 3 and 6 days past adult moult, with an average of 5.8 days; we found the same average onset of calling, but a larger range of onset of calling (2-13 days). In the earliest study of calling effort, Alexander & Meral (1967) found a few high-effort callers that called for ten hours per day, based on monitoring which males were calling every couple of hours in the field; our highest effort callers called for as much as fourteen hours per day based on continuous sampling. In the laboratory, Cade & Wyatt (1984) found that *G. veletis* males called for 7.5 hours on average per night; in contrast, French & Cade (1989) monitored

which males were calling every five minutes in the field, and found a mean time spent calling of 1.8 hours per night. Our results fall between these two studies, with a mean of 3.6 hours of calling per night. Our results are similar to those of Burpee & Sakaluk (1993), which reported that males called for 2.5 hours on average in the laboratory.

Only one previous study investigated whether time spent calling changes as males age in *G. veletis*. Cade & Wyatt (1984) recorded males for 45 days and found that there was no change in the amount of time spent calling as males aged; in contrast, we found that time spent calling peaked at day 20 and decreased thereafter. There is no previous information on how any of the other calling parameters measured change as *G. veletis* age, although recent studies have investigated these relationships in other cricket species. We found that, in general, older males called with reduced effort compared to young males. Older males had lower time spent calling, shorter song durations, quieter songs, and longer periods of silence within their chirps (longer interchirp durations and interpulse durations). However, older males also produced more pulses per chirp, and consequently longer chirp durations, as well as longer pulse durations, which reflect higher effort. Our results are quite similar to those of Verburgt et al. (2011), which reported that all *G. bimaculatus* song traits declined in energetic quality as males aged except pulses per chirp and chirp duration. Older male *G. bimaculatus* produced calling songs with decreased pulse durations, song durations, and amplitude, and increased interchirp and intersong intervals. Verburgt et al. (2011) note that the increase in number of pulses per chirp does not compensate for the decrease in pulse duration and increase in interchirp interval; the energetic content of the songs of *G. bimaculatus* decreases significantly with age.

What is the relationship between calling effort and longevity?

We found that lifetime calling effort was positively related to longevity, such that males that called the most over their life also lived longer than males that called less often. However, longevity was not related to mean daily calling effort, so calling effort is not predictive of longevity in *G. veletis*. Consequently, the relationship between lifetime calling effort and longevity may simply result from the longer-lived males living for more days on which to call, and thus a higher lifetime calling effort than males that died earlier. Hunt et al. (2004) found that *T. commodus* males in good condition called more often early in life, and had higher lifetime calling effort, but died sooner than males in poor condition. On the contrary, Judge et al. (2008) found that high-condition male *G. pennsylvanicus* called more and survived longer than low-condition males. While there are theoretical predictions for either a positive or negative relationship between signaling effort and longevity, the empirical evidence is not yet conclusive.

Interestingly, there was some disparity between the results of our population-level analyses and our individual-level analyses. Our population-level analyses revealed significant changes in all calling parameters as males age, generally reflecting decreased effort invested by older males, whereas only calling effort and chirp duration changed with male age according to the repeated-measures analyses. While our individual-level analyses were limited to the expected natural lifespan of male crickets in the field, we may have uncovered more age-related changes if we had acquired extended laboratory lifespan data for a sufficient number of males (Hoikkala et al. 2008). Although we did not find a direct relationship between daily calling effort and longevity, the disparity between our population- and individual-level analyses might indicate a change in population

structure over time, such that males that invest less effort into calling live longer than males that invest heavily into calling.

Now that we have found that the calls of male spring field crickets change as males age, and that females could potentially use calls to discriminate amongst males of different ages for mate choice, the next logical step is to develop a female preference function (Wagner 1998) by testing female preference for different male calling parameters in the laboratory. Studies of female preference are necessary to assess the biological significance of variation in the properties of calls. Of particular interest to our research group is female preference for calls from the same males at different ages to test whether females prefer younger or older males, which should be possible given the nature of our recording system. This is our next avenue of investigation with the calls of *G. veletis* and other field crickets.

Figures

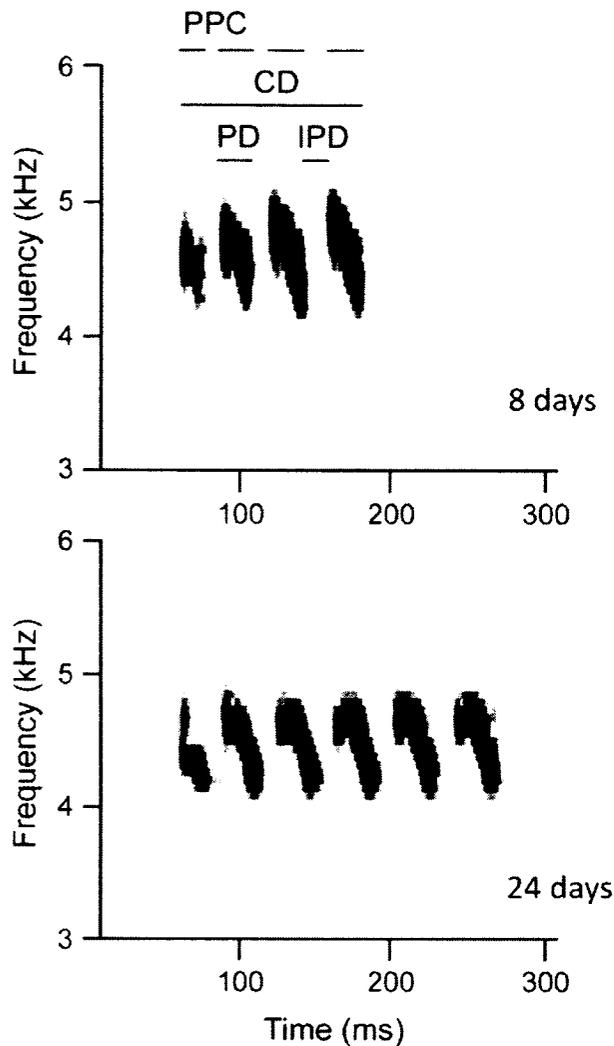


Figure 2.1. Spectrograms of example *G. veletis* acoustic mate attraction calls measured in this study. One chirp is shown at each of two ages (8 and 24 days); these two chirps were produced by the same male. The chirp produced at age 24 days has more pulses, a longer chirp duration, slower pulse rate, and a lower carrier frequency than the chirp produced at 8 days, as was typical for older males in our study. Four of the fine-structural characteristics we measured are indicated on the upper chirp. PPC: number of pulses per chirp; CD: chirp duration; PD: pulse duration; IPD: interpulse duration.

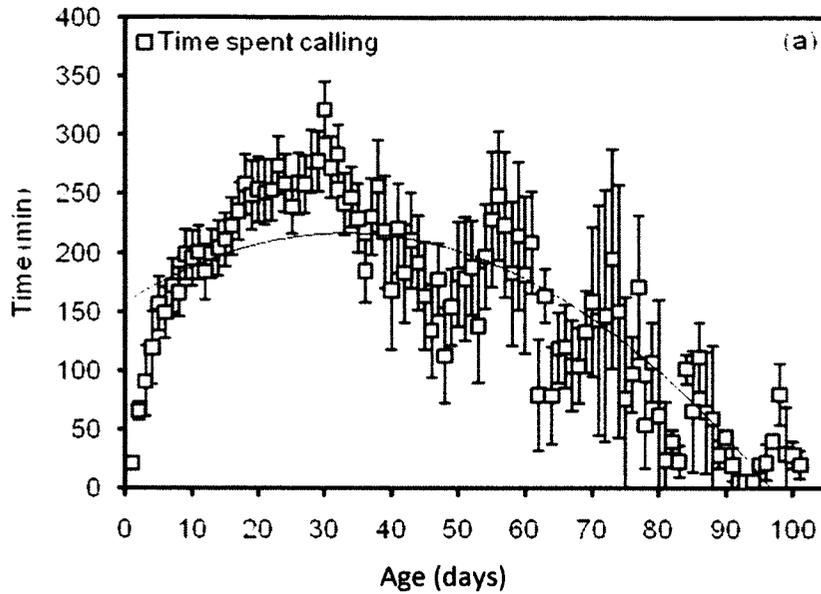
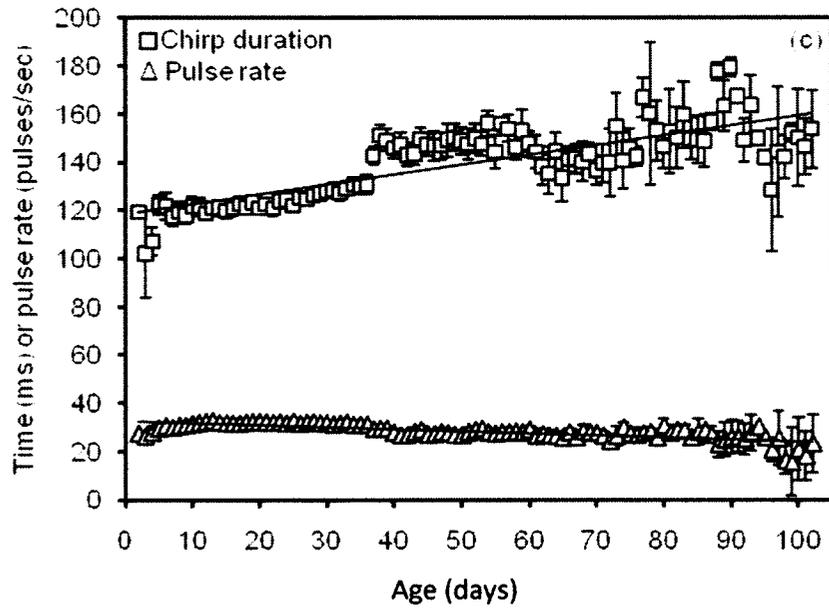
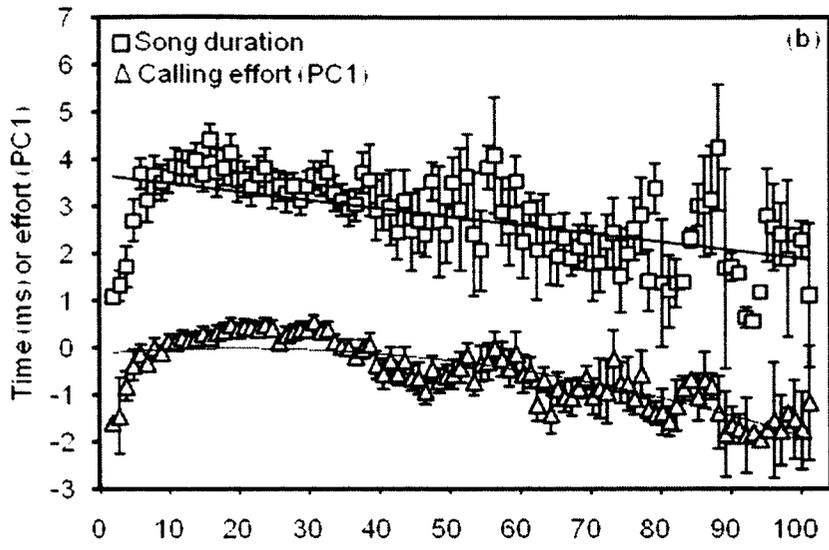
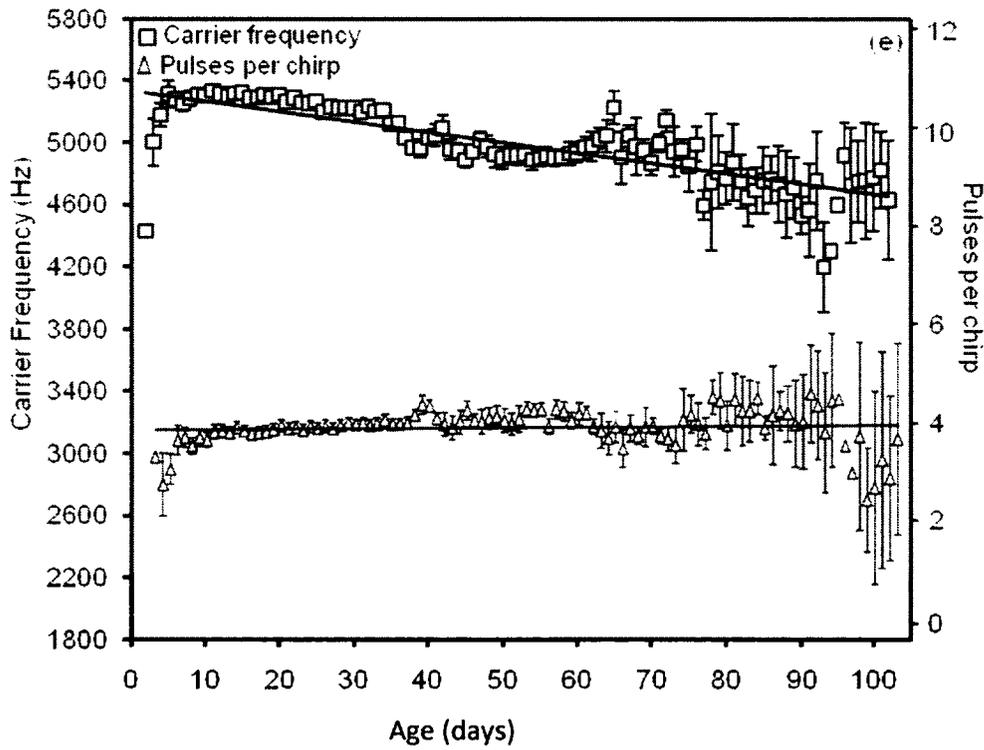
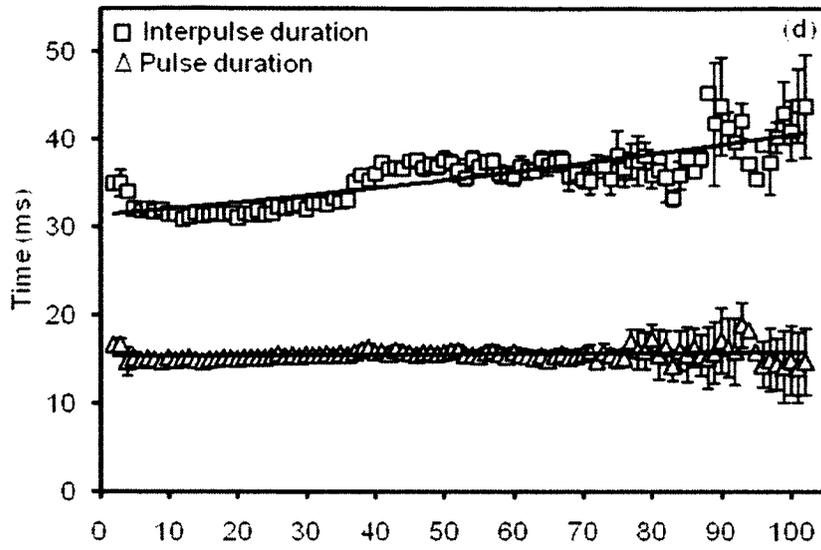
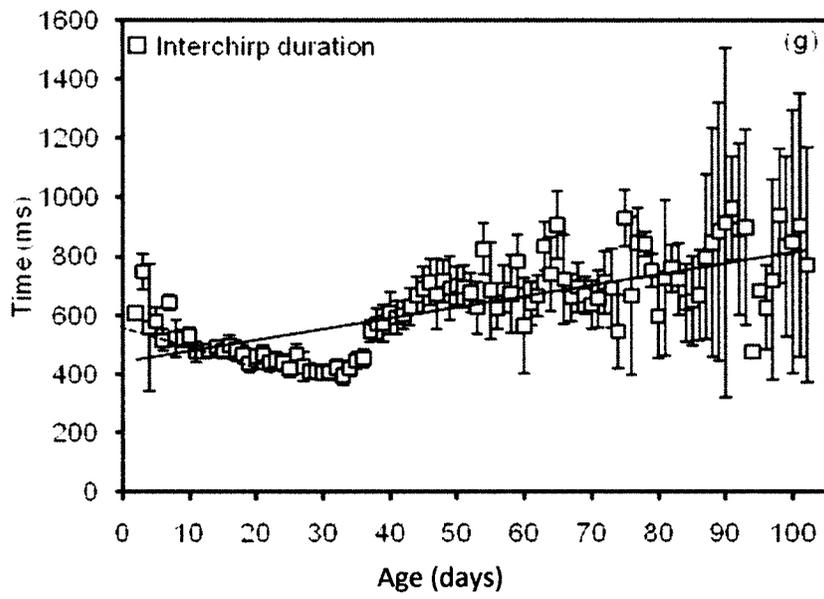
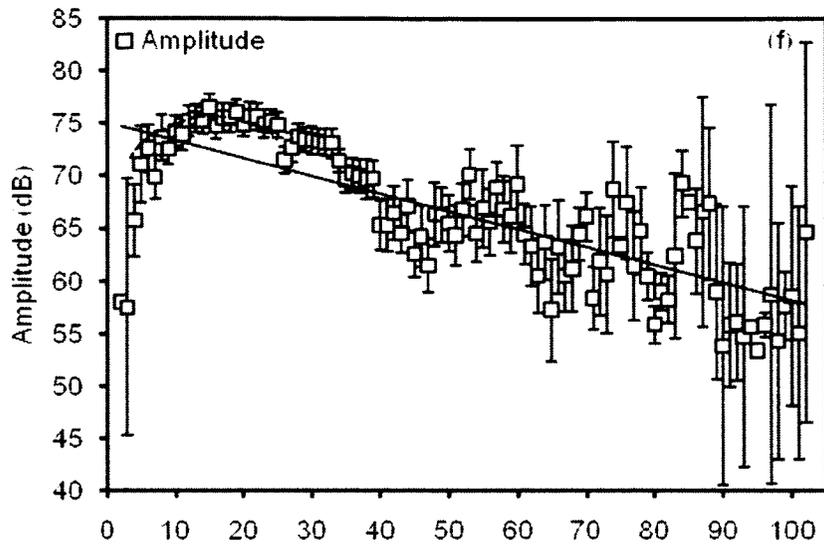


