SURVIVAL, SONG AND SEXUAL SELECTION

by

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ABSTRACT

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Darwinian sexual selection predicts that males with the most extravagant secondary sexual traits suffer elevated mortality. Although correlative evidence has generally not borne this idea out, recent research, including a field cricket study, showed that investment in sexually selected traits is costly to survival. I investigated male survival, ornamentation (song) and mating success in a North American grylline, *Gryllus pennsylvanicus*, to test the generality of previous work and highlight the importance of ecology differences to resource allocation. As the calling songs of older male *G. pennsylvanicus* are highly attractive to females, in Chapter 2 I tested whether male age correlated with calling song and found a weak but statistically significant correlation, thus leaving open the possibility that choosy females use an age-based indicator mechanism. In Chapter 3, I tested the condition dependence of male survival and calling effort. In contrast to previous work, I found that high condition males both called more and lived longer than low condition males, although there was no trade-off between survival and calling effort. The substantial condition dependence of calling effort suggests that calling effort is under strong directional selection. In Chapter 4 I tested whether female mating preferences resulted in strong selection on male calling effort. I also tested for the condition dependence of female mating preferences. I found that female choosiness was condition-dependent, but the rank of preferred male songs (preference function) was not. Both low and high condition females preferred high calling effort over low calling effort song. In Chapter 5 I tested for evidence of nonlinear selection on male survival that might explain the nonlinear pattern of male investment in survival seen in Chapter 3 (i.e. male survival leveled-off with increasing condition). I found that socially experienced females, but not virgin and naive females, exerted linear selection on male age. I discuss these and the other results of my thesis in the context of previous work on field crickets and condition-dependent ornamentation. Finally, Appendix A reports results that confirm ancient Chinese cultural knowledge that large headed male crickets are more successful in male-male combat.

ACKNOWLEDGEMENTS

For all the support that I've received during my PhD, I owe a debt of gratitude to a vast number of people. This section is my attempt to articulate that gratitude. Before I do, I should point out that the flaws in this thesis are mine, but all the good bits I attribute in large part to the influence of the following people.

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It seems a bit odd to thank my study animals, the fall field crickets, but they are the inspiration for this thesis and the source of countless future research paths. Their wonderfully exuberant song is music to my ears, and on a warm late summer evening there are few things I'd rather be doing than listening to that music.

I must extend my gratitude to the Natural Sciences and Engineering Research Council, The Orthopterists' Society and CUPE Local 3902 for funding during my degree.

Last, but by no means least, I thank my family for their love and support. They provide the bedrock on which I stand now. Most of all I thank my Mother, Jeannie Judge. In a thesis where half of the experiments involve the effects of diet quality on behaviour and lifespan, she made sure that I ate well and often so that I didn't have trade-off thesis completion against my own survival. This PhD was the hardest thing that I've ever done in my life, and I could not have done it without her care. Words cannot describe what I owe you – thanks for everything Mom.

TABLE OF CONTENTS

CHAPTERS

1 GENERAL INTRODUCTION.

2 DO MALE FALL FIELD CRICKETS, *G. PENNSYLVANICUS*, SIGNAL THEIR AGE

5 EXPERIENCED (BUT NOT VIRGIN AND SOCIALLY-NAIVE) FEMALE FIELD CRICKETS EXERT DIRECTIONAL SELECTION ON MALES.

6 GENERAL DISCUSSION.

APPENDIX

A MALE WEAPONRY IN A FIGHTING CRICKET.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

General Introduction.

Kevin A. Judge

> *"The development, however, of certain structures – of the horns, for instance, in certain stags – has been carried to a wonderful extreme; and in some instances to an extreme which, as far as the general conditions of life are concerned, must be slightly injurious to the male. From this fact we learn that the advantages which favoured males have derived from conquering other males in battle or courtship, and thus leaving a numerous progeny, have been in the long run greater than those derived from rather more perfect adaptation to the external conditions of life."*
>
> \- Charles Darwin (1871, p. 279)

> *"Now this extremely rigid action of natural selection must render any attempt to select mere ornament utterly nugatory, unless the most ornamented always coincided with 'the fittest' in every other respect; while, if they do so coincide, then any selection of ornament is altogether superfluous. If the most brightly coloured and fullest plumaged males are* not *the most healthy and vigorous, have* not *the best instincts for the proper construction and concealment of the nest, and for the care and protection of the young, they are certainly not the fittest, and will not survive, or be the parents of survivors."*
>
> \- Alfred Russell Wallace (1889, p. 295)

1.1 Background

Darwin's theory of sexual selection (1871) sought to explain a phenomenon, namely the existence of loud, colourful and otherwise encumbered males, which seemed inconsistent with natural selection (Darwin 1859). Because of strong competition between members of the same species for limited resources, natural selection was thought to eliminate waste and inefficiency by the differential survival and reproduction of individuals with traits that conferred an advantage in this competition (Darwin 1859). What could be more wasteful, inefficient and sure to bring about a swift end in the digestive tract of a predator than the gaudy plumage of male birds of paradise or the massive horns of male beetles; extravagant male traits seemed to hamper a male survival. Yet there is a profuse abundance of characteristics that might be considered detrimental to male survival. Sexual selection reconciled this apparent paradox – the existence of traits apparently maladaptive under natural selection – by positing that such traits were advantageous to males in the competition for mates *despite* hampering their survival (Darwin 1871, see quotation above). Thus there is an explicit negative correlation between male survival and ornamentation in Darwin's original formulation of the theory of sexual selection – the fittest males have the brightest ornaments but the poorest survival.

Debate over the sign of the survival/ornamentation correlation, negative or positive, has exercised the minds of evolutionists ever since Darwin (Cronin 1991). Wallace disagreed with Darwin on this point, instead suggesting that only the fittest males would be able to support costly ornaments and so should also be best able to survive the rigours of a hostile world (Wallace 1889; Cronin 1991). Subsequently, Fisher (1915, 1930) elegantly showed how male ornaments could evolve past their natural selection (survival) optima as long as the benefit to highly ornamented males of having highly ornamented sons outweighed the detriment of dying younger than drab males. However, the balance of empirical evidence (metaanalysed) supports Wallace: survival and ornamentation are positively correlated in males (Jennions et al. 2001). Furthermore, older males often have a mating success advantage over younger males (e.g. Robel 1967; Zuk 1987c, 1988; Connor 1989; Simmons and Zuk 1992; Simmons 1995; Dickinson 2001; but see Jones et al. 2000; reviewed in Brooks and Kemp 2001).

Thus, ornamentation, survival and mating success seem to be positively linked in males of a wide variety of species. However, recent experimental work has both supported (Kotiaho 2000, Pike et al. 2007) and conflicted with (Hunt et al. 2004; D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms) the general pattern outlined above. In lycosid wolf spiders (Kotiaho 2000) and stickleback fish (Pike et al. 2007), males in good condition both survived longer and had greater sexual ornamentation. However, the opposite pattern was true in grylline crickets (Hunt et al. 2004) and ambush bugs (D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms): males in good condition had better developed secondary sexual traits, but lived shorter lives.

In this thesis, I address the relationships between ornamentation, survival and mating success in another grylline, the fall field cricket, *Gryllus pennsylvanicus* Burmeister (Orthoptera: Gryllidae: Gryllinae). By doing so, I hope to: a) facilitate comparisons with the previous grylline work (Hunt et al. 2004, 2005), and b) clarify early work on this species (Zuk 1987c, 1988), thereby offering new insights into the interplay between life history and mating systems. In next sections of this introductory chapter, I give a brief background of my study organism. Following that is a more detailed outline of the thesis in which I examine the relationships between: a) song (ornament) and survival (Chapters 2 and 3), b) song and mating success (Chapter 4), and c) survival and mating success (Chapter 5).

***1.2 Biology of* G. pennsylvanicus**

Until the mid-1950s, native field crickets in eastern North America were all assigned to a single species, *Acheta assimilis* Fabricius. Although regional variation in calling song and life history were noted (Rehn and Hebard 1915; Fulton 1952), no morphological characters could be found to reliably distinguish these variants (Alexander 1957). Building upon the pioneering work of Fulton (1952), Alexander (1957) used male calling song, life history and crosses between putative species to revise the taxonomy of gryllines in the eastern United States, and recognized five species, one of which was *Gryllus pennsylvanicus* Burmeister (then *A. pennsylvanica*). Alexander (1957) described two populations of *G. pennsylvanicus* – one overwintering as a late-instar nymph and breeding in the spring and early summer, the other overwintering as an egg and breeding in the late summer until the first severe frost – which were later recognized as separate species, *G. veletis* and *G. pennsylvanicus* respectively (Alexander and Bigelow 1960).

Because the only reliable method of distinguishing these two species is based on the timing of their life history (Alexander 1957), Alexander and Bigelow (1960) proposed that *G. veletis* and *G. pennsylvanicus* were sister species and had diverged by a process of allochronic speciation, whereby a temporal separation between the breeding seasons of the two incipient species restricts gene flow (Alexander and Bigelow 1960). However, *G. veletis* and *G. pennsylvanicus* are not sister taxa; instead, *G. pennsylvanicus* form a well-supported clade with *G. ovisopis* and *G. firmus* (Harrison and Bogdanowicz 1995; Huang et al. 2000), the latter of which forms an extensive hybrid zone with *G. pennsylvanicus* in the eastern United States (Harrison and Arnold 1982). Currently, a major molecular phylogenetic revision of North American Gryllinae is underway (D. Gray, pers. comm.) that will include the approximately 20 (Capinera et al. 2004) western species as well as eastern species left out of earlier phylogenies (e.g. *G. vernalis*). This greatly anticipated work will undoubtedly provide ample diversity fodder for research into the evolution of life histories.

G. pennsylvanicus is a common in southern Ontario and is widespread across much of North America (Alexander 1968; Capinera et al. 2004). Individuals inhabit grassy disturbed areas

(Tennis 1983; Capinera et al. 2004) and are often found around areas of human habitation (Alexander and Meral 1967; K. A. Judge, pers. obs.). *G. pennsylvanicus* is omnivorous (Criddle 1925) and has been shown to be a significant predator of both seeds (Brust and House 1988; Carmona et al. 1999; O'Rourke et al. 2006) and invertebrates (Monteith 1971; Bechinski et al. 1983; Burgess and Hinks 1987; D. S. Phillip, H. Bhamber, and K. A. Judge, unpubl. data). The broad diet of *G. pennsylvanicus*, coupled with seasonal variation in the availability of different types of prey (plant or animal) could exert substantial diversifying selection on cricket life histories (i.e. the genotypes that are optimal in high seed abundance years are likely different from those that are most fit in years of high invertebrate prey – genotype by environment interactions; Reznick et al. 2000).

During the breeding season, the number of adult females captured in pitfall traps peaks approximately two weeks after the peak in the number of adult males captured (Carmona et al. 1999), which seems to indicate protandry. Breeding in some areas also coincides with the seed rain from certain agricultural weeds (O'Rourke et al. 2006), possibly providing females with food resources to increase their fecundity. Males call from the mouths of burrows or cracks in the ground into which they escape when disturbed. Calling males are separated from each other by approximately 7.7 to 10.3 m in the field (French et al. 1986), likely making it costly for females to sample large numbers of potential mates. Male *G. pennsylvanicus* calling song consists of short chirps – roughly two to three per second – each consisting of three to five pulses (each a single closure of the male forewings or tegmina) (Alexander 1957; Doherty and Storz 1992). Like most other gryllines, females are attracted to male calling song (Alexander 1961; Loher and Dambach 1989; Jeffery et al. 2005) and are attracted to higher calling effort at least when population density is low (French and Cade 1989). In an elegant series of field experiments, Zuk (1987c) showed that female *G. pennsylvanicus* were more attracted to calling song produced by older males than that of younger males. Males found paired with females in the field were also older than unpaired calling males from nearby (Zuk 1988). However, in the earlier experiment higher calling effort explained a small, but statistically significant proportion of the variance in female attraction (Zuk 1987c), raising the possibility that the apparent preference of females for the songs of older males might be due to differences in calling effort between older and younger males.

1.3 Outline of Thesis

My thesis seeks to explain the pattern of old male mating advantage in *G. pennsylvanicus* (Zuk 1987c, 1988) by considering: a) the correlation between male song and age (Chapter 2), b) the condition dependence of male survival and calling effort (Chapter 3), c) the condition dependence of female choice for higher male calling effort (Chapter 4), and d) the shape of selection exerted by females with different social experience on male traits, including age (Chapter 5).

Older male *G. pennsylvanicus* may have a mating advantage because females prefer to mate with them more than younger males. This hypothesis is predicated on an ability of females to distinguish among males of different ages. Although a number of authors have called into question the efficacy/generality of a female preference that relies on male age as an indicator of quality as opposed to assessing male quality using other phenotypic traits (Brooks and Kemp 2001) there is ample evidence of age changes in male secondary sexual characteristics such as plumage and song in birds (e.g. Hill 1996; Delhey and Kempenaers 2006; Doucet et al. 2007; Probst et al. 2007), body size in anurans (Howard 1978a), and song in insects (Hartley and Stephen 1989; Stiedl et al. 1991; Ritchie et al. 1995). Such changes would provide females with cues to the age of potential mates. Previous work on crickets has shown some evidence of age-related changes in male calling song (see Table 2.1), however the hypothesis that male age is reflected in his calling song has not been tested adequately. In Chapter 2 I use both longitudinal and cross-sectional approaches to test the hypothesis that the ages of male *G. pennsylvanicus* are reflected in their calling songs. In the longitudinal analysis I record the calling song of individual males at both young and old ages and tested for changes in a variety of song parameters. The cross-sectional analysis examines the correlations between male age and song parameters in a sample of males whose calling song was recorded at different ages. If: a) one or more parameters of calling song change with age (longitudinal study), and b) male age is correlated with those same parameters (cross-sectional study), then it is possible that female *G. pennsylvanicus* use calling song to discriminate among potential mates of varying ages.

An alternative hypothesis to explain the mating advantage of older male field crickets is that females are attracted to high quality males who also happen to survive longer than low quality males. Although male sexually selected traits and male survival are usually positively correlated (Jennions et al. 2001), recent work on field crickets (Hunt et al. 2004) and ambush bugs (D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms) highlighted that this is not always the case. In Chapter 3 I test the condition dependence of both male survival and calling effort in *G. pennsylvanicus* by raising crickets on high-, medium- and low quality diets. I measure experimental male calling effort every five days from sexual maturity until death. If the older male mating bias is simply a consequence of high quality males (who invest more in calling effort) surviving longer and thus being more likely found paired with a female, then males raised on high quality food should both call more and survive longer than males raised on low quality food. Although this prediction is not exclusive of the hypothesis that females also prefer older males per se, it does suggest that females should show strong preferences for greater calling effort as has been found in other gryllines (Cade and Cade 1992; Crnokrak and Roff 1995, 1998a,b; Holzer et al. 2003; Scheuber et al. 2003a,b; Hunt et al. 2004).

Mate choice is likely a costly trait, in terms of predation risk, the energetic cost of assessing potential mates and lost foraging opportunities. However, there are also a host of potential benefits to mate choice, both material (e.g. Howard 1978b; Downhower and Brown 1980; Gwynne 1988; Voigt et al. 2005) and genetic (e.g. Welch et al. 1998; Tregenza and Wedell 2002; Head et al. 2005), suggesting that mate choice is a trait under strong selection for optimization. There is ample evidence of variation in mate choice, so the question is posed as to why this variability exists (Jennions and Petrie 1997). One possibility is that mate choice is dependent upon the resources of the choosy individual – females in good condition should display stronger preferences and invest more resources in making mate choice decisions. In Chapter 4 I test the condition dependence of female choice for male calling effort by raising individual *G. pennsylvanicus* from hatching on high- and low quality diets. I then measure two components of female choice: a) preferences (rank order of preferred male phenotypes) and b) choosiness (effort expended in assessing potential mates; Jennions and Petrie 1997). If female choice for higher effort calling song is under directional selection then I predict that high condition females should display stronger preferences and be more choosy than low condition females.

Finally, although male age is positively correlated with mating success in *G. pennsylvanicus* (Zuk 1987c, 1988) and a number of other species (refs cited above), previous work has not examined the shape of selection (linear, quadratic or correlational) on male age (but see Jones et al. 2000, 2004). For example, do females prefer ever-increasing male age or, as has been shown in some insects (Jones 2000, 2004), do females most prefer males of intermediate age? Chapter 5 addresses this question by conducting a multivariate selection analysis (Lande and Arnold 1983; Philips and Arnold 1989) of a sample of males of varying ages, body sizes and body shapes. To increase the chances of detecting differences in the shape of selection on males, I also measured selection on males exerted by both virgin females and experienced females – two classes of females who are predicted to differ in the strength of selection that they exert on females (Halliday 1983; Gabor and Halliday 1997). If male age is positively correlated with some aspect of male quality as suggested by Zuk (1988), then selection on male age by females should primarily be directional and positive.

CHAPTER 2

Do male fall field crickets, *Gryllus pennsylvanicus*, signal their age?

Kevin A. Judge

2.1 Abstract

Older males are often reported to have higher mating success than younger males. To the extent that male quality and survival are positively correlated, this observation raises the possibility that females use male signals to assess age and thus quality. I tested this hypothesis in the fall field cricket, *Gryllus pennsylvanicus*, a species in which females are known to prefer older males, and males call to attract females. Tests were both longitudinal (males recorded early and late in life) and cross-sectional (males recorded once, each at different ages). I measured a variety of temporal and spectral calling song parameters and tested the predictions that: 1) calling song changes with age, and 2) variation in calling song explains variation in age. I found significant changes with increased age: calls showed decreased pulse period, decreased pulse duration, decreased pulse peak frequency, more pulses per chirp and increased pulse period variability. Although pulse period and pulse duration were negatively related with male age in bivariate correlations, canonical correlation failed to detect any significant relationship between song as a whole and male age. The majority of information in male song appears to be related to body size. Thus, there is signal information about male age in a few critical song parameters (pulse period and pulse duration), but not song as a whole. I discuss the results in relation to the auditory sensitivity of *G. pennsylvanicus*, and suggest a simple mechanism that explains both female preference for older males and female discrimination against heterospecific males.