Figure 2.2. The change in calling parameters changed as males aged: (a) time spent calling, (b) song duration and calling effort, (c) chirp duration and pulse rate, (d) interpulse duration and pulse duration, (e) carrier frequency and pulses per chirp, (f) amplitude, and (g) interchirp duration. A comparison of the different relationships for the expected lifespan of a cricket in the wild (28 days, plotted with a short dashed line) and for the extended lifespan of crickets in the laboratory (up to 102 days, solid line) is shown for amplitude and interchirp duration. Values are daily means and error bars represent standard error. Untransformed data shown.







Tables

Table 2.1. CricketSong recording settings and thresholds for *G. veletis*.

Acoustic parameter	Recording setting
Amplitude trigger level	55 dB
Trigger decay constant	10 ms
Maximum SPL	100 dB
Minimum intersong duration	30 sec
Minimum interchirp duration	100 ms
Minimum pulses per chirp	1
Minimum gap between pulses	1 ms
Typical pulse duration	14 ms
Typical interpulse duration	26 ms
Typical dominant frequency	5000 Hz

Table 2.2. The influence of male age, identity, condition, and body size on *G. veletis* acoustic mate attraction signal parameters using generalized linear mixed models (N=62 males).

Acoustic parameter	Effect	Estimated variance	DF	t/Wald Z	P	% Variance explained
Time spent calling	Age	28785.04		10.79	<0.001	92.44
	Identity	2105.16		1.03	0.30	
	Condition	-5.45	96.75	-0.38	0.71	
	Size	-0.74	55.85	-0.16	0.88	
	Condition*Age	-0.083	157.71	-0.29	0.77	
	Size*Age	-0.003	153.00	-0.31	0.76	
Calling effort	Age	0.96		15.36	<0.001	36.51
	Identity	0.64		4.10	<0.001	24.38
	Condition	-0.12	74.61	-0.91	0.36	
	Size	-0.007	52.66	-0.016	0.99	
	Condition*Age	0.005	196.68	2.36	0.019	0.18
	Size*Age	-0.002	169.40	-3.85	<0.001	0.008
Song duration	Age	2.75		21.52	<0.001	41.68
	Identity	1.93		4.50	<0.001	29.27
	Condition	-0.44	68.66	-2.14	0.036	6.63
	Size	0.062	52.85	0.84	0.40	
	Condition*Age	0.0070	308.60	2.50	0.013	0.10
	Size*Age	-0.0004	275.40	-5.46	<0.001	0.006
Pulse rate	Age	9.79		15.73	<0.001	18.52
	Identity	18.59		4.74	<0.001	35.15
	Condition	-0.47	61.74	-0.79	0.43	
	Size	-0.10	52.73	-0.46	0.65	
	Condition*Age	0.012	186.87	1.93	0.055	
	Size*Age	-0.0012	163.19	-7.48	<0.001	0.002
Pulses per chirp	Age	0.36		14.90	<0.001	7.20
	Identity	0.67		4.71	<0.001	13.36
	Condition	-0.065	62.53	-0.56	0.58	
	Size	0.013	52.97	0.31	0.76	
	Condition*Age	-0.0036	173.15	-0.28	0.78	
	Size*Age	-0.0006	150.58	-1.78	0.077	

Chirp duration	Age	297.14		13.85	<0.001	40.88
	Identity	349.42		4.38	<0.001	49.06
	Condition	0.067	67.21	0.024	0.98	
	Size	0.98	52.34	0.98	0.33	
	Condition*Age	-0.075	169.98	-2.02	0.045	0.010
	Size*Age	0.0045	143.70	4.56	<0.001	0.0006
Interchirp duration	Age	27059.00		14.09	<0.001	46.89
	Identity	25011.27		4.29	<0.001	43.34
	Condition	15.80	71.44	0.66	0.51	
	Size	-1.13	53.26	-0.13	0.90	
	Condition*Age	-0.40	168.98	-1.15	0.25	
	Size*Age	0.012	146.62	1.34	0.18	
Pulse duration	Age	1.62		17.83	<0.001	9.04
	Identity	2.03		4.68	<0.001	11.31
	Condition	-0.16	64.90	-0.80	0.43	
	Size	0.040	53.42	0.52	0.60	
	Condition*Age	0.0032	221.49	1.32	0.19	
	Size*Age	-0.0001	195.35	-1.72	0.086	
Interpulse duration	Age	8.15		15.19	<0.001	18.12
	Identity	5.55		4.09	<0.001	12.34
	Condition	0.46	74.06	1.24	0.22	
	Size	0.023	52.40	0.18	0.86	
	Condition*Age	-0.015	191.70	-2.55	0.012	0.033
	Size*Age	0.0015	167.50	9.90	<0.001	0.0034
Amplitude	Age	63.59		18.59	<0.001	31.32
	Identity	64.77		4.58	<0.001	31.90
	Condition	-0.76	65.40	-0.68	0.50	
	Size	0.022	52.47	0.052	0.96	
	Condition*Age	0.046	236.35	3.13	0.002	0.022
	Size*Age	-0.0026	209.06	-6.79	<0.001	0.0013
Carrier frequency	Age	29380.22		24.49	<0.001	32.89
	Identity	54649.89		4.86	<0.001	60.56
	Condition	-21.27	59.04	-0.67	0.51	
	Size	-11.43	52.41	-0.95	0.34	
	Condition*Age	0.60	295.94	2.02	0.044	<0.001
	Size*Age	-0.062	264.28	-8.25	<0.001	<0.001

Table 2.3. The influence of male age, identity, condition, and body size on *G. veletis* acoustic mate attraction signal parameters using generalized linear mixed models (N = 62 males; over expected natural lifespan of 28 days).

Acoustic parameter	Effect	Estimated variance	DF	t/Wald Z	P	% Variance explained
Time spent calling	Age	16865.77		9.08	<0.001	72.66
	Identity	6031.57		2.42	0.016	25.99
	Condition	15.20	77.94	0.98	0.33	
	Size	-4.73	51.31	-0.89	0.38	
	Condition*Age	-1.06	99.72	-2.00	0.048	0.0046
	Size*Age	0.21	98.08	12.11	<0.001	0.0009
Calling effort	Age	0.68		11.59	<0.001	22.32
	Identity	0.66		4.07	<0.001	21.72
	Condition	0.021	83.29	0.16	0.88	
	Size	-0.036	52.10	-0.79	0.43	
	Condition*Age	0.0083	113.59	0.22	0.83	
	Size*Age	0.0010	110.73	8.18	<0.001	0.033
Song duration	Age	2.56		14.05	<0.001	38.40
	Identity	2.01		4.13	<0.001	30.16
	Condition	-0.084	87.07	-0.36	0.72	
	Size	-0.004	53.30	-0.051	0.96	
	Condition*Age	-0.004	147.68	-0.53	0.60	
	Size*Age	0.0009	144.90	4.05	<0.001	0.013
Pulse rate	Age	7.47		15.00	<0.001	14.88
	Identity	20.26		4.79	<0.001	40.38
	Condition	0.032	63.99	0.050	0.96	
	Size	-0.12	52.28	-0.53	0.60	
	Condition*Age	-0.014	151.74	-1.24	0.22	
	Size*Age	0.002	149.17	5.61	<0.001	0.004
Pulses per chirp	Age	0.25		12.52	<0.001	4.70
	Identity	0.61		4.74	<0.001	11.38
	Condition	0.049	68.50	0.44	0.66	
	Size	-0.007	53.64	-0.17	0.86	
	Condition*Age	-0.002	121.22	-1.09	0.28	
	Size*Age	0.0004	118.40	5.51	<0.001	0.007

Chirp duration	Age	337.58		4.46	<0.001	29.80
	Identity	337.58		4.46	<0.001	54.31
	Condition	0.97	74.06	0.35	0.73	
	Size	0.39	53.49	0.40	0.69	
	Condition*Age	-0.020	105.63	-0.30	0.76	
	Size*Age	0.0066	101.97	3.07	0.003	0.001
Interchirp duration	Age	18910.52		10.48	<0.001	42.36
	Identity	20055.96		4.14	<0.001	44.93
	Condition	-3.16	86.62	-0.14	0.89	
	Size	1.18	54.90	0.15	0.88	
	Condition*Age	0.59	104.77	0.88	0.38	
	Size*Age	-0.18	101.60	-8.73	<0.001	0.0004
Pulse duration	Age	1.69		9.69	<0.001	8.77
	Identity	1.65		4.16	<0.001	8.54
	Condition	0.21	84.93	0.98	0.33	
	Size	0.0021	55.65	0.029	0.98	
	Condition*Age	-0.0093	84.97	-1.48	0.14	
	Size*Age	0.00007	82.38	0.36	0.72	
Interpulse duration	Age	5.15		10.42	<0.001	11.74
	Identity	4.89		3.94	<0.001	11.14
	Condition	0.038	85.24	0.10	0.92	
	Size	-0.0081	52.06	-0.006	0.99	
	Condition*Age	0.016	99.10	1.45	0.15	
	Size*Age	-0.0010	96.26	-2.84	0.005	0.002
Amplitude	Age	48.53		14.39	<0.001	25.95
	Identity	56.90		4.42	<0.001	30.42
	Condition	0.11	75.50	0.096	0.92	
	Size	-0.24	52.05	-0.59	0.56	
	Condition*Age	0.020	137.81	0.67	0.50	
	Size*Age	0.0052	135.48	5.57	<0.001	0.003
Carrier frequency	Age	27084.10		12.57	<0.001	33.84
	Identity	47078.76		4.60	<0.001	58.82
	Condition	-21.36	72.55	-0.66	0.51	
	Size	-11.26	53.46	-0.98	0.33	
	Condition*Age	0.18	118.28	0.25	0.81	
	Size*Age	0.0049	115.68	0.21	0.84	

Table 2.4. Mean and standard error values for *G. veletis* long-distance acoustic calling parameters at three ages. To account for potential change in the population structure over time, only males who were recorded at all three time points were included (N = 15 males). All calling parameters changed significantly as males aged (see Table 2).