2.2 Introduction

Older males often have higher mating success than younger males (e.g. Robel 1967; Zuk 1987c, 1988; Conner 1989; Simmons and Zuk 1992; Côté and Hunte 1993; Simmons 1995; Dickinson 2001; Felton et al. 2006; but see Jones et al. 2000, 2004; reviewed in Brooks and Kemp 2001). This pattern could arise due to a number of processes. For instance, high quality males may mature early and therefore have more time to acquire resources (e.g. territories) important for attracting a mate than low quality males. If age is measured as time since maturity, as is the case in many insects (e.g. Neville 1963; Zuk 1987a; Simmons 1995) then mate attraction will favour older males because they will, on average, have accumulated more or better quality resources. Another mechanism by which older males may be favoured is if male display increases in attractiveness with age. As sexually reproducing organisms age many aspects of their phenotype change (Rose 1991) including male display. To the extent that survival and quality are correlated in males, these phenotypic changes may provide a ready-made signal of quality. For example, the adult plumage of male birds often changes with age (e.g. Hill 1996; Delhey and Kempenaers 2006; Doucet et al. 2007; Probst et al. 2007), and females are known to prefer males that have “older” plumage (Hunt et al. 1998a,b; Sheldon et al. 1999). Other potential examples of age-related quality indicators are the attainment of silver back hair in male gorillas, *Gorilla gorilla* (Levréro et al. 2006) and large body size in male bullfrogs, *Rana catesbeiana* (Howard 1978a).

Male field crickets (Orthoptera: Gryllidae: Gryllinae) use forewing stridulation to produce an acoustic signal (calling song) that attracts females and wards off rival males (Alexander 1961; Loher and Dambach 1989). In related tettigoniid orthopterans the forewings wear with age, resulting in changes to song (Hartley and Stephen 1989; Stiedl et al. 1991; Ritchie et al. 1995). Thus wear-related (age-related) changes in grylline male song could form the basis for the observed mating bias in favour of older males (Zuk 1987c, 1988; Simmons and Zuk 1992; Simmons 1995; Chapter 6). As males age they may also increase energetic components of calling song in response to their decreasing residual reproductive value (Williams 1966).

In grylline species where females are not known to prefer older males, no age-related changes in calling song have been found to date (*Acheta domesticus* (Gray 1997), *Gryllus integer* (Hedrick 1986), *G. rubens* (Walker 2000), *G. texensis* (Cade and Wyatt 1984; Souroukis et al. 1992; Gray and Cade 1999; but see Bertram 2000), *Teleogryllus africanus* (Cade and Wyatt 1984), *T. oceanicus* (Walker and Cade 2003)) (Table 2.1). The evidence for age-related changes in calling song is somewhat equivocal for species in which older males are known to have a

Table 2.1: Summary of the studies that have looked for age-related changes in field cricket calling song.

Species	Older Male Bias	Song Parameters Examined[*]	Experimental Design	Relationship Found with Male Age	Reference
Acheta domesticus		AMP, PPC, CFQ, ICI	cross-sectional	none	Gray 1997
Gryllus bimaculatus	Yes[a]	CFQ, Q, CD, ICI, CR, PPC, PD, IPI, PR[**]	cross-sectional	older males had more variable PR (not significant after sequential Bonferroni correction)	Simmons and Zuk 1992
G. campestris	Yes[b]	CFQ, CR, PPC, CD, ICI, E	longitudinal	older males had lower CFQ and shorter CD with fewer PPC	Jacot et al. 2007
G. integer (California)		PSB	longitudinal	none	Hedrick 1986
G. rubens		PR	longitudinal	none	Walker 2000
G. pennsylvanicus	Yes[c]	NCE	longitudinal	none after 5 days post-adult moult	Cade and Wyatt 1984
		PPC	cross-sectional	older males had more PPC	M. Zuk unpubl. data, cited in Zuk 1988
		BD	longitudinal	older males had shorter BD	Zuk and Simmons 1997
		PR, PPC, ICI, CD	longitudinal	none	Ciceran et al. 1994
G. texensis (formerly *G. integer* Texas)		NCE	longitudinal	none after 5 days post-adult moult	Cade and Wyatt 1984
		NCE, TCP	longitudinal	middle-aged males had greater NCE than young or old males, and males initiate calling earlier in the night as they age	Bertram 2000
		PR, PPT, ITI, PMP	cross-sectional	none	Souroukis et al. 1992
		PPT	cross-sectional	none	Gray and Cade 1999

Table 2.1 continued

Species	Older Male Bias	Song Parameters Examined[*]	Experimental Design	Relationship Found with Male Age	Reference
G. veletis	Yes[c]	NCE	longitudinal	none after 5 days post-adult moult	Cade and Wyatt 1984
Teleogryllus africanus		NCE	longitudinal	none after 5 days post-adult moult	Cade and Wyatt 1984
T. oceanicus		for chirp portion: CD, PPC, PR, PD, IPI, CFQ for trill portion: TD, CPT, CR, PD, IPI, ICI, CFQ for entire song: SD, PSC	cross-sectional	none	Walker and Cade 2003

[a] (Simmons and Zuk 1992); [b] (Simmons 1995); [c] (Zuk 1987c, 1988)

[*] AMP (amplitude); BD (bout duration); CD (chirp/trill duration); CFQ (carrier frequency); CPT (chirps per trill); CR (chirp rate); E (chirp energy); ICI (interchirp interval); IPI (interpulse interval); ITI (intertrill interval); NCE (nightly calling effort); PD (pulse duration); PMP (proportion missing pulses); PPC (pulses per chirp); PPT (pulses per trill); PR (pulse rate); PSB (percent time spent calling in short bouts (<5s)); PSC (proportion of song that is chirp); Q (Q factor); SD (song duration); TCP (temporal calling pattern (start time, mean time, stop time)); TD (trill duration)

[**] song parameters examined also included coefficients of variation for all measured parameters

mating success advantage (*G. bimaculatus* (Simmons and Zuk 1992), *G. campestris* (Simmons 1995), *G. pennsylvanicus* (Zuk 1987c, 1988; Chapter 6) and *G. veletis* (Zuk 1987c, 1988)). Cade and Wyatt (1984) failed to detect any significant change in male *G. veletis* song with age, but they measured only one parameter of calling song (nightly calling effort). In *Gryllus bimaculatus*, variability in syllable (pulse) rate increased as males aged, but this relationship was not statistically significant after controlling for multiple tests (Simmons and Zuk 1992). Aging male *G. campestris* had significantly decreased carrier frequency, shortened chirp durations and fewer syllables (pulses) per chirp in their calling songs (Jacot et al. 2007).

In the fall field cricket, *G. pennsylvanicus*, age information is conveyed through calling song as females preferred older males when exposed to calling song and no other aspect of male phenotype (Zuk 1987c). A subsequent analysis of *G. pennsylvanicus* calling song did not detect any change in male song with age (Ciceran et al. 1994); however, this study failed to adequately control for male age, and analysed small sample sizes of both song parameters and individuals. Additionally, Zuk has reported that, as they age, male *G. pennsylvanicus* both shorten the duration of song bouts (Zuk and Simmons 1997) and increase the number of pulses per chirp (unpubl. data cited in Zuk 1988). As a result, the hypothesis that male *G. pennsylvanicus* signal their age has neither been conclusively eliminated nor convincingly supported.

I tested this hypothesis by conducting a longitudinal analysis in which I compared the songs of males recorded early and late in life. My experiment improves upon a previous attempt (Ciceran et al. 1994) by increasing the sample size, measuring more song parameters and controlling for male age more precisely. I also conducted a cross-sectional analysis to address the hypothesis that variation in male age is correlated with variation in male song. To do this I used a multivariate approach (canonical correlation analysis and redundancy analysis) to relate calling song to aspects of male phenotype, including age and morphology. This cross-sectional approach is important to ensure that there is sufficient variation in male song to enable females to choose males of different ages. For example, a longitudinal analysis may detect relatively subtle age-related changes in calling song, but these changes may not convey enough information for females to predict male age. Multivariate analyses also have the benefit of conserving statistical power to test the hypothesis of interest. Loss of statistical power has been an issue in previous studies examining correlations between song and other aspects male phenotype (e.g. Simmons and Zuk 1992).

Recent work on the neurobiology and phonotactic behaviour of *G. pennsylvanicus* has demonstrated that females are sensitive to variation in carrier frequency and pulse period (Jeffery et al. 2005) as are other gryllines (Schildberger et al. 1989). Jeffery et al. (2005) found that females are most attracted to calling song models with carrier frequency of 5 kHz and pulse period of 40 ms. So in addition to conducting multivariate tests, I also conducted univariate tests to determine whether these song parameters: a) change as individual males age (longitudinal study) and b) are correlated with male age across a sample of males (cross-sectional study).

2.3 Methods

2.3.1 Study Animals

I used first generation offspring of crickets captured in late August and early September 2002 from the grounds of the University of Toronto at Mississauga (43°32'50.51"N, 79°39'37.80"W).

I housed all juvenile crickets in large plastic containers (48 cm long, 35 cm wide, 31 cm high) at 25˚C, 70% relative humidity and a light cycle of 12hr light: 12hr dark. All crickets were fed Purina® cat chow (ground pellets for the first two to three weeks of life and whole pellets afterwards) and provided with water in cotton-plugged plastic vials. Layers of egg cartons provided shelter and a substrate on which to moult. I added new food every three to four days and changed water vials as needed. I moved larger nymphs to a separate bin to reduce cannibalism of smaller individuals. I isolated penultimate-instar nymphs in individual containers (polyethylene, 9 cm diameter, 8 cm high) with food (changed weekly), a cotton-plugged microfuge tube filled with water (changed biweekly or more frequently if needed), and a small piece of egg carton.

2.3.2 Recording Calling Song

All recordings were carried out in a sound-attenuating room, under red light and at 26ºC. When a male started to sing, I moved him gently to another table where I replaced the lid of his individual container (i.e. his rearing container) with one containing a screen window (to increase access to his song). I then placed his container under the microphone, which was positioned 2 cm directly above the screen of the container (approximately 10 cm above the floor of the container). When he resumed singing, I started recording his calling song using an Audio-Technica AT815b line cardioid/electret condenser microphone (±1 dB SPL [re: 20 µPa] between 3 and 6 kHz, K. A. Judge, unpubl. data) connected to a Tascam DA-P1 digital audio tape recorder (48 kHz sampling rate). Reverberation did not seem to be a problem (data not shown); this may have been because of the directionality of the microphone, the relatively short distance from cricket to microphone, and the thin (< 1 mm) container walls. Recordings were stopped after three to four minutes of continuous singing or at least one bout of continuous singing. If the male failed to resume singing, he was returned to the pool of unrecorded males. At the end of an evening's recording, all of the recorded males were weighed to the nearest 0.1 mg using a Mettler AE 50 balance.

The pool of males to be recorded on an evening was a haphazard selection of the population of individually housed males alive at that time. If a male's age at first recording was within 3 to 12 days post-adult moult, then I attempted to record his calling song 14 days later. A 14-day span was chosen for the longitudinal analysis because this enabled me to record males twice within approximately the natural age range for this species (Zuk 1987a; Murray and Cade 1995). Males who were successfully recorded twice were included in the longitudinal analysis, other males were included in the cross-sectional analysis. Thus males who were included in this study may represent a biased subset of relatively high effort callers, since they had to call on one or two separate occasions, and not all males called when selected for recording (K. A. Judge, pers. obs.). Most of the studies listed in Table 2.1 suffer from this problem. However, there is little that can be done to force a male to call (but see Wenzel et al. 1998). Furthermore, non-calling males may be displaying an alternative mating tactic (Cade 1979, 1981).

2.3.3 Measuring Song Parameters

All recorded songs were transferred to a PC using CoolEdit 2000, through a custom-built port connected to the digital input of a Creative SoundBlaster Audigy soundcard. Songs were saved as 48 kHz, 16-bit, mono .wav files. For each file, I selected a region of calling song that showed the greatest density and regularity of chirps and then saved a 16-chirp section from one bout of song from that region as an ASCII text file for transfer to a Macintosh computer for analysis. The filenames of these text files were encoded by a third party to allow me to remain blind to the song identity (and thus male age) while I measured song parameters. On the Macintosh, all manipulation and analysis of songs was done using Canary 1.2.4. (Cornell Laboratory of Ornithology). All text files were converted to Canary files and highpass filtered at 1 kHz to remove background noise.

I measured a range of temporal and spectral parameters for each song, including: chirp duration, chirp period, pulses per chirp, pulse duration, pulse period, chirp peak frequency, pulse peak frequency and pulse bandwidth –3dB from peak amplitude. I measured chirp peak frequency because carrier frequency varied slightly among pulses within a chirp and this represents a measure of carrier frequency integrated over the entire chirp. However, I also measured pulse peak frequency because I wanted to measure the properties of one individual wingstroke and thus be able to correlate measurements made on the same pulse (i.e. carrier frequency, pulse bandwidth, pulse duration, pulse period). Also, it is unclear which frequency measure may be more relevant to female evaluation of calls – "instantaneous" (pulse by pulse) or "average" (chirp) frequency. I also included in analyses the standard deviation of both chirp period and pulse period as estimates of the rhythmicity or cadence of male song. Pulse measurements were carried out on the second pulse of the chirp (Fig. 2.1). To ensure a high degree of consistency in the measurement of temporal parameters, I standardized the waveform window within Canary to an on-screen resolution of 0.10 s/inch and 0.50 Pa/inch (temporal and amplitude dimensions respectively) for chirp duration and interchirp interval, and 0.03 s/inch and 0.20 Pa/inch for pulse duration and interpulse interval. To generate frequency spectra, I used a 42.67 ms analysis frame, with an FFT of 2048 points and a Hamming window function. I set the on-screen amplitude resolution to 0.1 dB/inch and frequency resolution to 100 Hz/inch to standardize measurements of peak frequency and bandwidth. Temporal parameters were measured on each of the first 15 chirps, but spectral parameters were measured on only the first five chirps since these measurements were much less variable than temporal parameters (K. A. Judge, pers. obs.). Therefore, all measurements used in the analyses are means (median in the case of pulses per chirp) of either 15 or five measurements.

2.3.4 Morphological Measurements

I used NIH Image (version 1.62 for Macintosh) to measure: head width, pronotum width, pronotum length, left and right femur length, and left and right harp area on all individuals. The harp is a modified area of the male forewing that serves to radiate song (Bennett-Clark 1989). I did not use the method of Simmons and Ritchie (1996) to estimate harp area, but instead measured it more directly using six easily recognized landmarks around the perimeter of the harp. To increase the accuracy of harp area measurements, I measured each harp three times and used the mean area for all analyses. I calculated mean femur length, femur length asymmetry (left – right), mean harp area, and harp area asymmetry (left – right). Harp area asymmetry has been found to be related to cricket calling song (Simmons and Ritchie 1996) and femur length asymmetry may reflect an individual's ability to buffer developmental instability (Van Valen 1962). Therefore, including mass, each male had measurements for eight morphological variables to include in the cross-sectional analysis.

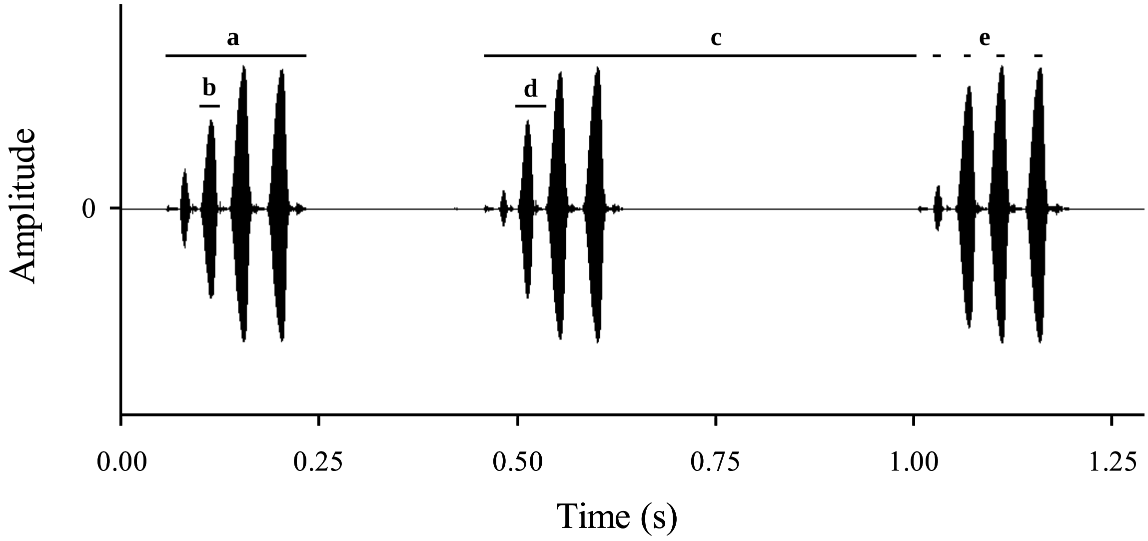

Figure 2.1: Amplitude by time plot of *G. pennsylvanicus* calling song illustrating the temporal parameters that were measured: a) chirp duration, b) pulse duration, c) chirp period, d) pulse period, and e) pulses per chirp.

2.3.5 Statistical Analysis

For the longitudinal analysis, I conducted pairwise analyses to test for age-related changes in the ten song parameters. Then, because many of the song parameters were highly intercorrelated, I also conducted a principal components analysis to reduce the ten song parameters to a set of orthogonal song principal components. I followed this with a multivariate repeated measures GLM with age class (older versus younger) as the within-subjects factor and the song principal components as dependent variables.

To test the hypothesis that male age is conveyed in calling song (cross-sectional analysis), I first carried out a series of pairwise correlations on male age, the morphological variables and the ten male song variables to identify univariate correlations between song and age, age and male morphology and song and male morphology. I then carried out a canonical correlation analysis (CCA) and redundancy analysis with age and the eight morphological variables as dependent variables, and the ten song parameters as independent variables. CCA is the full multivariate extension of univariate correlation analysis (Tabachnick and Fidell 2001). In CCA, one set of variables (often referred to as dependents) is related to another set of variables (independents) through the computation of canonical variates, which are linear combinations of the dependent and independent variables that maximize variance shared between canonical variate pairs (i.e. one independent canonical variate and one dependent canonical variate). CCA computes as many pairs of canonical variates (CVPs) as there are variables in the smaller set, either dependent or independent. Each subsequent CVP maximizes the proportion of variance explained from that left over from the previous CVP. Redundancy analysis allows for the evaluation of the proportion of variance in one set of variables that is explained by the canonical variate of the other set (Tabachnick and Fidell 2001).

To examine the amount of information about male age relative to male morphology that is conveyed in song, I conducted three sequential CCA and redundancy analyses. The first analysis had only male age as the dependent variable (equivalent to multiple regression, Tabachnick and Fidell 2001), the second had only male morphology as the dependent variables, and the third had both male age and morphology. The amount of variance in the dependents explained by the song variables for each of the different CCAs will reveal how important song is to predicting age (see Rotenberry et al. 1996 for a similar use of CCA and redundancy analysis).