Acoustic parameter	Day 10	Day 30	Day 50
Daily time spent calling (min)	100.82 ± 34.28	259.23 ± 58.64	114.90 ± 45.39
Daily calling effort (PC1)	-0.30 ± 0.40	0.50 ± 0.31	-0.47 ± 0.41
Song duration (min)	3.76 ± 0.94	4.65 ± 0.89	2.52 ± 0.72
Pulse rate (pulses/sec)	31.23 ± 1.52	31.28 ± 1.07	26.49 ± 2.27
Pulses per chirp	3.86 ± 0.27	4.39 ± 0.16	3.88 ± 0.37
Chirp duration (ms)	123.08 ± 7.95	142.20 ± 6.68	144.63 ± 7.83
Interchirp duration (ms)	572.88 ± 70.81	507.26 ± 58.31	739.81 ± 90.69
Pulse duration (ms)	15.18 ± 0.66	16.31 ± 0.44	15.35 ± 0.53
Interpulse duration (ms)	32.07 ± 1.13	33.33 ± 0.73	36.40 ± 0.90
Amplitude (dB)	71.06 ± 4.05	73.76 ± 2.27	66.29 ± 3.54
Carrier frequency (Hz)	5215.03 ± 117.74	5077.82 ± 107.01	4912.32 ± 59.21

Chapter 3: Signaling effort does not predict aggressiveness in male spring field crickets, *Gryllus veletis*

Abstract

The relationship between signaling and aggression has rarely been investigated, even though both types of behaviour are important aspects in determining which males will be successful in mating. However, there is a growing interest in studying signals that predict aggressive behaviour or fighting ability. The goals of our study were three-fold: we investigated the relationship between signaling effort and aggression, the influence of body condition on aggression, and the effect of fighting experience on subsequent signaling behaviour in wild-caught and laboratory-reared male spring field crickets (*Gryllus veletis*). We found that aggressive behaviour was not influenced by signaling effort, body condition, or whether the males were field-captured or lab-reared. Signaling effort was highly repeatable within individuals, but aggressive behaviour had low repeatability. We found no evidence for a winner or loser effect on signaling; there was no change in signaling effort when we compared contest winners and losers before and after they participated in aggressive contests. We suggest that future studies investigate the fine-scale components of acoustic signals produced immediately prior to, during, and immediately following aggressive behaviour to test whether these signals indicate male fighting ability or aggressive intent in crickets. Long-distance acoustic signaling and aggressive behaviour appear to be independent of one another in spring field crickets, perhaps serving different functions in female attraction and male-male competition, respectively.

Introduction

Non-lethal patterns of settling disputes, such as agonistic displays and ritualized fighting, have likely evolved because such behaviours benefit the individuals engaged in the disputes (Maynard Smith and Harper 2003). Aggression is generally regarded as a behavioural strategy for asserting claim to a limited resource, such as a territory or a mate, to increase reproductive success (Cunningham and Birkhead 1998). For agonistic displays to be reliable, only those individuals who can back them up with physical displays should use them. Enquist (1985) suggested that honest signaling can occur in competitive situations due to the potential cost of fighting against a strong individual, contrary to earlier models that emphasized that such displays could not form evolutionary stable strategies due to the ease of bluffing (e.g. Maynard Smith 1974). While selection should favour reliable displays that signal information about the fighting ability or aggressive intent of competitors, low levels of deception are expected to occur (Számadó 2008).

Recently, Searcy and Beecher (2009) highlighted that while there are many animal signals that are thought to function as agonistic displays, there is little empirical evidence that these signals provide information about the fighting ability or aggressive intent of individual signalers. For example, there is mixed, but predominately negative, evidence that song type matching or song overlapping predicts aggression in songbirds (Searcy and Beecher 2009). Notable exceptions to this generalization include changes in call frequency as an honest indicator of fighting ability in frogs (Wagner 1992), and low-amplitude song as a threat signal predicting imminent attack in songbirds (Searcy et al. 2006; Ballentine et al. 2008; Hof and Hazlett 2010). Researchers have thus been

prompted to provide evidence that behaviours we assume to serve as aggressive signals do indeed predict fighting ability or aggressive intent (van Staaden et al. 2011).

Most studies examine the mechanisms of sexual selection in isolation, but this approach can result in a misleading understanding of the total strength and form of sexual selection on male traits (Hunt et al. 2009). Our study focuses on whether acoustic signaling behaviour is related to fighting ability in crickets. It is important to examine how signaling is related to male-male competition to more accurately assess some of the selective forces acting on male signaling. Research on the signaling behaviour of crickets has been extensive, but studies of agonistic behaviour are relatively less common. Further, the evidence for a relationship between long-distance mate attraction signaling and aggression is mixed and comes from only two recent studies. Wilson et al. (2010) found no relationship between signaling effort and aggression in domesticated European house crickets (*Acheta domesticus*). Similarly, Bertram and Rook (in press) found no relationship between signaling effort and aggression in laboratory-reared Jamaican field crickets (*Gryllus assimilis*). However, Bertram and Rook (in press) found a positive relationship between fine-scale components of signaling (such as signal amplitude and frequency) and aggression in laboratory-reared *G. assimilis* males. Because these studies reveal inconsistent patterns between signaling and aggression, there is a need to examine this relationship in other cricket species. Further, given previous studies were performed on domesticated and laboratory-reared individuals, there is an even stronger need to examine this relationship in field-captured individuals to determine whether these relationships occur in nature.

In crickets, both signaling and fighting are important aspects in determining which males will be successful in mating. Male crickets signal acoustically to attract females and repel rival males (Alexander 1961). They rub their raised forewings together; each closing stroke produces a pulse of sound, and pulses are concatenated into chirps (Pfau and Koch 1994). Females use the acoustic properties of the male's long-distance signal to distinguish between potential mates. Female crickets tend to prefer males who invest the most effort into acoustic signaling. For example, female *Teleogryllus commodus* prefer males that signal most often (Hunt et al. 2004). In several cricket species, males that signal most through the night tend to mate most often (Cade and Cade 1992; Rodríguez-Muñoz et al. 2010). Signaling requires a territory, and males frequently compete for territorial signaling sites and access to females using discrete agonistic displays (fights; Alexander 1961; Hack 1997; Hofmann and Schildberger 2001; Jang et al. 2008; Bertram et al. 2011). Winning fights increases male mating success *via* male-male competition and possibly through female choice favouring dominant males (Simmons 1986a). Females of many animal species select dominant males as mates (fish: Doutrelant and McGregor 2000; birds: Mennill et al. 2002; e.g. crustaceans: Aquiloni and Gherardi 2010), and thus fighting ability can also directly influence reproductive success.

Our study had three goals: to investigate the relationship between signaling and aggression, the influence of body condition on aggression, and the effect of fighting experience on subsequent signaling behaviour. First, we address the question of whether high-effort signalers are also highly aggressive in male-male contests or whether there a trade-off between mate attraction ability and fighting ability. Understanding how sexual traits are related can reveal whether genetic quality or trade-offs prevail in the allocation

of energy to sexual behaviours (Andersson and Simmons 2006). Using wild-caught and lab-reared spring field crickets (*Gryllus veletis*), we quantified male signaling behaviour on nights before, during, and after they participated in staged aggressive contests to investigate the relationship between signaling effort and aggressiveness. We predicted that signaling would be an honest indicator of fighting ability, such that males that signaled with high effort also displayed high levels of aggression during male-male contests.

Sexual signals are predicted to exhibit condition-dependent expression (genetic capture hypothesis; Rowe and Houle 1996) whereby males in good condition are able to simultaneously maximize multiple sexual signals. Given that aggressive behaviour is costly (Hack 1997), males in good condition should have more energy to sustain agonistic displays and therefore experience higher success in contests than males in poor condition (Shackleton et al. 2005; Brown et al. 2006). A review of the sexual selection literature revealed that body size is the trait most likely to influence the outcome of both male-male competition and female mate choice (Hunt et al. 2009). Studies in several cricket species have found that larger males and males in better body condition (e.g. larger males, or males with higher size-corrected mass) typically win aggressive contests against smaller opponents (e.g. *A. domesticus*: Hack 1997; *G. pennsylvanicus*: Judge and Bonanno 2008), but other studies have found no relationship between size or condition and aggressiveness (e.g. *G. assimilis*: Bertram et al. 2011). Therefore, our second goal was to examine whether body condition influences aggressive behaviour in *G. veletis*. We predicted that body condition would be related to aggressive behaviour, such that males

in good condition would display higher levels of aggression compared to males in poor condition due to increased resource-holding potential.

Finally, we compared signaling behaviour before and after aggressive contests to ask whether contest outcome affects the signaling behaviour of winners and losers differently. Biogenic amines, such as serotonin and dopamine, have been shown to play a role in aggressive behaviour in invertebrates (e.g. Adamo et al. 1995). A recent study in *G. bimaculatus* found that the experience of winning a fight leads to an increase in motivation *via* the octopaminergic system, an invertebrate equivalent to the adrenergic system of vertebrates (Rillich and Stevenson 2011). We may thus expect differences in signaling behaviour in winners and losers following aggressive contests due to changes in internal chemical balances. We predicted that winners would increase signaling effort following their victory in an aggressive contest, and similarly that losers would decrease signaling effort following their contest loss.

Methods

Experimental animals

We captured adult male and female field crickets (*G. veletis*) in Ottawa, ON, Canada (45°19' N, 75°40' W) in May and June 2008. Field-captured males were held individually for three days prior to participating in trials to record their acoustic signals and also to minimize effects of previous fighting or mating experience. We also used males from the first generation of lab-reared offspring. Late-instar nymph males were removed from the colony and checked daily for adult eclosion. Adult males were individually housed in circular, transparent plastic 540 mL containers. Crickets were

provided with water and food (Harlan Teklad Laboratory Rodent Diet #8604: 24% crude protein, 4% crude fat, 4.5% crude fibre) *ad libitum* and were held in a temperature-controlled room ($30 \pm 2^\circ\text{C}$).

Acoustic recording

We placed males in an electronic acoustic recording system to record their long-distance mate attraction calling (see Bertram and Johnson 1998 for details). The recording system determines when each male signals, how much time he spends signaling, and the duration of his signaling bouts. We recorded males for three nights prior to their first fight, for the three nights following the days they participated in fights, and for three additional nights following their last fight. Lab-reared males were placed in the recording system when they were seven days post-eclosion and field-captured males were recorded beginning the night after their capture. Each day, males were recorded for 18 hours, from 1600h - 1000h. Recording was paused at 1000h to remove males for contests on the appropriate days and to replenish food and water.

Agonistic contests

Males were weighed using a Denver Instruments analytical balance (Pinnacle Series model PI-314; precision ± 0.1 mg). Wild-caught males were weighed the morning after we captured them; lab-reared males were weighed on day nine post-imaginal molt. Males were weight-matched for contests so there was no more than a 25% difference in body mass between opponents. Opponents were weight-matched to increase the probability that they would fight because several studies have shown that opponent size difference can influence aggression and fight outcome in crickets (e.g. Jang et al. 2008).

Immediately following weighing, we placed a dot of coloured nail polish on each male's pronotum to allow for identification during trials.

Each male participated in three fights, one per day for three consecutive days. Thirty-six wild-caught male *G. veletis* were paired in 54 experimental trials and 32 lab-reared males were paired in 48 trials. As part of a separate experiment, a third-party receiver (audience) was placed adjacent to the arena (Fitzsimmons & Bertram, in review). The order of presentation of audience type (male, female, none) was systematically varied between males.

Agonistic trials were conducted in a small Plexiglas arena (15 cm L × 15 cm W × 21 cm H) with two compartments separated by a removable divider and a sand-covered floor. All trials were filmed from above using a colour video camera (Canon Vixia HG10) mounted on a tripod. One male was placed on each side of the divider and given two minutes to acclimatize to the arena and detect the audience. The divider was then removed allowing the males to come into contact. Because agonistic behaviours are initiated after body contact (Jang et al. 2008), we defined a 'contest' as the period of time from when males first came into body contact through to the establishment of clear dominance. Winners are easily identified because they typically chase the loser and often perform post-conflict victory displays (judders/body jerks and aggressive stridulations; Bertram et al. 2010; Logue et al. 2010), while losers retreat upon further contact (Alexander 1961; Hofmann and Schildberger 2001; Judge and Bonanno 2008; Bertram et al. 2010). We defined the end of the contest operationally as when one individual retreated in two consecutive encounters, because we observed instances of dominance reversals in successive encounters in several contests. Cricket contests are typically

comprised of short bursts of combat (termed ‘encounters’) followed by longer periods of relative inactivity (Hack 1997). Thus, a trial was the entire observation period, a contest was the period within a trial in which the crickets were interacting and performing agonistic behaviours, and most contests were comprised of several agonistic encounters before clear dominance was established. Trials were terminated after the conclusion of the contest: once clear dominance was established between males, fighting had ceased, and all post-conflict displays had been completed. Once a trial was completed, the walls of the arena were wiped with 95% ethyl alcohol, and the sand raked to minimize the chance of pheromones affecting future fights (Judge and Bonanno 2008).

We conducted trials between 1000h and 1200h. Calling and mating activity has been shown to peak at and after dawn in several cricket species, including *G. veletis* (French and Cade 1987), and thus the morning represents a time of day when motivation to fight is expected to be high.

The duration and frequency of all agonistic behaviours were scored for each individual by replaying the videos in slow motion on a monitor. Our scale of aggressive behaviours ranged from zero to four: withdraw = zero; antennal fencing = one; kick = two; mandible spread, chase, mandible engagement, or bite = three; and grapple = four (Jang et al. 2008; Bertram et al. 2010; Bertram et al. 2011).

For each male, we quantified three values of aggressiveness per trial: total aggression score, time spent performing aggressive behaviours, and aggression intensity score (Bertram et al. 2010; Bertram et al. 2011). For total aggression score, we calculated the time each male spent performing various aggressive acts and then multiplied the

aggression score for each behaviour by the total number of seconds that behaviour was performed, resulting in weighted aggression scores for each act. These weighted aggression scores were then summed across all behaviours each individual performed in a contest, resulting in a total aggression score for each male for each trial. To calculate aggression intensity score, we divided total aggression scores by the contest duration to account for variable contest duration.

Body size measures

We measured head width, pronotum length, and pronotum width for each cricket following natural death. Measurements were taken using a Zeiss Axio Observer inverted dissecting microscope and highly magnified photographs (AxioVision v4.8, Carl Zeiss, Jena, Germany; magnification approx. $8.5\times$, resolution $\sim 1.60\ \mu\text{m}$).

Statistical methods

Due to colinearity of the calling variables, the aggression metrics, and the body size metrics, we used principle components analysis (PCA) to reduce the number of factors in our models (Table 3.1). We also performed analyses involving condition with the more commonly-used residuals of mass on body size. Our results did not differ when analyzed with residuals of mass, and given the problems associated with use of residuals as a response variable (Freckleton 2002), we present results obtained from PCA scores only.

We assessed within-individual variation in calling and aggression scores by quantifying the repeatability of the PC using intraclass correlation coefficients following Falconer and Mackay (1996). Repeatability scores were calculated from the mean square

values generated by ANOVA with individual as the predictor variable. Repeatability scores above 0.50 were considered high (Boake 1989).

To assess whether calling parameters were related to aggression metrics, we took mean values of the calling principle components (PC) scores over three nights for each period (pre-fight, fight, post-fight) for each male, and the mean of each male's aggression PC scores from their three fights. We then built general linear models (GLM) to examine whether aggression was influenced by calling metrics, whether the males were field-captured or lab-reared, and/or male body condition. We performed separate GLM for each calling period (pre-fight, fight, post-fight).