I used SPSS 10 for Windows for all statistical analyses. I tested the assumption of normality for all variables using Kolmogorov-Smirnov tests with Lilliefors significance correction. All tests were two-tailed and had an a priori Type I error rate of 5%.

2.4 Results

<u>2.4.1 Longitudinal Analysis</u>

I recorded 28 males at both a young (mean ± SE = 7.1 ± 0.6 days post adult moult) and old (mean ± SE = 21.4 ± 0.6) age, which is well within the age range found in wild populations of *G. pennsylvanicus* (Zuk 1987a; Murray and Cade 1995). Normality tests revealed that several of the song parameters (chirp period, chirp period standard deviation, pulses per chirp, pulse period standard deviation and pulse bandwidth) deviated significantly from normality (K-S Lilliefors tests, all $p < 0.005$). Transformations failed to restore normality for all of these variables, so I carried out non-parametric tests for all variables. Results are consistent regardless of whether the data are analyzed with parametric or non-parametric statistics (data not shown). Because this analysis is exploratory in nature I did not correct for multiple comparisons.

Males lost weight as they aged (paired t-test: $t = 5.492$, $df = 25$, $p < 0.001$), dropping from a mass of 431 mg (± SE = 17 mg) when young to 397 mg (± SE = 16 mg) at an older age. To test whether the age-related change in mass confounded age-related changes in any of the song parameters, I correlated change in mass with change in each song parameter. Change in mass was significantly correlated with change in only one song parameter, pulse period (Spearman's rho = 0.446, $p = 0.023$, for all others $p > 0.284$).

Given that I could not transform all of the song variables to conform to the assumption of normality, and that mass change correlated with change in pulse period, I could not test the overall hypothesis that song as a whole changed with age by using a multivariate repeated measures GLM. Consequently I tested each song parameter individually using Wilcoxon signed rank tests. Song changed significantly with age. In particular, old males had shorter pulse periods, shorter pulse durations, lower pulse peak frequencies, more pulses per chirp and more variable pulse periods compared to when they were young (Table 2.2, Fig. 2.2). However, given that change in mass also correlated with change in pulse period, this result cannot be exclusively attributed to aging. I did not detect any effect of age on: chirp duration, pulse bandwidth, chirp period standard deviation, chirp peak frequency or chirp period (Table 2.2).

To further test the prediction that song changes with age in *G. pennsylvanicus*, I conducted principal components analysis (PCA) on the ten song parameters. Although normality tests revealed that many of the original song parameters were not normally distributed (see above), PCA does not make assumptions about the distribution of variables as long as principal components are not tested for statistical significance (Tabachnick and Fidell 2001). I included songs for males when they were both young and old since I wanted to compare changes in song within males. PCA extracted five song principal components (SPCs) with eigenvalues greater than one that together explained 88.5% of the variation in the original ten song parameters. SPC loadings for each song parameter and the variance explained by each SPC are given in Table 2.3. Normality tests revealed that SPC1 and SPC5 significantly deviated from normal distributions (SPC1: test statistic = 0.118, $p = 0.051$; SPC5: test statistic = 0.125, $p = 0.030$). However, given that visual inspection of boxplots showed that both variables were symmetric around the median and had few outliers, and transformations failed to improve the normality of these two variables (data not shown), I proceeded with parametric tests.

Change in mass was not significantly correlated with any of the SPCs (all $|r| < 0.266$, all $p > 0.190$). I tested for age-related changes in the five SPCs by conducting a multivariate repeated-measures GLM with male age (young or old) as the within-subjects variable and the five SPCs as dependent variables. Song changed significantly with age (Pillai's trace = 0.445, $F_{5,23} = 3.691$, $p = 0.013$). Univariate analyses revealed that only SPC1 changed significantly with age ($F_{1,27} = 16.619$, $p < 0.001$): males had higher values of SPC1 when they were older than when they were younger (mean difference ± SE = 0.691 ± 0.169) meaning that they tended to increase pulses per chirp, decrease pulse duration, decrease pulse period, increase chirp duration and increase pulse

Table 2.2: Medians and interquartile ranges (IQR) for each of the song parameters for male *G. pennsylvanicus* at younger and older ages.

	Younger		Older			
Parameter	Median	IQR	Median	IQR	WSR Z[a]	p
Chirp Duration (ms)	178.6	168.7-186.0	179.6	172.1-190.6	1.252	0.210
Chirp Period (ms)	631.2	562.2-693.8	600.0	498.9-696.8	0.660	0.509
Chirp Period Variability (S.D. ms)	86.4	54.9-119.8	90.4	49.1-168.3	1.002	0.316
Pulses Per Chirp (pulses)	4	3-5[b]	4	4-6[b]	2.333	0.020
Pulse Duration (ms)	21.6	19.4-24.1	18.2	16.3-21.2	2.892	0.004
Pulse Period (ms)	40.2	36.7-42.4	38.0	34.5-40.8	3.393	0.001
Pulse Period Variability (S.D. ms)	1.4	1.1-2.2	2.0	1.5-2.7	2.049	0.040
Chirp Peak Frequency (kHz)	4884	4646-5096	4861	4717-4990	0.934	0.350
Pulse Peak Frequency (kHz)	4838	4604-5041	4774	4577-4875	2.517	0.012
Pulse Bandwidth (Hz)	121	88-158	114	88-134	1.207	0.227

[a] Wilcoxon signed rank test, normal approximation

[b] Minimum to maximum instead of IQR

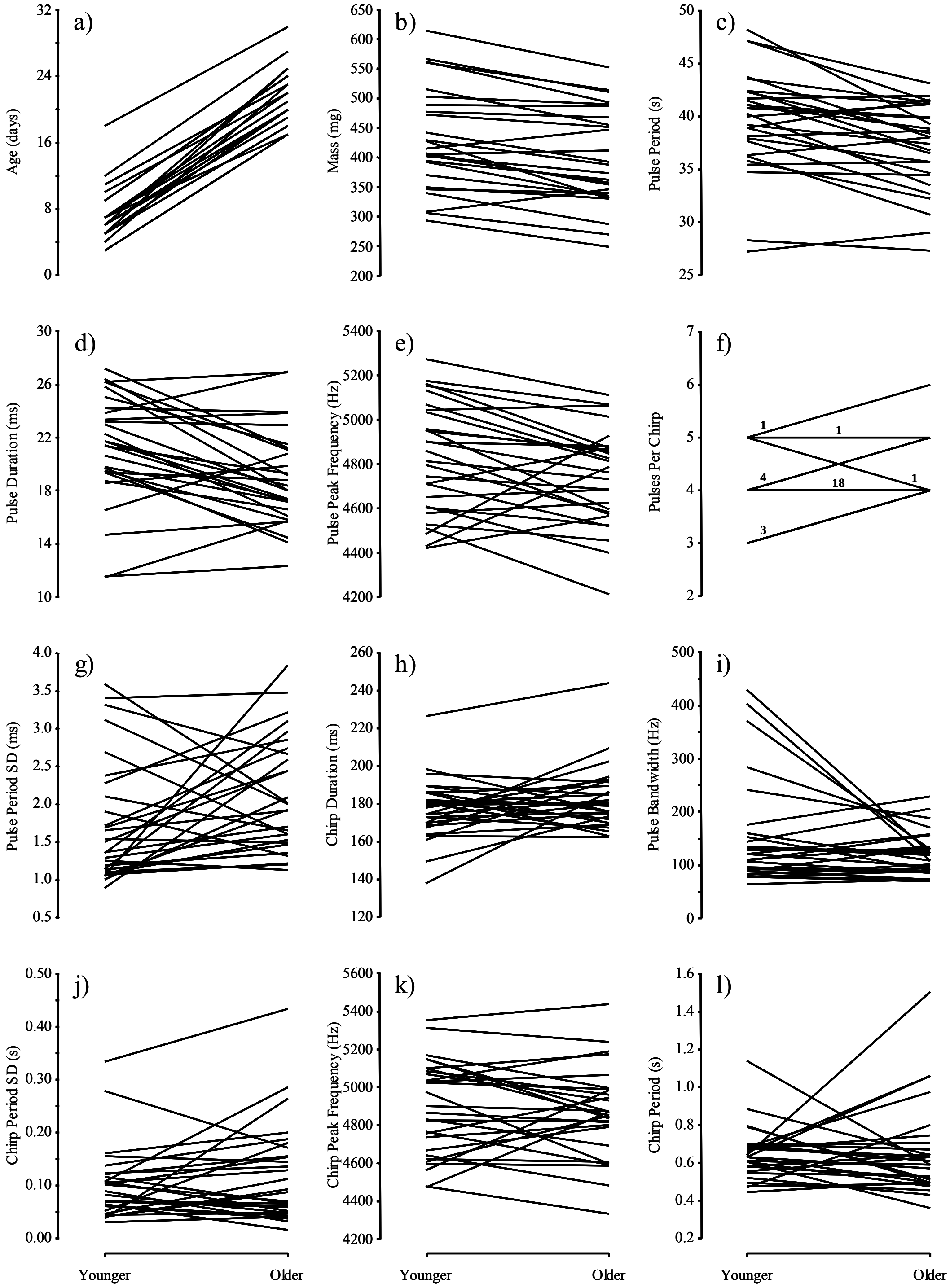

Figure 2.2: Plot of changes in *G. pennsylvanicus*: a) age, b) mass, and c to l) calling song parameters from the longitudinal analysis. The nine song parameters are in order of decreasing statistical significance based on Wilcoxon signed rank tests: c) pulse period, d) pulse duration, e) pulse peak frequency, f) pulses per chirp, g) pulse period standard deviation, h) chirp duration, i) pulse bandwidth, j) chirp period standard deviation, k) chirp peak frequency, l) chirp period. Each line represents the change in calling song for an individual male.

Table 2.3: Principal components analysis of male song in the longitudinal analysis. Principal component loadings greater than 0.5 are bolded.

	Song Principal Component				
Parameter	1	2	3	4	5
Chirp Duration	**0.594**	0.332	0.365	0.428	0.380
Chirp Period	0.324	**0.669**	**0.511**	-0.152	-0.146
Chirp Period Variability	0.298	**0.502**	0.411	**-0.520**	-0.340
Pulses Per Chirp	**0.852**	0.061	0.133	0.383	0.143
Pulse Duration	**-0.735**	0.382	0.088	0.217	0.323
Pulse Period	**-0.734**	0.428	0.346	-0.060	0.196
Pulse Period Variability	**0.558**	-0.191	-0.416	-0.343	0.173
Chirp Peak Frequency	0.078	**-0.782**	**0.576**	-0.144	0.126
Pulse Peak Frequency	-0.149	**-0.760**	**0.585**	-0.123	0.146
Pulse Bandwidth	-0.141	-0.242	0.180	**0.544**	**-0.698**
Eigenvalue	2.711	2.427	1.588	1.124	0.998
% of Variance	27.106	24.269	15.882	11.237	9.981

period variability when they aged (see Table 2.3). I did not detect differences in any of the other SPCs (SPC2: $F_{1,27} = 0.221$, p = 0.642; SPC3: $F_{1,27} = 2.430$, p = 0.131; SPC4: $F_{1,27} = 0.923$, p = 0.345; SPC5: $F_{1,27} = 0.490$, p = 0.490).

2.4.2 Cross-sectional Analysis

I recorded 104 male *G. pennsylvanicus* for the cross-sectional analysis, however upon measurement of morphology, two males were excluded from analysis because their left forewing was either missing or grossly deformed. The remaining 102 males ranged in age from 4 to 27 days post-adult moult (mean ± SE = 14.1 ± 0.6 days). Male *G. pennsylvanicus* were directionally asymmetric for femur length (one sample Kolmogorov-Smirnov test: Z = 1.426, p = 0.034), but not harp area (Z = 0.948, p = 0.311), although femur length asymmetry and harp area asymmetry were positively correlated (Spearman's rho = 0.245, p = 0.013). Males had longer right than left femurs ($\text{median}_{L\text{-}R} = -0.03$ mm, interquartile range = -0.07 to 0.04 mm). Given that field cricket stridulation is inherently directionally asymmetric, with males usually stridulating with the right forewing on top of the left (Masaki et al. 1987; K. A. Judge, pers. obs.), I retained the directionality of harp area asymmetry measurements in all subsequent analyses even though I did not detect directional asymmetry in harp area.

Normality tests revealed that many of the song variables (chirp duration, chirp period, chirp period standard deviation, pulses per chirp, pulse period standard deviation and pulse bandwidth) and several of the morphological variables (harp area asymmetry, femur length asymmetry and body mass) deviated significantly from normality (K-S Lilliefors tests, all $p < 0.016$). Transformations failed to normalize many of the variables (data not shown), so I tested for pairwise correlations using non-parametric Spearman's rank correlations. Male age was significantly correlated with only pulse period (Spearman's rho = -0.255) and pulse duration (Spearman's rho = -0.222) (Table 2.4). Pulse duration was positively correlated with all of the measures of body size, and pulse period was negatively correlated with harp area asymmetry (Table 2.4). Both chirp peak frequency and pulse peak frequency were negatively correlated with all of the measures of body size, but most strongly with mean harp area (Table 2.4).

Canonical correlation analysis, like multiple regression, is sensitive to multicolinearity (Tabachnick and Fidell 2001). Therefore, I reduced five of the measures of body size (body mass, head width, pronotum width, pronotum length and mean femur length) to a summary measure of body size using principal components analysis (PCA). Although harp area is strongly positively correlated with these five measurements (all Pearson $r > 0.690$, all $p < 0.001$) I did not include harp area in the PCA because this structure is directly related to production of calling song (Bennett-Clark 1989). PCA resulted in one principal component with an eigenvalue greater than 1 (PC1 eigenvalue = 4.626) and that explained 92.5% of the variation in the original five variables. Variable loadings on PC1 (henceforth called body size) were as follows: body mass (0.961), head width (0.977), pronotum width (0.955), pronotum length (0.961) and mean femur length (0.955).

When age, body size, harp area, harp area asymmetry and femur length asymmetry (collectively male phenotype) were entered into a canonical correlation analysis (CCA) as dependents, calling song correlated significantly with male phenotype (age and morphology) on two canonical variate pairs (CVPs) (CVP1: $F_{50,400.15} = 2.363$, $p < 0.001$; CVP2: $F_{36,331.51} = 1.477$, $p = 0.043$). The first canonical correlation was 0.681 (46.3% shared variance) and the second was 0.500 (25.0% shared variance). Redundancy analysis showed that within the first two CVPs, calling song accounted for 18.0% of the variance in the dependent variables (Table 2.5). Following Tabachnick and Fidell (2001), I considered only variable loadings of 0.3 or greater when interpreting the CVPs. Within the first CVP: lower pulse peak frequency (-0.861), lower chirp peak frequency (-0.839) and longer pulse duration (0.360) were positively correlated with harp area (0.980) and body size (0.650) (Table 2.5). And within the second CVP: shorter pulse duration (-0.547), shorter pulse period (0.530), greater pulse period variability (0.491) and more pulses per chirp (0.342) were negatively correlated with harp area asymmetry (-0.721, i.e. right harp increasing in area relative to the left) and body size (-0.521) (Table 2.5). Male age did not load strongly (i.e. above 0.3, Tabachnick and Fidell 2001) on either of the two CVPs (Table 2.5).

Table 2.4: Matrix of Spearman rank correlations among male phenotypic variables and song parameters. Bolded correlations are statistically significant at $p < 0.05$ (N = 102 for all cells).

	Phenotypic Variables									Song Variables								
	AGE	MASS	HW	PW	PL	MHA	ASHA	MFL	ASFL	CD	CP	CPSD	PPC	PD	PP	PPSD	CPKF	PPKF
Phenotypic Variables																		
MASS	-0.025																	
HW	-0.037	**0.922**																
PW	0.056	**0.895**	**0.922**															
PL	-0.024	**0.872**	**0.913**	**0.882**														
HAM	-0.047	**0.699**	**0.729**	**0.681**	**0.648**													
ASHA	0.044	0.089	0.077	0.062	0.088	0.134												
FLM	-0.079	**0.884**	**0.891**	**0.834**	**0.899**	**0.707**	0.107											
ASFL	0.127	0.069	0.025	0.007	-0.008	-0.116	**0.245**	-0.022										
Song Parameters																		
CD	0.010	0.150	0.163	0.156	0.144	0.172	-0.063	0.193	-0.047									
CP	0.083	0.065	0.013	0.000	-0.016	0.038	**0.195**	-0.010	-0.077	0.135								
CPSD	0.009	-0.017	-0.067	-0.036	-0.112	-0.062	0.076	-0.063	-0.025	0.025	**0.684**							
PPC	0.163	-0.128	-0.080	-0.051	-0.092	-0.048	-0.179	-0.069	-0.029	**0.640**	0.062	0.053						
PD	**-0.222**	**0.298**	**0.309**	**0.197**	**0.277**	**0.259**	0.147	**0.324**	0.046	-0.014	**-0.252**	-0.160	**-0.276**					
PP	**-0.255**	0.182	0.119	0.098	0.119	0.037	**0.207**	0.146	0.028	-0.005	0.044	0.113	**-0.532**	**0.504**				
PPSD	-0.136	-0.152	-0.170	-0.134	-0.149	-0.015	-0.129	**-0.212**	0.000	0.031	-0.030	0.137	0.120	**-0.239**	**-0.221**			
CPKF	-0.092	**-0.344**	**-0.295**	**-0.307**	**-0.310**	**-0.516**	-0.122	**-0.373**	0.124	-0.056	-0.120	-0.089	0.070	-0.115	-0.032	0.024		
PPKF	-0.096	**-0.314**	**-0.261**	**-0.287**	**-0.282**	**-0.518**	-0.046	**-0.322**	0.173	-0.078	-0.130	-0.103	0.000	-0.054	0.035	-0.050	**0.943**	
PBW	-0.024	0.028	0.067	0.126	0.050	-0.081	-0.116	0.002	0.074	-0.087	-0.056	0.039	0.052	**-0.253**	-0.074	0.092	0.029	0.014

[*] Abbreviations are as follows (units of measurement at the end of parentheses): AGE (age at recording, days), MASS (mass at recording, mg), HW (head width, mm), PW (pronotum width, mm), PL (pronotum length, mm), MHA (mean harp area, mm^2), ASHA (asymmetry in harp area, mm^2), MFL (mean femur length, mm), ASFL (asymmetry in femur length, mm), CD (chirp duration, ms), CP (chirp period, ms), CPSD (chirp period standard deviation, ms), PPC (pulses per chirp, pulses), PD (pulse duration, ms), PP (pulse period, ms), PPSD (pulse period standard deviation, ms), CPKF (chirp peak frequency, Hz), PPKF (pulse peak frequency, Hz), PBW (pulse bandwidth, Hz).