Using matched-pairs analysis, we examined whether calling parameters changed from the night before males first fought to the night after their first fight and whether this difference was influenced by whether the males won or lost their fight.

We performed statistical analyses in JMP v9.0.2 (SAS, Cary, NC, USA). Data are presented as mean \pm standard error.

Results

Over the course of the experiment, males signaled acoustically for 60.14 ± 3.45 minutes per night, with an average of 11.95 ± 0.47 signaling bouts per night, and average signaling bout duration of 4.42 ± 0.30 minutes. Three males never signaled, and the three highest effort signalers spent more than five hours per night signaling. Full descriptive statistics are presented in Table 3.2. Signaling PC scores were highly repeatable within individuals ($r = 0.62$).

Males displayed aggressive behaviour in 78% of trials. Fights averaged 181.7 ± 14.5 s in duration and males spent 87.0 ± 9.8 s performing aggressive behaviours during contests (Table 3.2). Aggression PC scores were moderately repeatable within males across fights ($r = 0.32$).

Our GLM revealed that aggression scores were not influenced by signaling scores, body condition, or whether males were field-captured or lab-reared (Figure 3.1; Pre-fight GLM: whole model: $r^2_{\text{adj}} = 0.013$, $F_{4,61} = 1.21$, $P = 0.32$; Fight GLM: whole model: $r^2_{\text{adj}} = -0.040$, $F_{4,61} = 0.38$, $P = 0.82$; Post-fight GLM: whole model: $r^2_{\text{adj}} = -0.026$, $F_{4,61} = 0.59$, $P = 0.67$).

We found that time spent signaling did not change from the night before fighting to the night after fighting, and was not influenced by whether males won or lost their fight (Figure 3.2; matched-pairs t-test: $t = 1.49$, $P = 0.14$, $df = 53$; winner/loser: $F = 0.009$, $P = 0.92$). Similarly, there was no significant difference in the number of signaling bouts (matched-pairs t-test: $t = -0.31$, $P = 0.76$, $df = 53$; winner/loser: $F = 0.57$, $P = 0.45$) nor signaling bout duration (matched-pairs t-test: $t = 1.53$, $P = 0.13$, $df = 53$; winner/loser: $F = 0.016$, $P = 0.90$).

Discussion

Our results indicate that signaling effort is not related to aggressive behaviour in spring field crickets. We found neither a positive relationship, which would indicate an honest signaling scenario (Zahavi 1975; Zahavi 1977), nor a negative relationship, which would indicate a potential trade-off between investment in signaling and aggression. Our results are consistent with those of Wilson et al. (2010) and Bertram and Rook (in press)

who found no relationship between signaling effort and aggression in European house crickets (*A. domesticus*) and Jamaican field crickets (*G. assimilis*), respectively. Together these findings suggest that signaling effort is not indicative of aggressive behaviour in crickets.

In our examination of signaling, we only quantified gross temporal aspects of acoustic signaling (total time spent signaling, number of bouts, bout duration). It is possible that fine-scale aspects of the signals, including temporal and frequency characteristics, may contain information about fighting ability. Bertram and Rook (in press) revealed a positive relationship between the fine-scale components of signals and aggression in *G. assimilis*. Male *G. assimilis* that produce more attractive, higher effort signals (e.g. with more pulses per chirp, lower carrier frequency, and louder chirps) are more aggressive in male-male contests (Bertram and Rook in press). To date, this is the only study to explore the relationship between fine-scale signal components and aggression in crickets. Future work is required to determine if Bertram and Rook's finding is indicative of a general pattern in crickets or whether the relationship varies between species.

A second avenue for future research is to examine the signals produced immediately prior to agonistic interactions to address whether cricket acoustic signals communicate aggressive intent. This is an exciting and growing area of current research in animal behaviour (Searcy and Beecher 2009; van Staaden et al. 2011), and studies of multiple signaling systems across taxa will improve our understanding of the functions of agonistic signals. For example, it is possible that features of aggressive songs produced immediately prior to an aggressive contest predict a male's aggressiveness, such as the

number of songs, the fine-scale components of the songs (Wagner 1992), or the song amplitude (Hof and Hazlett 2010) predict a male's aggressiveness in crickets.

The results of our repeatability analyses reveal that while signaling behaviour is highly repeatable within individuals, aggressive behaviour is not. Our finding lies in direct contrast to a meta-analysis of the repeatability of various animal behaviours that concluded that aggression is one of the most repeatable behaviours for individuals across species (Bell et al. 2009). Since repeatability of aggression was low in our study, the lack of predictive relationship between signaling effort and aggression is perhaps unsurprising. Behaviours that are influenced by energy stores or the social environment may be relatively variable compared to behaviours under morphological or physiological constraint (Bell et al. 2009). In crickets, Briffa (2008) found that male *A. domesticus* that won fights had higher muscular glucose levels than losers, and a recent study revealed that male *G. veletis* display different levels of aggressive behaviour in different social environments (Fitzsimmons and Bertram in review). Thus aggressive behaviour may be phenotypically plastic and highly variable within individuals, depending largely on both intrinsic and external factors. In order to estimate a more controlled metric of aggression, we suggest that future studies consider using techniques that remove confounding effects such as opponent size, condition, and behaviour, perhaps by assessing male aggression in simulated contests using a mirror instead of an actual opponent (e.g. Desjardins and Fernald 2010).

Our finding that winners and losers did not change their signaling behaviour when comparing the night before they fought to the night after their first fight is also perhaps unsurprising in light of the low repeatability of aggression scores and the high

repeatability of signaling scores. Further, we compared total signaling behaviour over an 18 hour period before and after fights, whereas a winner or loser effect may be apparent on a much shorter time-scale. For example, winner effects in *G. bimaculatus* disappear after 20 minutes (Rillich and Stevenson 2011), and both winner and loser effects last only an hour or two in jumping spiders (*Phidippus clarus*; Kasumovic et al. 2010).

Traditionally, the winner and loser effects that are studied after aggressive contests are on subsequent aggressive behaviour, but we predicted that an aggressive experience might also have effects on subsequent signaling behaviour. In future studies we will examine the signaling behaviour of contest participants, including metrics of both signaling effort and fine-scale properties of signals, immediately following aggressive contests to examine whether there is a short-term winner or loser effect on signaling behaviour.

The ‘aggressive songs’ of crickets are produced prior to and throughout agonistic contests, and recently Logue et al. (2010) revealed that the use of signals during agonistic interactions mitigates the costs of fighting. We investigated a separate acoustic signal, the long-distance mate attraction signal, but it is possible that aggressive songs, which are used only in the context of male-male contests, are more relevant to the question of whether the signals contain information about fighting ability or aggressive intent. Brown et al. (2006) studied aggressive songs in *A. domesticus* and found that temporal aspects of songs were indicative of male body size, and that male size affected the intensity and outcome of fights. Our result that body condition did not influence aggression lies in contrast to the majority of studies in crickets that show a positive relationship between body condition and aggression (e.g. *A. domesticus*: Hack 1997; *G. pennsylvanicus*: Judge and Bonanno 2008). However, the evidence is certainly not unequivocal on this

relationship; several other studies have found no relationship between size or condition and aggressiveness (e.g. *G. assimilis*: Bertram et al. 2011). Similarly, the evidence for a relationship between male size or condition and signaling effort is mixed. In a previous study of *G. veletis*, Fitzsimmons and Bertram (2011) found that neither male size nor condition influenced the fine-scale components of signaling or signaling effort, while Bertram and Rook (in press) and Judge et al. (2008) found that males in good condition (higher residual mass, and larger males raised on a high quality diet, respectively) called with higher effort than males in poor condition in *G. assimilis* and *G. pennsylvanicus*. In sum, and in contrast to our prediction, it appears that body condition does not influence aggressive behaviour (present study) or signaling behaviour (Fitzsimmons and Bertram 2011) in spring field crickets. Long-distance acoustic signaling and aggressive behaviour appear to be independent of one another in spring field crickets, perhaps serving different functions in the two arenas of sexual selection, female attraction and male-male competition.

Figures

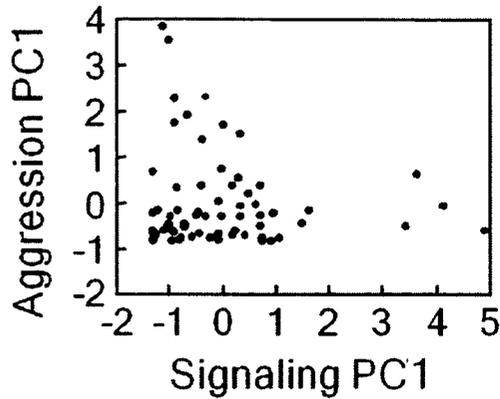


Figure 3.1. We found no relationship between male signaling behaviour and aggressive behaviour in *Gryllus veletis*. This plot shows mean signaling PC scores for the three nights before males fought, and typifies the lack of relationships found in the fight nights and the post-fight nights.

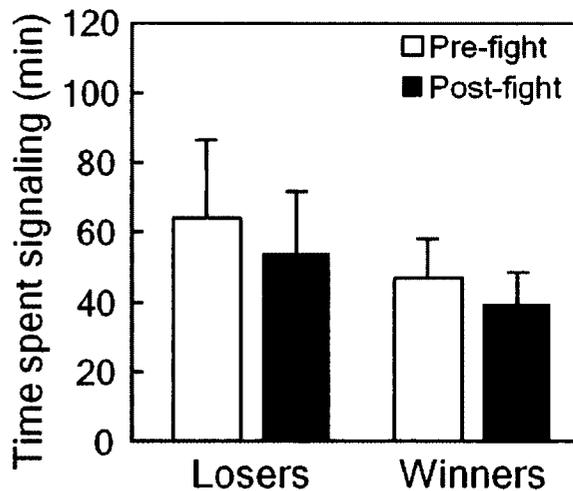


Figure 3.2. Neither losers nor winners changed their signaling behaviour after participating in an aggressive contest. Values represent total time spent signaling (mean \pm SE) for the night immediately preceding and following the first day of aggressive contests.

Tables

Table 3.1. Principle component analysis results for signaling, aggression, and body size metrics.

PCA	PC	% variance explained	Factors	Loadings
Signaling	PC1	63	Time spent calling	0.70
			Bout duration	0.56
			Number of bouts	0.46
	PC2	30	Number of bouts	0.79
			Bout duration	-0.61
Aggression	PC1	94	Time spent calling	-0.02
			Total aggression score	0.71
			Time spent aggressive	0.71
Body condition	PC1	88	Mass	0.51
			Head width	0.50
			Pronotum length	0.50
			Pronotum width	0.50

Table 3.2. Metrics of signaling effort and aggressive behaviour. Signaling was recorded for males aged 7 - 15 days post-eclosion for 18 hours per day (1000 - 0400h), and aggressive behaviour was scored for three fights per male conducted on three consecutive days.

Behavioural parameter	Mean \pm SE	Median	Minimum	Maximum
Time spent calling (min)	60.14 \pm 3.45	24.88	0	539.97
Number of signaling bouts	11.95 \pm 0.47	9	0	77
Signaling bout duration (min)	4.42 \pm 0.30	2.18	0	94.95
Total aggression score	152.3 \pm 20.74	41.5	0	2227
Time spent performing aggressive behaviours (s)	87.0 \pm 9.80	25	0	821
Aggression intensity score (adjusted for variable contest duration)	0.82 \pm 0.084	0.32	0	8.83

Table 3.3. Results of general linear models indicating there is no effect of signaling effort, body condition, or rearing environment (field or lab) on aggressiveness (PC1) in *G. veletis*.

Time period	r^2_{adj}	F	P	Effects	F	P
Pre-fight	0.013	1.21	0.32	Signaling PC2	3.90	0.053
				Body condition	0.30	0.58
				Field or lab	0.19	0.66
Fight	-0.040	0.38	0.82	Signaling PC2	0.032	0.86
				Body condition	0.87	0.35
				Signaling PC1	0.38	0.54
				Signaling PC2	0.19	0.66
Post-fight	-0.026	0.59	0.59	Field or lab	0.058	0.81
				Signaling PC1	1.42	0.24
				Body condition	0.76	0.39
				Field or lab	0.22	0.64
				Signaling PC2	0.020	0.89

Chapter 4: Playing to an audience: Aggressiveness and victory displays vary with the sex of audience members

Abstract

Studies of animal behaviour have recently begun to incorporate the influence of the social environment, providing new opportunities for the study of signaling strategies and signal evolution. While there is some evidence in vertebrate taxa that audiences gather information from social interactions and change the behaviour of signallers, little is known about whether audiences influence behaviour in invertebrates. We examined whether the presence and sex of an audience influenced contest and post-contest behaviour in male field crickets (*Gryllus veletis*). Males were more aggressive in the presence of a female audience, and produced more victory display behaviour in the presence of a male audience. Thus, the sex of the audience may impose different costs and benefits for competing males depending on the behaviour observed. Our results suggest that male crickets dynamically adjust their contest behaviour to gain a reproductive benefit *via* female eavesdropping and may also deter future aggression from potential rivals *via* the advertisement of the contest victory.

Introduction

The social environment may provide a template for the action of social and demographic factors on the evolution of animal behaviours (Moore et al. 1997; Danchin et al. 2004; Powers et al. 2011; Perrin et al. 2012). In nature, many animals engage in signaling contests for dominance, territories, resources, and mates, and contests often occur within a communication network with several signalers and receivers within range of one another (McGregor 2005). The communication network model expands the scope

of studies of animal contest behaviours, including eavesdropping (Peake 2005), victory displays (Bower 2005), and audience effects (Matos and Schlupp 2005). Of particular interest here is audience effects, defined as changes in signaling behaviour during interactions caused by the mere presence of an audience: an individual that is present during, but does not directly take part in, signaling interactions between others (Matos and Schlupp 2005). An audience may introduce extra costs or benefits to signalers (Zahavi 1979); for example, eavesdropping fish are more likely to initiate aggressive interactions with a loser than a winner (Oliveira et al. 1998), imposing an immediate cost to the loser, and female fish and birds may copy the mate choice of other females, resulting in mating benefits to the chosen male (Galef Jr and White 1998; Godin et al. 2005).