Table 2.5: Results of the canonical correlation analysis, including: canonical loadings of each of the dependent and independent variables on their respective canonical variates, the percent variance of each canonical variate that is explained by variables from the other set (Redundancy), and the canonical correlation for each pair of canonical variates.

	Canonical Variate Pair	
Canonical Variate	1	2
Dependent Variables		
Age	-0.038	0.210
Body Size	0.650	-0.521
Mean Harp Area	0.980	-0.134
Harp Area Asymmetry	0.031	-0.721
Femur Length Asymmetry	-0.257	-0.243
Redundancy (%)	13.4	4.6
Independent Variables		
Chirp Duration	0.164	0.028
Chirp Period	-0.049	-0.154
Chirp Period Variability	-0.040	-0.009
Pulses Per Chirp	-0.076	0.342
Pulse Duration	0.360	-0.547
Pulse Period	0.059	-0.530
Pulse Period Variability	0.029	0.491
Chirp Peak Frequency	-0.839	-0.021
Pulse Peak Frequency	-0.861	-0.189
Pulse Bandwidth	-0.270	-0.238
Redundancy (%)	16.9	10.6
Canonical Correlation	0.681	0.500

CCA between one dependent and several independent variables is equivalent to multiple regression (Tabachnick and Fidell 2001); therefore, I conducted a multiple regression of age on the ten song parameters. This multiple regression was not statistically significant ($F_{10,91} = 1.592$, $p = 0.121$). Removal of age from the full CCA did not greatly change canonical correlation between song and male phenotype (canonical correlation$_{\text{morphology}} = 0.680$, canonical correlation$_{\text{age+morphology}} = 0.681$). Redundancy analysis showed that calling song accounted for 16.9 % of variation in the first canonical variate when just male morphology was included, whereas it accounted for 13.5 % of variation in the first canonical variate when both age and morphology were included.

2.5 Discussion

Older male *G. pennsylvanicus* field crickets have been found to be more attractive to females than younger males (Zuk 1987c, 1988; Chapter 6), even when females had only calling song on which to base their mate choice (Zuk 1987c). This result demonstrates that the calling song of older males differs from that of younger males. This may be the result of: a) ontogenetic changes in male song, b) differential survival by a subset of males with calling songs different from other males in the population, or c) both. In this study, I tested the hypothesis that male song changes with age in *G. pennsylvanicus*. Males recorded at young and then older ages (7 and 21 days post adult moult respectively) showed significant changes in song. When they were older, males had shorter pulse period, shorter pulse duration, lower pulse peak frequency, more pulses per chirp and more variable pulse period than when they were younger (Fig. 2.2). Males also weighed less when they were older, and change in mass correlated significantly with change in pulse period. However, mass loss likely doesn't confound age-related changes in song because there were no correlations between mass loss and any song principal component, and the first song principal component changed significantly with age. Moreover, in a separate cross-sectional analysis, pulse period and pulse duration were negatively correlated with male age (Table 2.4). However, male song as a whole did not explain a significant portion of the variance in male age when correlations with male morphology were taken into account (Table 2.5).

2.5.1 Do male G. pennsylvanicus *signal their age?*

Recently, Jacot et al. (2007) found that, when they were older, individual male *G. campestris* produced calling song with lower carrier frequency, and more pulses per chirp than when they were younger. Thus, the older male mating advantage in this species (Simmons 1995) may be at least partially explained by this age-related decrease in carrier frequency (Jacot et al. 2007) since females prefer lower carrier frequencies (Scheuber et al. 2004). Previous work on *G. pennsylvanicus* calling song (Ciceran et al. 1994) failed to detect statistically significant age-related changes in song. However, Ciceran et al. (1994) reported increases in pulses per chirp, interchirp interval, chirp duration and pulse rate (inverse of pulse period) as males grew older. All of these effects, except increased interchirp interval (not analyzed), were statistically significant in the current study (Table 2.3).

The results of the current longitudinal study are consistent with the hypothesis that female preference for older males is based on ontogenetic changes in calling song. However, for this preference to work, females must be able to discriminate male age on the basis of calling song.

Although age was related to both pulse period and pulse duration in bivariate correlations (Table 2.4), song as a whole did not significantly predict male age. Furthermore, removal of age from the CCA did not decrease the amount of variance in male phenotype explained by calling song. Thus, if female *G. pennsylvanicus* listen to male calling song as a whole, there seems to be little information about age on which females could base a mating preference for older males. However, if females pay attention to just pulse period or pulse duration, then it may be possible for females to choose older males.

Interestingly, Jeffery et al. (2005) found that the attractiveness of *G. pennsylvanicus* calling song varied with pulse period, attaining peak attractiveness at 40ms. In the current study, individual males decreased the pulse period of their calling song from 40 ms when they were younger to 38 ms when they were older. This might suggest that age-related changes in calling song may make older males less attractive. However, as Jeffery et al. (2005) tested females with calling song models with pulse periods of 35 and 40 ms, the true peak in attractiveness may lie between 35 and 40 ms, close to the older male pulse period of 38 ms found in the current study. Furthermore, other age-related changes, such as increases in pulses per chirp (e.g. Fig. 2.2), which increases the duty cycle (proportion of time filled with male signal) of male song, may interact with decreases in pulse period and pulse duration, to cause male *G. pennsylvanicus* to increase in attractiveness with age. Future work on the phonotactic preferences of female *G. pennsylvanicus* should present females with song models that represent the full range of song parameter combinations to more fully describe the selection that females exert on all song parameters (e.g. Brooks et al. 2005).

2.5.2 What information is conveyed in male song?

Of the phenotypic variables considered in the cross-sectional analysis (male age, body size, harp area, harp area asymmetry, femur length asymmetry), *G. pennsylvanicus* calling song appears to convey information mostly about male body size, particularly harp area. Males with both lower carrier frequency (both pulse and chirp peak frequency) and longer pulse duration were larger in both harp area and body size (Tables 2.4 and 2.5), although song was most strongly associated with harp area within the first canonical variate pair. This is perhaps not surprising since the harp is directly involved in radiating calling song (Bennett-Clark 1989), and larger harps resonate at a lower frequency (Nocke 1971). In addition, pulse duration is controlled by the duration of contact between forewings during the closing stroke of stridulation (Bennett-Clark 1989). Larger male *G. pennsylvanicus* have larger forewings and thus longer files (part of the stridulatory apparatus on the forewing), and so may have longer duration pulses because their forewings are in contact for longer during stridulation. This assumes that males do not vary the proportion of their forewings used or the speed of forewing closure during stridulation.

Why do male *G. pennsylvanicus* reliably signal harp area and body size in calling song? In most Gryllidae, calling song attracts females and can ward off rival males (Alexander 1961; Loher and Dambach 1989), so reliable information about body size in calling song may be important in both competition with rivals and signalling male quality. Jeffery et al. (2005) found that a carrier frequency of 5 kHz was most attractive to female *G. pennsylvanicus*. Given that the males in the cross-sectional analysis had a mean pulse peak frequency of 4920 ± 30 Hz, carrier frequency and thus harp area may be under stabilizing selection by female choice, contrary to the usual pattern of females favouring larger males (see below). In contrast, male-male competition likely exerts directional selection on harp area since larger male *G. pennsylvanicus* win more fights than smaller males (Souroukis and Cade 1993). Field crickets stridulate during aggressive interactions (Alexander 1961), and reliable information about body size conveyed through aggressive song may help males reduce the costs of fighting when body size differences are great. Therefore, male-male competition may maintain the correlation between body size and carrier frequency (i.e. positive correlational selection), so selection for larger body size would result in selection for larger harps and lower carrier frequency.

Evidence that larger male body size is favoured by sexual selection in *G. pennsylvanicus* is mixed. In a field enclosure experiment where sex ratio was manipulated, Souroukis and Cade (1993) detected significant total selection and directional selection on male weight, although the result depended on sex ratio. In the wild, there is some evidence for directional sexual selection: males found paired with females were significantly larger in only one of two body size dimensions than males found alone (Zuk 1988). However, in studies where males were prevented from competing physically, there was no significant selection for larger males (Zuk 1987c; Chapter 6). These results suggest that male-male competition may be more important than female choice in determining male mating success in the field.