Because animal contests are costly (injury risk, predation risk, energy expenditures, lost opportunities; Riechert 1988), attending to and gaining information from interactions between others (eavesdropping; Peake 2005) is a relatively low-cost strategy that allows individuals to modify their behaviour toward the participants and potentially avoid the costs of conflict. Game theory models predict that eavesdroppers influence the costs and benefits of contests, potentially enhancing the value of victory and promoting aggressive behaviour (Johnstone 2001), or alternatively reducing the frequency of aggressive behaviour, depending on model assumptions (Mesterton-Gibbons and Sherratt 2007). Quantitative genetic approaches highlight the importance of considering the social environment as a potential indirect genetic effect on interacting phenotypes; the evolution of traits, such as aggression, whose expression is influenced by interactions with other individuals can be enhanced or inhibited depending on the nature

of the social environment (Moore et al. 1997). Because contest behaviour is necessarily expressed in a social context, our understanding of behavioural strategies and the costs and benefits of conflict will be enhanced if we consider the potential effects of eavesdroppers and audiences. Audiences may be important not only in influencing the costs and benefits to individual signallers in daily interactions, but also as a evolutionary force acting on the form and content of signals and signaling strategies (Matos and Schlupp 2005).

Studies of audience effects are rare despite their potential influence on signaling strategies of individuals. The few studies that have investigated the influence of the social environment on signaling behaviour have found audience effects in several contexts. For example, male domestic chickens (*Gallus gallus domesticus*) increase alarm call rate in the presence of an audience (Gyger et al. 1986), and also produce more food calls when a female audience is present than with a male audience or no audience (Marler et al. 1986). A male audience influences male mate choice in Atlantic molly (*Poecilia mexicana*), such that males change their female preference in the presence of a potential competitor (Plath et al. 2008). In vervet monkeys (*Cercopithecus aethiops*), a species with biparental care, males increase infant care when a female is present, and females in turn prefer males they have observed displaying care (Hector et al. 1989). Although the social context of interactions can affect animal behaviour, we still know little about how individuals assess and respond to different types of audiences in different behavioural contexts. Such information should enhance our understanding of the selective forces that shape behavioural variation within social networks. Further, because existing studies

have only focused on vertebrates, the ubiquity and diversity of audience effects is not yet known.

Here we provide the first investigation of audience effects on contest and post-contest behaviour in an invertebrate, the spring field cricket (*Gryllus veletis*). Male field crickets frequently engage in aggressive contests (fights) over resources, which comprise a series of discrete and easily scored behaviours that increase in aggression level and energy expenditure as the contest progresses (Alexander 1961; Hofmann and Schildberger 2001; Jang et al. 2008). Winning a contest increases a male's mating success either through male-male competition by establishing dominance and providing access to mate attraction territories (Alexander 1961) or *via* female choice for dominant males (Simmons 1986a). Cricket contest winners often advertise their success *via* victory displays that incorporate the use of aggressive songs and body jerks (Alexander 1961; Tachon et al. 1999; Jang et al. 2008; Logue et al. 2010). Given cricket densities can be high in field crickets (Alexander 1961; Ritz and Köhler 2007) and mate attraction signaling and male-male contests occur in close proximity (Rodríguez-Muñoz et al. 2011), many contests are likely to occur with female and male audiences nearby.

Few studies have focused on how animals behave after signaling contests, despite the significant consequences that post-conflict behaviour may have on the individuals involved in the contest and on bystanders. Victory displays are behaviours performed by contest winners after the conclusion of contests; these displays may serve several functions, including reinforcing the victory to the loser and highlighting the victory for bystanders, such as prospective breeding partners and potential rivals (Bower 2005). Recent evidence suggests that aggressive songs and body jerks function as victory

displays in crickets (Bertram et al. 2010), but whether such displays serve to reinforce the dominance of the winner (browbeating) or to communicate the victory to nearby prospective mates or potential rivals (advertising; Bower 2005; Mesterton-Gibbons and Sherratt 2006) or both is unknown. Following cricket contests we compared the behaviour of winners to examine whether audiences influence victory displays.

Methods

Experimental animals

We captured adult male and female field crickets (*G. veletis*) in Ottawa, ON, Canada (45°19' N, 75°40' W) in May and June 2008. Because captured males were of unknown age, mating status, and fighting status, they were held individually for three days prior to participating in trials to minimize effects of previous experience. We also used males from the first generation of lab-reared offspring. Late-instar nymph males were removed from the colony and checked daily for adult eclosion. Adult males were individually housed in circular, transparent plastic 540 mL containers. Crickets were provided with water and food (Harlan Teklad Laboratory Rodent Diet #8604: 24% crude protein, 4% crude fat, 4.5% crude fibre) *ad libitum* and were held in a temperature-controlled room (30 ± 2°C).

Agonistic contests

Agonistic trials were conducted in a small Plexiglas arena (15 cm L × 15 cm W × 21 cm H) with two compartments separated by a removable divider and a sand-covered floor. A third-party receiver (audience) was placed adjacent to the arena. The audience was separated from the subject males by a transparent partition, which contained small

holes to allow visual, olfactory, and auditory information to be transmitted between the audience and the subject males. The interacting males were pre-exposed to the audience for 2 minutes, and then the divider was removed to allow interaction between subject males. The pre-exposure period was intended to ensure that the males were aware of the presence of an audience (e.g. Matos and McGregor 2002). All trials were filmed from above using a colour video camera (Canon Vixia HG10) mounted on a tripod. We conducted trials between 1000h and 1200h. Calling and mating activity has been shown to peak at and after dawn in several cricket species, including *G. veletis* (French and Cade 1987), and thus the morning represents a time of day when motivation to fight is expected to be high.

Males were weighed using a Denver Instruments analytical balance (Pinnacle Series model PI-314). Wild-caught males were weighed the morning after we captured them; lab-reared males were weighed on day nine post-imaginal molt. Males were weight-matched so there was no more than a 25% difference in body mass between opponents. Opponents were weight-matched to increase the probability that they would fight because several studies have shown that opponent size difference can influence aggression and fight outcome in crickets (e.g. Jang et al. 2008). Immediately following weighing, we placed a dot of coloured nail polish on each male's pronotum to allow for identification during trials. Males were paired in agonistic trials beginning the day after they were weighed and marked, and they participated in one trial per day for three consecutive days (one trial per audience type, each male with a different opponent). The order of presentation of audience type (male, female, none) was systematically varied between males.

Thirty-six wild-caught male *G. veletis* were paired in 54 experimental trials and 32 lab-reared males were paired in 48 trials. One male was placed on each side of the divider and given two minutes to acclimatize to the arena and detect the audience. The divider was then removed allowing the males to come into contact. Because agonistic behaviours are initiated after body contact (Jang et al. 2008), we defined a ‘contest’ as the period of time from when males first came into body contact through to the establishment of clear dominance. Winners are easily identified because they typically chase the loser and often perform post-conflict victory displays (judders/body jerks and aggressive stridulations; Bertram et al. 2010; Logue et al. 2010), while losers retreat upon further contact (Alexander 1961; Hofmann and Schildberger 2001; Judge and Bonanno 2008; Bertram et al. 2010). We defined the end of the contest operationally as when one individual retreated in two consecutive encounters, because we observed instances of dominance reversals in successive encounters in several contests. Cricket contests are typically comprised of short bursts of combat (termed ‘encounters’) followed by longer periods of relative inactivity (Hack 1997). Thus, a trial was the entire observation period, a contest was the period within a trial in which the crickets were interacting and performing agonistic behaviours, and most contests were comprised of several agonistic encounters before clear dominance was established. Trials were terminated after the conclusion of the contest: once clear dominance was established between males, fighting had ceased, and all post-conflict displays had been completed. Once a trial was completed, the walls of the arena were wiped with 95% ethyl alcohol, and the sand raked to minimize the chance of pheromones affecting future fights (Judge and Bonanno 2008).

The duration and frequency of all agonistic behaviours were scored for each individual by replaying the videos in slow motion on a monitor. Our scale of aggressive behaviours ranged from zero to four: withdraw = zero; antennal fencing = one; kick = two; mandible spread, chase, mandible engagement, or bite = three; and grapple = four (Adamo and Hoy 1995; Jang et al. 2008; Bertram et al. 2010; Bertram et al. 2011). We also noted when males performed aggressive stridulation and body jerks, behaviours thought to function as victory displays (Bertram et al. 2010; Logue et al. 2010), and they were scored as three and one, respectively (Jang et al. 2008).

For each male, we quantified three values of aggressiveness per trial: total aggression score, time spent performing aggressive behaviours, and aggression intensity score (Bertram et al. 2010; Bertram et al. 2011). For total aggression score, we calculated the time each male spent performing various aggressive acts and then multiplied the aggression score for each behaviour by the total number of seconds that behaviour was performed, resulting in weighted aggression scores for each act. These weighted aggression scores were then summed across all behaviours each individual performed in a contest, resulting in a total aggression score for each male for each trial. To calculate aggression intensity score, we divided total aggression scores by the contest duration to account for variable contest duration. We similarly calculated total victory display aggression scores and aggression intensity scores for behaviours performed by winners following the conclusion of the contests.

Statistical methods

We used generalized linear mixed models (GLMM) with gamma error structure and log link function to examine the factors influencing total aggression, time spent being aggressive, and aggression intensity. Male identity was included as a random effect to control for multiple contests per individual. Wald F tests were used to calculate P values (Bolker et al. 2009). The explanatory variables were audience (male, female, or none), participant (winner or loser), and the interaction audience*participant.

To address whether audience type was related to contest length, we ran a generalized linear model (GLM) with Gamma error structure and log link function, and tested for the effect of audience type with Wald χ^2 .

Wild-caught and lab-reared males did not differ significantly in three of the four metrics of aggression: contest length ($F_{1, 66} = 0.050$, $P = 0.824$), total aggression ($F_{1, 66} = 0.102$, $P = 0.751$), and time spent aggressive ($F_{1, 66} = 0.052$, $P = 0.821$). Data were pooled from contests of wild-caught and lab-reared males for these three metrics. Aggression intensity, a measure of aggression controlling for variable contest lengths, was significantly higher for wild-caught than lab-reared males ($F_{1, 66} = 8.46$, $P = 0.0049$), and we thus ran two separate GLMM for this metric.

To analyze victory display behaviour, we used GLM with Gamma error structure and log link functions. We tested for the effect of audience type on total victory display aggression, victory display length, and victory display aggression intensity with Wald χ^2 .

We performed statistical analyses in JMP v9.0.2 (SAS, Cary, NC, USA) and SPSS Statistics v.20.0.0 (IBM, Armonk, NY, USA).

Results

Males displayed aggressive behaviour in 78% of trials, and they were neither more nor less likely to fight in the three audience conditions ($\chi^2 = 0.80$, $P = 0.67$, $N = 102$, $DF = 2$). However, the total aggression males displayed during contests was explained by audience type and whether the male was the winner or loser of the contest (GLMM: Winner/Loser $F_{2, 195} = 252.26$, $P < 0.001$; Audience $F_{2, 195} = 3.94$, $P = 0.021$). Similarly, the time males spent performing aggressive behaviours was explained by audience type and whether the male was the winner or loser (Winner/Loser $F_{2, 195} = 282.53$, $P < 0.001$; Audience $F_{2, 195} = 3.34$, $P = 0.037$). For both of these metrics, males performed between 54% and 84% more aggressive behaviour when a female audience was present than with a male audience or no audience (Figure 4.1), and winners were more aggressive than losers. Contest duration was not influenced by audience type (GLM: Wald $\chi^2 = 2.16$, $P = 0.340$). Aggression intensity, a measure that controls for variable contest duration, was influenced by audience type in wild-caught males only (GLMM: Audience $F_{2, 100} = 4.41$, $P = 0.015$), and whether the male was the winner or loser in both wild-caught and lab-reared males (GLMM: Winner/Loser $F_{2, 100} = 122.02$, $P < 0.001$; Winner/Loser $F_{2, 87} = 627.64$, $P < 0.001$, respectively).

Winners performed victory displays after 98% of the aggressive trials. Males were neither more nor less likely to perform victory displays in the three audience conditions (Fisher exact test: $P = 0.21$), but the amount of victory display behaviour was explained by audience type (GLM: Wald $\chi^2 = 6.92$, $P = 0.031$). Males displayed 55% and 64% more victory display behaviour in the presence of a male audience than in the presence of a female audience or no audience, respectively (Figure 4.2). The duration and intensity of

victory displays were not however related to audience type (GLM: Wald $\chi^2 = 3.67$, $P = 0.16$; Wald $\chi^2 = 1.42$, $P = 0.49$, respectively).

Discussion

If female crickets use information from male contests to make mating decisions and prefer contest winners, as in other taxa (e.g. Mennill et al. 2002), then the incentive for males to display more aggressively with a female audience may be higher than with a male audience or no audience due to the potential reproductive gain. An audience effect on victory display behaviour suggests that male crickets perform victory displays to advertise their victory to potential rival males, potentially reducing the likelihood of audience males initiating contests in the future (Mesterton-Gibbons and Sherratt 2006). Whether audience males gain information through eavesdropping on signaling interactions in crickets is unknown, but our results suggest a potential payoff for both victorious males and eavesdropping males if audiences do indeed avoid future costly physical conflict with observed contest winners.

Our study provides the first evidence that invertebrates modify their contest behaviour in the presence of an audience. The ability to perceive the presence and sex of an audience and adjust behaviour accordingly is thus not restricted to vertebrates and may be more common across animal taxa than previously recognized. Our study also provides the first evidence of an audience effect on victory display behaviour. Our results confirm previous observations of increased aggression in the presence of a female in *G. bimaculatus* (Tachon et al. 1999), and support the idea that the sex of the audience is important to aggressive interactions (e.g. Matos and McGregor 2002). Audiences may also influence signal evolution, potentially driving the evolution of separate signals for

public and private information transfer (Dabelsteen 2005), and favouring flexible behavioural strategies (plasticity or bet hedging). Because most animal behaviour occurs in a social context, indirect genetic effects arising from the social environment may provide a source of heritable variation on which selection can act (Moore et al. 1997; Wilson et al. 2009). To form biologically-relevant models of animal behaviour and study the evolution of signals and signaling strategies, we must explicitly consider the social environment and incorporate the costs and benefits of signaling in the presence of audiences (Johnstone 2001).

Figures

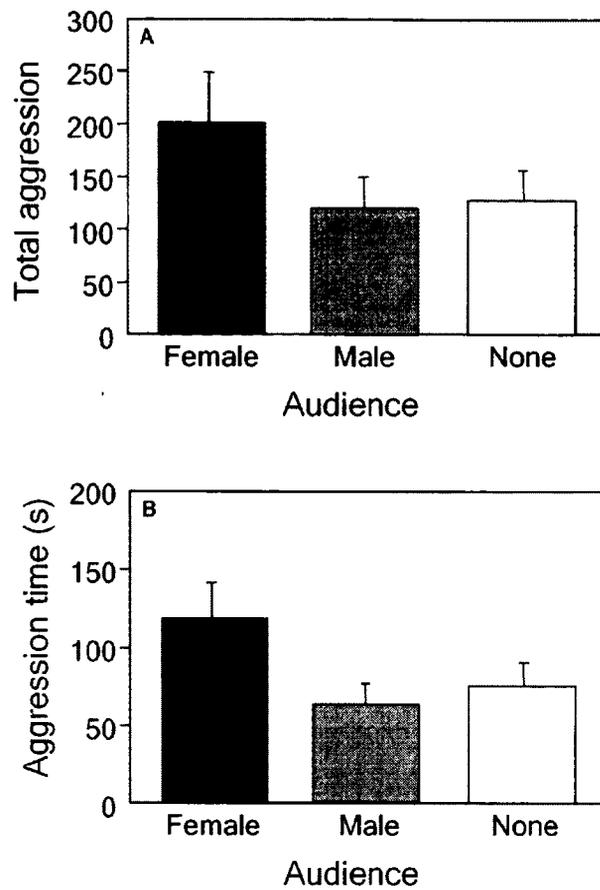


Figure 4.1. Males displayed the highest level of aggression (A) and spent more time performing aggressive behaviours (B) in contests with a female audience than with a male or no audience (means \pm SE).

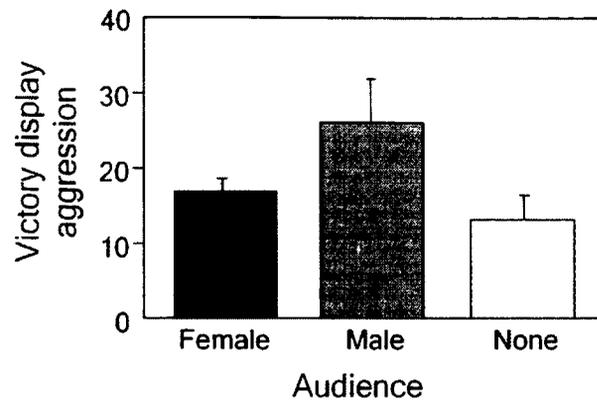


Figure 4.2. Males displayed the highest levels of victory display behaviour after contests with a male audience than with a female or no audience (means \pm SE).

Chapter 5: No relationship between long-distance acoustic mate attraction signals and sperm quality in spring field crickets (*Gryllus veletis*)

Abstract

To father offspring a male must succeed at two processes of sexual selection: 1) mate with a female, and 2) fertilize her eggs. The relationship between pre- and postcopulatory sexual selection is the subject of much debate. In many animal taxa, females mate multiply and receive nothing but sperm and seminal fluid from their mating partners, which can lead to sexual selection continuing beyond copulation in the form of sperm competition. The sperm competition hypothesis and the phenotype-linked fertility hypothesis predict opposite association patterns between precopulatory and postcopulatory sexually selected traits. Empirical studies have found evidence consistent with both hypotheses, highlighting the difficulty in predicting the relationship between investment in mate acquisition and sperm quality. We investigated the relationship between the quality and quantity of acoustic mate attraction signals and sperm quality in wild-captured and laboratory-reared spring field crickets, *Gryllus veletis*. We found no relationship between pre- and postcopulatory traits, providing no evidence for either the sperm competition or phenotype-linked fertility hypothesis. It appears that while female crickets may receive benefits by choosing males based on acoustic signal characteristics, whether the benefits are a result of genetic quality, seminal fluid contents, or some other male trait remains unknown.

Introduction

Behavioural studies of sexual selection tend to focus on events that lead up to copulation and the transfer of sperm, and consequently we know most about how the selective forces prior to mating act on female choice and male-male competition. However, females in many species mate multiply, which can lead to sexual selection continuing beyond copulation *via* sperm competition and cryptic female choice (Parker 1970; Eberhard 1996; Simmons 2001). Thus, male mating success does not necessarily reflect reproductive success in promiscuous species where paternity success is influenced by traits related to both precopulatory mate attraction and postcopulatory fertilization success. Postcopulatory sexual selection can counteract mating success (e.g. Preston et al. 2001) or can amplify mating success (e.g. Thomas and Simmons 2009) which may lead to inaccurate estimates of total sexual selection (Hunt et al. 2009).

The sperm competition hypothesis and the phenotype-linked fertility hypothesis predict opposite association patterns between precopulatory and postcopulatory sexually selected traits. Sperm competition predicts a negative association between traits involved in mate acquisition and sperm quality due to the allocation of limited resources to these traits (Parker 1998). Conversely, phenotype-linked fertility predicts that mate attraction traits and sperm quality will covary positively and females select for exaggerated male phenotypes to ensure fertilization success (Sheldon 1994). Empirical studies have found evidence consistent with both hypotheses, adding to the difficulty in predicting the relationship between investment in mate acquisition and sperm quality. For example, in support of sperm competition, Simmons et al. (2011) found that men with higher pitched (less attractive) voices had lower sperm concentrations in their ejaculates, Rowe et al.

(2010) found a trade-off between plumage colouration and sperm number and motility in red-backed fairy wrens (*Malurus melanocephalus*), and Demary and Lewis (2007) found a trade-off between bioluminescent signaling and paternity in fireflies (*Photinus greeni*). Conversely, in support of phenotype-linked fertility, Beausoliel et al. (in press) found males with more red colouration have higher sperm velocity in redbreast dace (*Clinostomus elongates*), Ruther et al. (2009) found pheromone strength correlates with sperm number in parasitic wasps (*Nasonia vitripennis*), and Skinner and Watt (2007) found that larger males produce longer sperm in guppies (*Poecilia reticulata*). Several studies have also found no relationship between mate attraction and sperm traits. For example, blue nuptial colouration is not related to sperm number in Moor frogs (*Rana arvalis*; Hettyey et al. 2010), and song characteristics are not related to sperm number or morphology in sedge warblers (*Acrocephalus schoenobaenus*; Birkhead et al. 1997). There is thus a lack of consensus across taxa about the association patterns between precopulatory and postcopulatory sexual traits.

Here we investigate the relationship between long-distance mate attraction signals and sperm traits in the spring field cricket, *Gryllus veletis*. Male crickets signal acoustically to attract females and repel rival males (Alexander 1961). Females use the acoustic properties of the male's long-distance signal to distinguish between potential mates, and tend to prefer males who invest the most effort into acoustic signaling (e.g. Cade and Cade 1992; Hunt et al. 2004; Rodríguez-Muñoz et al. 2010). Crickets provide a useful system to study pre- and postcopulatory traits because females mate multiply, female receive only ejaculates from their mates, and we know how ejaculate quality influences fertilization success in several species (e.g. Sakaluk and Eggert 1996; Schaus

and Sakaluk 2001). Previous studies examining the relationship between precopulatory mate attraction traits and sperm traits in crickets have found mixed results in terms of the patterns of association between traits (Table 5.1). In some species, males experience trade-offs between sperm quality and traits such as song attractiveness and limb asymmetry (Mallard and Barnard 2003), providing support for the sperm competition hypothesis. Conversely, several other studies have found positive associations between sperm traits and traits such as male body size (Sakaluk 1985; Simmons 1986b), attractiveness (Bussière et al. 2006), and dominance, providing support for the phenotype-linked fertility hypothesis (Thomas and Simmons 2009; Table 5.1). Finally, Ketola et al. (2007) found no relationship between courtship song rate and spermatophylax size. In sum, it appears there is no clear trend between mate attraction traits and sperm traits across cricket species.

The only study to date to investigate the relationship between long-distance acoustic signals and sperm traits in crickets was performed on the domesticated European house cricket (*Acheta domesticus*; Klaus et al. 2011). Klaus et al. (2011) examined sperm number, sperm viability, and the number of living sperm in *A. domesticus* and found no relationships between sperm traits and long-distance acoustic signal parameters. However, they found that lighter males produced significantly more sperm than heavy males, suggesting that the reproductive success of light males may depend on postcopulatory sexual traits whereas the success of heavy males is related to precopulatory sexual traits. Since the Klaus et al. (2011) study focused on domesticated crickets, we know relatively little about how acoustic mate attraction traits covary with sperm traits in natural cricket populations. Here we examine the association between

long-distance acoustic signals and sperm quality in both wild-captured and first-generation laboratory-reared crickets to test the predictions of the sperm competition and phenotype-linked fertility hypotheses. Our study is the first to examine this relationship in a natural cricket population, as well as the first to examine whether wild-captured males exhibit different sperm traits than laboratory-reared males.

Methods

Experimental animals

We captured adult male and female *G. veletis* in Ottawa, ON, Canada (45°19'N, 75°40'W) in May and June 2010. We also used the first generation of laboratory-reared offspring from the wild-captured individuals. Late-instar nymphs were removed from the colony, isolated by sex, and checked daily for adult eclosion. Adults were individually housed in circular, clear plastic 540 mL containers. Crickets were provided with water and food (Harlan Teklad Laboratory Rodent Diet No. 8604: 24% crude protein, 4% crude fat, 4.5% crude fibre) *ad libitum* and were held in a temperature-controlled room (30 ± 2°C).

Acoustic recording

Wild-captured males were placed into individual containers in an electronic acoustic recording system on the morning after capture. Lab-reared males were placed in the recording system when they reached day 7 of adulthood. The recording system consisted of 32 individually recording microphones (electret condenser type KECG2742PBL-A; Kingstate Electronics, Tamshui, Taipei, Taiwan), each with a single LED light that provided males with a 12-h/12-h L/D cycle, positioned 6.6 cm above the

top of the male's container. Each male was separated from its neighbours by an acoustically isolated enclosure (a 7-cm-thick Styrofoam box internally lined with 3.5-cm-thick acoustic foam) that contained the microphone and the LED light. This design minimized the likelihood of individuals detecting their neighbours' signals. Further, although the signals could be heard faintly through the acoustic foam, the minimum amplitude threshold of the recording system ensured that neighbouring microphones did not mistakenly record non-focal males. Microphones were calibrated relative to a known signal level so that input values from different channels could be compared.

We continuously recorded long-distance acoustic mate attraction signals for seven days for each male before conducting mating trials. Recording was paused for 15 min each day while we replenished food and water supplies. The microphones were continuously monitored and analyzed using CricketSong software (Cambridge Electronic Design, Cambridge, UK). Sounds were recorded at 31.25 Hz. We used an amplitude threshold of 55 db to determine pulse onset. However, this threshold was dynamically adjusted by CricketSong to account for males that called at higher than average amplitudes. For these individuals, the threshold was raised to a level proportional to the amplitude of the pulse and decayed back to the original value within 1–8000 ms (the system is self-scaling and, thus, the exact rate of decay is proportional to the size of the pulse). For very quiet individuals, the minimum threshold was manually set below the species-specific value to ensure that all of their pulses were scored correctly. We established species-specific thresholds to classify pulse, interpulse, chirp, and interchirp periods (see Fitzsimmons and Bertram 2011 for details).

Crickets produce sound by rubbing their forewings together. The fine-scale structure of cricket signals is determined by wing properties and the rate and pattern of wing movement. Each closing stroke produces a pulse of sound, and pulses are concatenated together to produce chirps (Pfau and Koch 1994). Because our recording system continuously monitored males' acoustic behaviour, our measure of daily time spent signaling quantifies the total amount of time each male spent signaling during a 24-h period. Hourly signaling parameter averages were weighted by the number of pulses produced in the hour. In this way, hours with many pulses were given heavier weight than hours when relatively few pulses were produced. Pulse rate (No. pulses/s) was calculated at a later date using the equation: (No. pulses per chirp/chirp duration) \times 1000.

Mating trials

We placed one male in an empty 540-mL container with a virgin female of known mass. The cricket pair was observed for up to an hour to determine whether mating occurred. We recorded the time it took for the male to begin producing courtship songs and the time it took for the female to mount the male and successfully mate, from their initial meeting to the transfer of a spermatophore. We calculated mating latency as the time from when the male began courting to when the female mounted the male and a spermatophore was successfully transferred. We used latency to mate in no-choice tests because Shackleton et al. (2005) outlined the benefits of using no-choice tests and highlighted the difficulty in controlling for male–male competition when using two-choice tests. No-choice tests measure the time it takes a female to mate when she is placed with a single male. This approach can be beneficial because the female's choice is

based on the full complement of chemical, acoustic, and physical mating cues that are provided by a male. Further, female choice is determined from actual mating, not quantified indirectly from time spent near a male or first male approached. Shackleton et al. (2005) provide an extensive review of the benefits of using no-choice tests and the extensive number of taxa where no-choice tests are utilized.

Sperm viability and number assays

We immediately removed the spermatophore from the female and placed it into 20 μL of Beadle saline (128.3 mM NaCl, 4.7 mM KCl, 23 mM CaCl_2). We then immediately ruptured the spermatophore and gently forced it through a pipette five times to prevent sperm agglutination. To assess the number of sperm produced, we counted sperm from the spermatophore solution using a 'improved Neubauer chamber' haemocytometer under $40\times$ magnification (Zeiss AxioImager.M2m; Pitcher et al. 2007; Klaus et al. 2011). Sperm count was expressed as the total number of sperm per mL for each male. To assess sperm viability, we used a live/dead sperm viability assay (Invitrogen Molecular Probes; Garcia-Gonzalez and Simmons 2005; Thomas and Simmons 2007). Five microlitres of the spermatophore solution was mixed with 5 μL of 1:50 diluted 1 mM SYBR-14 stain and left in the dark for 10 min before 2 μL of 2.4 mM propidium iodide was added. The sample was then incubated in the dark for an additional 10 min before being observed under a fluorescence microscope with a blue excitation filter ($\lambda = 470 \text{ nm}$ at 60%). This assay stained live (i.e. viable) sperm green with SYBR-14, a membrane-permeant nucleic acid stain; dead sperm with damaged membranes were stained red with propidium iodide. On very rare occasions,

sperm were stained both colours and, as the meaning of these moribund cells is unclear, they were not counted (Pitcher et al. 2007; Klaus et al. 2011). To minimize the effect of loss of viability that occurs with sperm age, images of the sperm were taken immediately after staining, and we determined the live/dead ratio by viewing the images after the assay was completed (Holman 2009). We counted 100 sperm per male, and sperm viability was calculated as the proportion of live sperm.

Thus, the measures we used to quantify sperm quality were total number of sperm, sperm viability, and the number of living sperm. To quantify the number of living sperm, we multiplied sperm viability by the total number of sperm. We included number of living sperm as one of our measures because sperm viability and sperm number are suspected to be non-independent of one another as they could share dependence on environmental and genetic factors (Holman 2009; Gress and Kelly 2011). As such, combining the two measurements may offer a more accurate measure of ejaculate quality than either assay alone.

Body size measurements

Males were weighed on the day they mated (day 14 for lab-reared males) using a Denver Instruments balance (Pinnacle Series model PI-314; precision ± 0.1 mg). We also measured head width, pronotum length, and pronotum width for each cricket.

Measurements were taken using a Zeiss Axio Observer inverted dissecting microscope and highly magnified photographs (AxioVision v4.8, Carl Zeiss, Jena, Germany; magnification approx. $8.5\times$, resolution approx. $1.60\ \mu\text{m}$).

Statistical methods

Due to colinearity of the fine-scale signaling variables and body size metrics, we used principal components analysis (PCA) to reduce the number of factors in our models (Table 5.2).

To assess whether signaling parameters were related to sperm metrics, we took mean values of the daily signaling principal components (PC) scores for each male. We then built general linear models (GLM) to examine whether sperm number, sperm viability, or the number of living sperm was influenced by fine-scale signaling metrics (PC1, PC2, PC3), time spent signaling, whether the males were wild-captured or lab-reared, mating latency (as an indirect measure of female choice), male body condition (PC1), male mass, female mass, and the interaction male mass*female mass. We performed separate GLM for each sperm metric. We also performed analyses involving body condition with the more commonly-used residuals of mass on body size. Our results did not differ when analyzed with residuals of mass, and given the problems associated with use of residuals as a response variable (Freckleton 2002), we present results obtained from PCA scores only. We performed statistical analyses in JMP v9.0.2 (SAS, Cary, NC, USA).

Results

Our PCA for signaling produced three PC factors with eigenvalues above 1.0 that together explained 75% of the variance in signal structure (Table 5.2). Examining the most heavily loaded signaling parameters for each component reveals that males with high PC1 scores had long chirp durations and a high number of pulses per chirp, whereas

males with high PC2 scores had signals with long interchirp and interpulse durations produced at low amplitudes. Males with high PC3 scores had signals with high carrier frequencies and short pulse durations (Table 5.2). Our body condition PCA resulted in one PC factor that explained 80% of the variation in body size and was loaded positively and equally by head width, pronotum length, and pronotum width (Table 5.2).

Over the course of the experiment, males signaled acoustically for an average of 107.37 minutes per night at an average carrier frequency of 5051 Hz and an amplitude of 63.5 dB (Table 5.3). Three males never signaled, and the three highest effort signalers spent more than five hours per night signaling. Descriptive statistics for fine-scale signal parameters are reported in Table 5.3. Males produced 9.61×10^5 sperm per mL on average, with an average viability rate of 75%, and 7.59×10^5 living sperm per mL (Table 5.3).

Our GLM revealed that sperm number, sperm viability, and the number of living sperm were not influenced by fine-scale signaling scores, time spent signaling, whether the males were wild-captured or lab-reared, mating latency, male body condition, male mass, or female mass (all $F \leq 1.02$, all $P \geq 0.44$; Figure 5.1; Table 5.4).

Discussion

We investigated the relationship between sperm quality and the quality and quantity of acoustic mate attraction signals in field crickets to test the predictions of sperm competition and the phenotype-linked fertility hypothesis. We found no evidence for either hypothesis, as sperm number, viability, and number of viable sperm were not influenced by the fine-scale components of signaling or signaling effort. Further, sperm

quality measures were not influenced by male mass or condition, or whether males were wild-captured or laboratory-reared. Our robust sample sizes and sophisticated acoustic recording equipment provide confidence in our conclusion that the lack of relationships are real and not due to limitations of our acoustic data. However, because we mechanically ruptured spermatophores, our sperm viability estimates may be lower than if sperm was allowed to evacuate freely without rupturing the spermatophores (Gress and Kelly 2011).

A requirement of the phenotype-linked fertility hypothesis is that the precopulatory traits of interest are selected for by females in mate choice (Sheldon 1994). We have not quantified female preference functions in *G. veletis*, but based on mate preference studies in closely-related species of cricket we believe the traits we studied are likely to be used by females in mate choice decisions. Female crickets tend to prefer males who invest the most effort into acoustic signaling. For example, female *Teleogryllus commodus* prefer males that signal most often (Hunt et al. 2004), and in several cricket species, males that signal most through the night tend to mate most often (e.g. Cade and Cade 1992; Rodríguez-Muñoz et al. 2010). Females prefer high chirp rates and long chirp durations in variable field crickets (*G. lineaticeps*; Wagner 1996a; Wagner and Hoback 1999), and shorter intercall intervals in Australian field crickets (*T. commodus*; Brooks et al. 2005). In playback experiments with *G. lineaticeps*, four times as many females were attracted to loud signals than to quiet signals (Wagner 1996b); female *G. texensis* also prefer louder signals when given the choice (Cade 1981). Thus we believe that the suite of traits that comprise the multicomponent acoustic mate

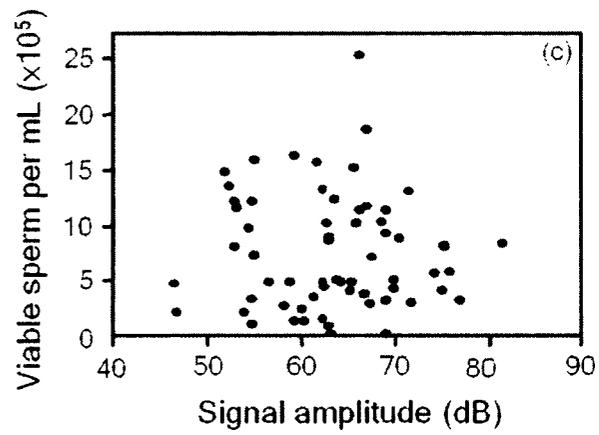
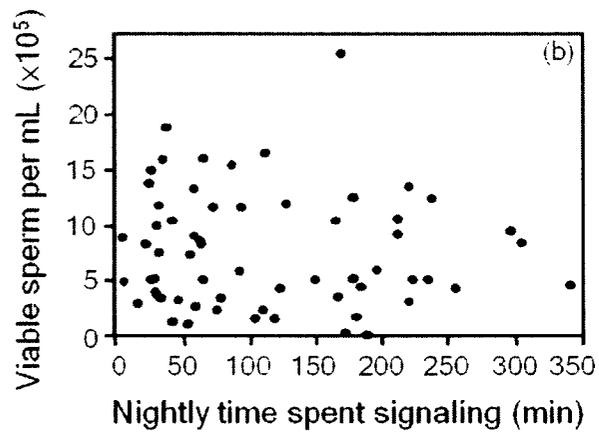
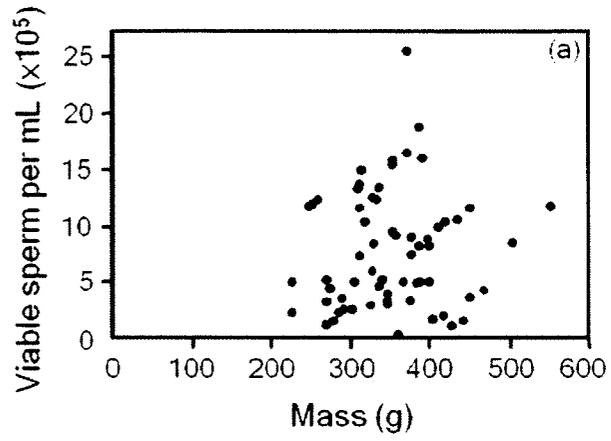
attraction signal in crickets are likely to be targets of selection by females and potential candidates for honest signals of sperm quality.

It is possible that acoustic mate attraction signals function to communicate information other than sperm quality to females. Perhaps most importantly, the characteristics of the signal may benefit females in locating mates (Kirkpatrick and Ryan 1991; Hack 1998) and preventing hybridization (Tynkkynen et al. 2006). In addition to the benefit of locating conspecific mates, previous research indicates that female field crickets receive indirect benefits from selecting amongst males based on their acoustic signal structure. For example, in *G. lineaticeps*, females receive fecundity and fertility benefits from mating with males with higher chirp rates and lifespan benefits from mating with males with longer chirp durations (Wagner et al. 2001; Wagner and Harper 2003). We did not measure female fertility or lifespan in the present study, but it would be interesting to replicate the studies on *G. lineaticeps* to assess whether females in other species of field cricket experience benefits based on male sperm quality and/or ejaculate content.

Our review of previous studies of relationships between mate attraction and sperm traits in crickets (Table 5.1) reveals that the precopulatory traits that show a positive relationship, supporting phenotype-linked fertility (Sheldon 1994), are different across species. For example, in *T. oceanicus*, dominant males have higher sperm viability, whereas in *Gryllodes supplicans* and *G. bimaculatus* larger males transfer more sperm to females as the result of producing larger spermatophores (Sakaluk 1985; Simmons 1986b). Conversely, in *G. bimaculatus*, males with asymmetrical limbs have higher numbers of sperm (Mallard and Barnard 2003), supporting the sperm competition

hypothesis (Parker 1998). The only studies to investigate relationships between acoustic signals and sperm traits report mixed results. Males with attractive courtship songs produce less viable sperm in *T. oceanicus* (Simmons et al. 2010), but courtship song rate is unrelated to spermatophylax size in *G. sigillatus* (Ketola et al. 2007). Our finding of no relationship between long distance acoustic signals and sperm quality agree with those of Klaus et al. (2011) for *A. domesticus* in terms of signal characteristics, but we did not find an effect of body size on sperm metrics as in Klaus et al. (2011). Overall, it appears that while female crickets may receive benefits from choosing males based on signal characteristics (Wagner et al. 2001; Wagner and Harper 2003), whether the benefits are a result of genetic quality, seminal fluid contents (e.g. nutrients; Gwynne 1988), or other factors remains unknown.

Figures



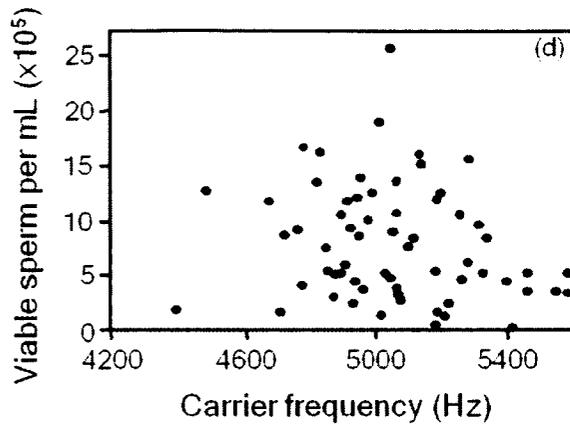


Figure 5.1. The lack of relationship between the amount of viable sperm males produced in a mating trial and (a) his mass, (b) how much time he spent producing long-distance mate attraction signals, (c) how loud he signaled, and (d) at what carrier frequency he signaled.

Tables

Table 5.1. Previous studies have found mixed results in the directionality of association between mate attraction traits and sperm traits in crickets.

Species	Finding	Reference	Supports sperm competition (SC) or phenotype-linked fertility (PLFH)?
<i>Acheta domesticus</i>	No relationship between sperm number or viability and long-distance acoustic signals	Klaus et al. 2011	---
<i>Teleogryllus oceanicus</i>	Males with more attractive courtship songs have less viable sperm	Simmons et al. 2010	SC
<i>Teleogryllus oceanicus</i>	Dominant males have higher sperm viability rates	Thomas and Simmons 2009	PLFH
<i>Gryllodes sigillatus</i>	Courtship call rate is unrelated to spermatophylax size	Ketola et al. 2007	---
<i>Teleogryllus commodus</i>	Attractive males (males that were mounted fastest by females) transfer more sperm as a result of females leaving spermatophore attached longer	Bussière et al. 2006	PLFH
<i>Gryllus bimaculatus</i>	Males with more asymmetrical limbs have higher numbers of sperm	Mallard and Barnard 2003	SC
<i>Gryllodes sigillatus</i>	Males with more asymmetrical limbs have lower numbers of sperm	Farmer and Barnard 2000; Mallard and Barnard 2003	PLFH
<i>Gryllus bimaculatus</i>	Larger males produce spermatophores that females leave attached longer	Simmons 1986b	PLFH
<i>Gryllodes supplicans</i>	Larger males produce bigger spermatophores and transfer more sperm as the result of females leaving spermatophore attached longer	Sakaluk 1985	PLFH

Table 5.2. Principal component analysis results for signaling and body size metrics.

Analysis of signaling included seven parameters (amplitude, carrier frequency, pulses per chirp, chirp duration, interchirp duration, pulse duration, interpulse duration); we list the three parameters with the heaviest loadings for each signaling principal component.

PCA	PC	Eigenvalue	% variance explained	Factors	Loadings
Signaling	PC1	2.08	30	Chirp duration	0.66
				Pulses per chirp	0.66
				Interpulse duration	0.26
	PC2	1.73	25	Interchirp duration	0.60
				Interpulse duration	0.53
				Amplitude	-0.51
	PC3	1.42	20	Carrier frequency	0.71
				Pulse duration	-0.68
				Chirp duration	0.15
Body condition	PC1	3.18	80	Head width	0.55
				Pronotum length	0.55
				Pronotum width	0.55
				Mass	0.28

Table 5.3. Descriptive statistics for *G. veletis* signaling parameters and sperm metrics.

Parameter	Mean	SE	Minimum	Maximum
Pulse duration (ms)	15.98	0.16	12.49	18.59
Interpulse duration (ms)	34.56	0.28	29.97	42.34
Pulses per chirp	3.54	0.055	3	5
Chirp duration (ms)	117.91	1.97	89.59	159.3
Interchirp duration (ms)	574.49	18.50	257.5	1009
Carrier frequency (Hz)	5050.66	30.09	4387	5581
Amplitude (dB)	63.46	0.92	46.52	81.23
Daily time spent calling (min)	107.37	10.55	0	341.3
Sperm viability	0.75	0.025	0.08	1.00
Sperm per mL	9.61×10^5	6.98×10^4	0	3.70×10^6
Viable sperm per mL	7.59×10^5	6.41×10^5	0	2.55×10^6

Table 5.4. Results of GLM reveal that *G. veletis* sperm metrics are not related to signaling metrics, male body condition, male and female mass, or whether males were wild-captured or lab-reared.

GLM	F _a	r ² _{adj}	P	Effects	F ₁	P
Sperm number	0.99	0.001	0.46	Body condition	1.03	0.31
				Male mass	0.96	0.33
				Mating latency	0.86	0.36
				Field/lab	0.77	0.38
				Female mass*Male mass	0.67	0.42
				Signaling PC1	0.64	0.43
				Signaling PC2	0.60	0.44
				Time spent signaling	0.17	0.68
				Female mass	0.03	0.86
				Signaling PC3	< 0.01	0.97
Sperm viability	1.04	-0.01	0.42	Female mass*Male mass	4.07	0.05
				Signaling PC3	1.82	0.18
				Signaling PC2	0.90	0.35
				Female mass	0.51	0.48
				Mating latency	0.48	0.49
				Signaling PC1	0.42	0.52
				Male mass	0.28	0.60
				Time spent signaling	0.09	0.77
				Body condition	< 0.01	0.95
				Field/lab	< 0.01	0.95
Number of viable sperm	0.91	-0.02	0.53	Female mass*Male mass	1.39	0.24
				Male mass	1.14	0.29
				Body condition	1.03	0.32
				Field/lab	0.77	0.38
				Mating latency	0.70	0.41
				Signaling PC1	0.61	0.44
				Female mass	0.18	0.68
				Signaling PC3	0.15	0.70
				Time spent signaling	0.04	0.84
				Signaling PC2	0.01	0.91

^aDF: Sperm number: 10, 50; sperm viability and number of viable sperm: 10, 48

Conclusions

The overarching goal of my dissertation was to investigate the relationships between multiple sexual traits in the spring field cricket, *Gryllus veletis*. I first examined the relationship between male age and acoustic mate attraction signal structure (Chapter 2). I found that the acoustic signals of male spring field crickets change with age; the signals of older males were quieter, with more silent periods within and between chirps, and produced less often than those of younger males. As males aged most of the changes in call structure reflect decreased calling effort. My results suggest that females may be able to distinguish between males of different age classes based on male mate attraction signals. I also investigated the relationship between signaling effort and longevity, and found that males that called the most over their life lived longer than males that called less.

I then investigated the relationship between signaling and aggression, the influence of body condition on aggression, and the effect of fighting experience on subsequent signaling behaviour in field crickets (Chapter 3). I found that aggressive behaviour was not influenced by signaling behaviour. Signaling behaviour was highly repeatable within individuals, but aggressive behaviour had low repeatability. I found no evidence for a winner or loser effect on signaling; there was no change in signaling effort when I compared contest winners and losers before and after they participated in aggressive contests. These results suggest that signaling and aggression are independent of one another in crickets, perhaps serving different functions in female attraction and male-male competition, respectively.

In my fourth chapter I examined whether the presence and sex of an audience influenced contest and post-contest behaviour in male field crickets. Males were more aggressive in the presence of a female audience, and produced more victory display behaviour in the presence of a male audience. My results suggest that male crickets dynamically adjust their contest behaviour to gain a reproductive benefit *via* female eavesdropping and may also deter future aggression from potential rivals *via* the advertisement of the contest victory.

In my fifth chapter, I examined the relationship between male acoustic signaling traits and sperm traits, to ask whether males honestly indicate their sperm quality to females (as we would expect from the phenotype linked fertility hypothesis), or whether there is a trade-off between signaling effort and sperm quality (as predicted by sperm competition hypothesis). I found no relationship between pre- and postcopulatory traits, providing no evidence for either the sperm competition or phenotype-linked fertility hypothesis. In sum, I found a relationship between male age and acoustic signal properties (Chapter 2) and between the social environment and aggressive behaviour (Chapter 4), but no relationship between signaling and aggression (Chapter 3) or signaling and sperm traits (Chapter 5).

Based on the genic capture hypothesis (Rowe and Houle 1996), I expected that the sexual signals I measured would exhibit condition-dependent expression. This hypothesis assumes that signals are dependent on overall condition because they are costly to produce (Zahavi 1977; Kotiaho 2000; Gil and Gahr 2002; Hunt et al. 2004). However, I found no effect of male size on acoustic signal characteristics, and body condition was only significant in explaining 7% of the variation in 1 of the 11 acoustic

parameters I measured (song duration; Chapter 2; Fitzsimmons and Bertram 2011). Similarly, I found no effect of body condition on aggressiveness (Chapter 3) or sperm quality (sperm number, sperm viability, and the number of living sperm; Chapter 5).

The lack of relationships between body condition and sexual traits should not be interpreted to mean that sexual traits are not influenced by condition because several studies have shown that when diet is manipulated, several traits respond (e.g. Hedrick 2005; Whattam and Bertram 2011). I provided all of my animals with unlimited access to high-quality food, so the spectrum of variation in naturally-occurring body condition, as much as it reflects the ability to acquire resources, was likely narrowed by this approach. Future studies should explore whether dietary limitations can influence the relationships between body condition and sexual traits to assess whether traits experience condition-dependent expression as expected by genic capture.

I was also surprised to find no differences between wild-captured and laboratory-reared males in sexual traits. I found no effect of rearing environment on aggressive behaviour (Chapter 3) or sperm traits (Chapter 5). I expected differences between wild-captured males and laboratory-reared males because I did not know the age or reproductive history of wild-captured males and because we do not know what effects rearing in the laboratory environment may have on many aspects of cricket life-history and behaviour. Several studies have found differences in important behaviours, such as mating success and oviposition behaviour, between wild-captured and laboratory-reared individuals (e.g. Glas et al. 2007; Calatayud et al. 2008). I did not know what effect breaking the natural overwintering diapause would have on *G. veletis*, but it appears that the relationships between their sexual traits are comparable to those of wild-captured

individuals. Further, while I found no differences in the relationships between traits for wild-captured and laboratory-reared individuals, this does not preclude the possibility that there are differences in the absolute values of the traits themselves. This is an area I hope to pursue in my future research.

In all of my data chapters I studied the long-distance acoustic mate attraction signal of male *G. veletis* in relation to other traits (age, aggression, sperm). The lack of relationships between this mate attraction signal and other traits lead me to suggest that future studies investigate the fine-scale components of acoustic signals produced immediately prior to, during, and immediately following aggressive behaviour and courtship to test whether these signals indicate male fighting ability, aggressive intent, or sperm quality in crickets. This is an exciting and growing area of current research in animal behaviour in the context of aggressive interactions (Searcy and Beecher 2009; van Staaden et al. 2011), and studies of multiple signaling systems across taxa will improve our understanding of the functions of agonistic signals. For example, it is possible that features of aggressive songs produced immediately prior to an aggressive contest predict a male's aggressiveness, such as the number of songs, the fine-scale components of the songs (Wagner 1992), or the song amplitude (Hof and Hazlett 2010). In the context of mating behaviour, there may be relationships between courtship song, produced immediately prior to mating, and sperm traits. For example, in *Teleogryllus oceanicus*, males with more attractive courtship songs have less viable sperm, suggesting a trade-off between allocation of resources into these two types of sexual traits (Simmons et al. 2010).

A final promising avenue for future research is to investigate female preference functions for mate attraction signals in crickets and to relate them to benefits females receive by being choosy. Quantifying female fertility and lifespan would be especially useful to assess whether females experience benefits from mating with preferred males based on sperm quality, ejaculate content (Wagner and Harper 2003), or by some indirect genetic mechanism (Zahavi 1975; Zahavi 1977). Using a framework that combines measuring multiple male sexual traits in several signaling modalities, assessing female preference functions for these male traits, and assessing the benefits to females for investing in mate choice will improve our understanding of pre- and postcopulatory sexual selection and allow us to examine whether trade-offs or honest signaling dominates in sexual signaling systems.

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Appendices

Appendix 1. Coauthor and journal permission

Susan M. Bertram, coauthor for Chapters 2, 3, 4, 5

Sue_Bertram <Sue_Bertram@carleton.ca> Sun, Mar 11, 2012 at 8:40 PM

Hi Lauren and whoever else this may concern,

I hereby grant Lauren Fitzsimmons permission to use all chapters of her dissertation entitled "The relationships between acoustic mate attraction signaling, age, aggressiveness, and sperm quality in spring field crickets" which I am a co-author on, as well as my permission to claim copyright for said chapters.

Sincerely,

Susan M. Bertram

Brill, publisher of Behaviour (Chapter 2)

Michiel Thijssen <Thijssen@brill.nl> Tue, Feb 28, 2012 at 2:06 AM

Dear Lauren,

You are herewith allowed to include your Behaviour paper in your PhD thesis, in the way you suggest.

Thanks for asking. I'm cc-ing our Rights & Permissions department to suggest explicitly listing 'PhD thesis' in the RightsLink module, or be more explicit of this obvious complimentary use.

Kindest regards,

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Leiden, the Netherlands

Appendix 2. Additional articles published related to dissertation

1. Citation: Bertram, S.M., Fitzsimmons, L.P., McAuley, E.M., Rundle, H.D., and R. Gorelick. 2012. Phenotypic covariance structure and its divergence for acoustic mate attraction signals among four cricket species. *Ecology and Evolution*, 2:181-195.

Abstract: The phenotypic variance–covariance matrix (P) describes the multivariate distribution of a population in phenotypic space, providing direct insight into the appropriateness of measured traits within the context of multicollinearity (i.e., do they describe any significant variance that is independent of other traits), and whether trait covariances restrict the combinations of phenotypes available to selection. Given the importance of P , it is therefore surprising that phenotypic covariances are seldom jointly analyzed and that the dimensionality of P has rarely been investigated in a rigorous statistical framework. Here, we used a repeated measures approach to quantify P separately for populations of four cricket species using seven acoustic signaling traits thought to enhance mate attraction. P was of full or almost full dimensionality in all four species, indicating that all traits conveyed some information that was independent of the other traits, and that phenotypic trait covariances do not constrain the combinations of signaling traits available to selection. P also differed significantly among species, although the dominant axis of phenotypic variation (p_{\max}) was largely shared among three of the species (*Acheta domesticus*, *Gryllus assimilis*, *G. texensis*), but different in the fourth (*G. veletis*). In *G. veletis* and *A. domesticus*, but not *G. assimilis* and *G. texensis*, p_{\max} was correlated with body size, while p_{\max} was not correlated with residual mass (a condition measure) in any of the species. This study reveals the

importance of jointly analyzing phenotypic traits.

2. Citation: Bertram, S.M., Rook, V.L.M., Fitzsimmons, J.M., and **L.P. Fitzsimmons**. 2011. Fine- and broad-scale approaches to understanding the evolution of aggression in crickets. *Ethology*, 117:1067-1080.

Abstract: Male field crickets frequently engage in agonistic contests to establish dominance in social interactions and gain access to mate attraction territories. Crickets (Orthoptera: Gryllidae) are often used as a model taxon to study aggression, but limited documentation of aggression in some cricket species hinders our understanding of its evolutionary costs and benefits. Our study investigated cricket aggression at two scales: the within-species scale for two cricket species, *Gryllus assimilis* and *G. veletis*, whose aggression had not been adequately documented and the among-species scale to detect evolutionary patterns in species' levels of aggression. In both *G. veletis* and *G. assimilis*, winners spent more time being aggressive than losers, but they were not larger or heavier. Collectively, our results reveal that *G. veletis* males are more aggressive than *G. assimilis*. Male *G. veletis* had higher aggression scores than male *G. assimilis*. The majority of *G. veletis* contests escalated to grappling (a highly aggressive behavior), while less than one quarter of *G. assimilis* contests escalated to grappling. Further, *G. veletis* males transitioned between two of the most aggressive behaviors most often while *G. assimilis* transitioned between two of the least aggressive behaviors most often. We integrate this new information on aggression for *G. assimilis* and *G. veletis* with previously documented aggression data for many cricket species to investigate aggression in a broader evolutionary context than previously possible. Within a phylogenetic

context, we test the hypothesis that species whose males use burrows from which to call and attract females are more aggressive than species with non-burrowing males. We found evidence consistent with this hypothesis; species with burrowing males tended to be more aggressive than species with non-burrowing males. Together, our study provides fine-scale understanding of aggression in two cricket species and broad-scale evolutionary context for aggression across cricket species.

3. Citation: Klaus, S.P., Fitzsimmons, L.P., Pitcher, T.E., and S.M. Bertram. 2011. Song and sperm in crickets: a tradeoff between pre- and post-copulatory traits or phenotype-linked fertility? *Ethology*, 117:154-162.

Abstract: When females mate multiply (polyandry) both pre- and post-copulatory sexual selection can occur. Sperm competition theory predicts there should be a trade-off between investment in attracting mates and investment in ejaculate quality. In contrast, the phenotype-linked fertility hypothesis predicts a positive relationship should exist between investment in attracting mates and investment in ejaculate quality. Given the need to understand how pre- and post-copulatory sexual selection interacts, we investigated the relationship between secondary sexual traits and ejaculate quality using the European house cricket, *Acheta domesticus*. Although we found no direct relationship between cricket secondary sexual signals and ejaculate quality, variation in ejaculate quality was dependent on male body weight and mating latency: the lightest males produced twice as many sperm as the heaviest males but took longer to mate with females. Our findings are consistent with current theoretical models of sperm competition. Given light males may have lower mating success than heavy males because

females take longer to mate with them in no-choice tests, light males may be exhibiting an alternative reproductive tactic by providing females with more living sperm. Together, our findings suggest that the fitness of heavy males may depend on pre-copulatory sexual selection, while the fitness of light males may depend on post-copulatory fertilization success.

4. Citation: Bertram, S.M., Rook, V.L.M., and L.P. Fitzsimmons. 2010. Strutting their stuff: victory displays in the spring field cricket, *Gryllus veletis*. *Behaviour*, 147:1249-1266.

Abstract: Contest winners may perform victory displays at the conclusion of agonistic contests. Victory displays are hypothesized to function in browbeating or advertisement. To date, victory displays have received little attention. Following agonistic contests, several field cricket species produce aggressive songs and shake their body forwards and backwards (body jerks). We examined 20 agonistic contests between field-captured adult male spring field crickets, *Gryllus veletis*. We characterized the aggressive songs and body jerks that occurred both during and immediately following conflicts to evaluate whether these behaviours should be classified as victory displays. Aggressive songs and body jerks were observed throughout the contests, not just immediately following the conclusion of the fight. Winner aggressive song and body jerk rates were higher during the post-conflict period than during the fight period. Further, while both winners and losers performed aggressive songs and body jerks, winners performed them at five times the rate of the losers during the post-conflict period. We conclude that aggressive songs

and body jerks should be considered victory displays, and that these victory displays may function as both browbeating and advertisement.

5. Citation: Fitzsimmons, J.M., and L.P. Fitzsimmons. 2010. Pre-copulatory behavior of the wheel bug *Arilus gallus* (Hemiptera: Reduviidae). *Entomological News*, 121:304-307.

Abstract: We describe the pre-copulatory behavior of a pair of *Arilus gallus* Stål (Heteroptera: Reduviidae) observed in a tropical dry forest in Sector Santa Rosa of Area de Conservación Guanacaste, northwestern Costa Rica (10°40'N, 85° 30'W). To our knowledge this is the first description of mating behavior in the genus *Arilus* other than several notes on the time of year *A. cristatus* (Linné) mates in the USA.