Body size has been shown to be an important determinant of male mating success in other grylline crickets. In *Acheta domesticus*, larger males win more fights (Hack 1997; Savage et al. 2005), are preferred by females (Gray 1997; Savage et al. 2005) and sing calling songs with more pulses per chirp than smaller males (Gray 1997). Larger male *G. campestris* are more attractive to females (Simmons 1992, 1995) and information about body size is conveyed through carrier frequency: larger males have larger harps and lower carrier frequencies (Simmons 1995; Simmons and Ritchie 1996). Additionally, male *G. campestris* that were nutritionally stressed as juveniles (Scheuber et al. 2003b) and had their immune system challenged (Jacot et al. 2005), moulted with smaller harps than males that had not been nutritionally stressed or immune challenged. So females may gain more information about males than simply body size from calling song carrier frequency (Scheuber et al. 2004). Larger male *G. bimaculatus* win more fights (Simmons 1986a, Hofmann and Schildberger 2001) and are preferred by females (Simmons 1986b). Simmons and Zuk (1992) found that larger males had longer pulse durations than smaller males. Another gryllid (Oecanthinae) in which larger male body size is sexually selected is the black-horned tree cricket, *Oecanthus nigricornis*. Larger males win more aggressive contests (Brown 1994) and are preferred by females (Bussière et al. 2005), who receive higher quality proteinaceous food gifts from larger males (Bussière et al. 2005). Larger male *O. nigricornis* have songs with lower carrier frequency and shorter pulse duration, although only the former was important in attracting females (Brown et al. 1996). In each of these studies, sexual selection seems to consistently favour larger males through both male-male competition and female choice. Clearly, further work is needed on the extent to which fitness of male *G. pennsylvanicus* is determined by male-male interactions versus female choice.

2.5.3 An alternative hypothesis to explain increased attractiveness of older male G. pennsylvanicus*?*

Female *G. pennsylvanicus* that appear to be choosing older males may actually be discriminating against heterospecific matings with *G. firmus*. These two closely-related gryllines hybridize across a wide area in the northeastern United States (Harrison and Arnold 1982). *G. pennsylvanicus* matures earlier than *G. firmus*, and the extent of overlap in breeding phenology between the two species varies across the hybrid zone (Harrison 1985). *G. pennsylvanicus* has both shorter pulse periods and shorter pulse durations than *G. firmus* (Alexander 1957; Doherty and Storz 1992). Thus, in areas of sympatry, female *G. pennsylvanicus* that prefer shorter pulse periods and/or shorter pulse durations and thus older (earlier-maturing) male *G. pennsylvanicus*, are less likely to hybridize with *G. firmus* than females that don't express the preference. However, in areas of allopatry such as my study area, why is the preference for shorter pulse period and thus older males maintained? Such a preference, if exercised, is likely to be costly since the oldest males represent a small fraction of males in the wild (Zuk 1987a) and calling males are relatively widely dispersed (French et al. 1986). Gene flow between areas of sympatry and allopatry (Walker 1974) may maintain the preference, although this is unlikely due to potential costs of the preference and limited dispersal since most *G. pennsylvanicus* lack flight wings (Harrison 1979). Alternatively, females may gain direct benefits by choosing older males if these males defend territories rich in food resources (Rost and Honegger 1987). High condition male *G. pennsylvanicus* (as manipulated through diet) lived longer and called more per day (i.e. higher duty cycle) than low quality males (Judge et al. 2008, Chapter 3), and females are attracted to higher duty cycle calling song (Chapter 4). Thus, if the ability to acquire larval resources (condition) is heritable (Rowe and Houle 1996), then females may gain indirect benefits by mating with older males. These benefits may play a role in keeping *G. pennsylvanicus* and *G. firmus* from introgressing into a single species, since matings between female *G. pennsylvanicus* and male *G. firmus* produce viable and fertile hybrid offspring (Harrison 1983). Clearly further experiments using female *G. pennsylvanicus* from the hybrid zone that examine phonotaxis to calling songs of varying pulse periods, coupled with studies of age-related changes in male calling song from these populations would clarify the role that phonotactic preferences play in preventing hybridization between *G. pennsylvanicus* and *G. firmus*.

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CHAPTER 3

Condition dependence of male lifespan and calling effort in a field cricket.

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3.1 Abstract

Sexually selected traits are thought to impose survival costs on showy males. Recent empirical work found a negative relationship between male display and survival in a field cricket species (Orthoptera, Gryllidae, Gryllinae) where there is no evidence of a mating bias towards older males. In most species, however, male survival and ornamentation are positively correlated, and older males often have a mating success advantage over younger males. These findings suggest that male quality and survival are positively correlated, but more tests of this hypothesis are needed. We measured the condition dependence of male survival and calling effort in another grylline, *Gryllus pennsylvanicus*, where older males have previously been shown to have greater mating success. We varied condition by manipulating diet, and measured male lifespan and calling effort to assess the relative condition dependence of these traits. High and medium condition males survived longer than low condition males, and high condition males called more than medium and low condition males. Differences in calling effort among the condition treatments were not apparent early in life, but emerged as males aged. We discuss possible explanations for the differences between our study and contrasting results such as the previous grylline work.

3.2 Introduction

Darwin initially proposed the theory of sexual selection to account for male traits, like the peacock's tail, that appeared to reduce survival (Darwin 1871). He proposed that the survival cost of these extravagant male traits was compensated by the advantages that their bearers gained in mating success (Darwin 1871). Contemporaries of Darwin, including Wallace, doubted that an adaptation could ever evolve that would reduce the survival of its bearer. Wallace proposed instead that only the most vigorous and fit males could develop extravagant traits and thus the most elaborated males would also survive the longest (Cronin 1991). Darwin's hypothesis was elegantly validated in theory by Fisher (1915, 1930), who showed how a male trait could evolve past its natural selection optimum, as long as females preferred males with elaborate traits, and both preference and trait became genetically correlated in future generations. Empirical findings of a negative relationship between male ornamentation and survival are thus often taken as evidence of the Darwin-Fisher process (e.g. Brooks 2000). Wallace's view that male ornamentation and survival should be positively correlated has been supported by empirical research – most studies report that males with the most elaborate ornaments also survive longest (meta-analysis by Jennions et al. 2001). Moreover, it is often concluded that older males have an advantage in mating success (e.g. Robel 1967; Zuk 1987c, 1988; Connor 1989; Simmons and Zuk 1992; Simmons 1995; Dickinson 2001; but see Jones et al. 2000; reviewed in Brooks and Kemp 2001), suggesting that male survival may be positively correlated with male quality. However, as several authors have pointed out, indicator mechanisms like the handicap hypothesis (Zahavi 1975, 1977) do not require that ornaments and survival be correlated, only that ornaments be correlated with male quality (Grafen 1990; Getty 1998; Kokko et al. 2002; Getty 2006). Thus, for any individual species, the direction of the survival/ornamentation correlation is likely determined by details of its life history.

One of the hallmarks of life history theory is the assumption that individuals are faced with decisions on how to allocate resources among competing life history traits (Stearns 1992). Whether these trade-offs (e.g. survival versus ornamentation) manifest themselves at the level of the population is determined by the relative variation in allocation versus acquisition of resources (van Noordwijk and de Jong 1986; Grafen 1990). For instance, if males vary widely in the rules that they use to allocate resources to either survival or ornaments, but don't vary much in how many resources they have to allocate, then the relationship will tend to be negative. On the other hand, if males vary widely in their acquisition of resources, but have similar allocation rules, then it will tend to be positive (van Noordwijk and de Jong 1986). By varying the amount of resources available to males (i.e. manipulating acquisition), we may be able to reveal a trade-off between survival and ornamentation (e.g. Hunt et al. 2004), and with some caution, be able to infer which life history trait is more important in determining male fitness.

The resource pool that an individual has available to allocate to competing life history traits has been referred to as condition (Rowe and Houle 1996). Fitness-determining traits, i.e. those under directional selection such as male ornaments, are predicted to be condition-dependent, that is, trait development is expected to vary positively with the acquisition of condition (e.g. Andersson 1982; Nur and Hasson 1984). Of the few studies that have examined the condition dependence of male ornaments experimentally (reviewed in Cotton et al. 2004), even fewer have manipulated condition and examined its effects on both male lifespan and level of male sexual traits. Male *Hygrolycosa rubrofasciata* wolf spiders fed *ad libitum* signaled (drummed their abdomens) at a higher rate than food-restricted males, and males fed *ad libitum* increased their signaling rate more than food-restricted males when in the presence of a female. In addition, as diet quality increased, so too did male survival, and there was a significant but weak positive correlation between signaling rate and survival (Kotiaho 2000). Similarly, male three-spined sticklebacks, *Gasterosteus aculeatus*, fed a diet high in carotenoids had greater red nuptial colouration and survived longer than males fed a diet low in carotenoids (Pike et al. 2007). In contrast, in the black field cricket, *Teleogryllus commodus*, males reared on a high quality diet signaled (produced calling song) at a higher rate, but died younger than males reared on a low quality diet, who signaled at a lower rate. The within treatment relationship between signaling and survival varied among diet treatments, being negative for high and medium condition males, but positive for low condition males (Hunt et al. 2004). Likewise, male ambush bugs, *Phymata americana*, fed a higher quantity of prey had greater lateral colouration, but tended to have higher mortality than males fed a lower quantity of prey (D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms). In each taxon, the male secondary sexual character is under directional positive sexual selection, either through female preferences as in the spider (Parri et al. 1997), stickleback (e.g. Bakker 1993) and cricket (Hunt et al. 2004), or through competitive advantage as in the bug (Punzalan et al. 2008). However, it is not known in these organisms whether older males have higher mating success, as is the case in many other species (reviewed in Brooks and Kemp 2001).

We investigated the condition dependence of male lifespan and calling effort in another grylline, the fall field cricket, *G. pennsylvanicus*, a species in which older males are more attractive to females than younger males (Zuk 1987c, 1988). Female gryllines are attracted to greater calling effort (Cade and Cade 1992; Crnokrak and Roff 1995, 1998a,b; Hunt et al. 2004), and males fed higher quantity or quality diets put more effort into calling (Crnokrak and Roff 1998a; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a,b; Hunt et al. 2004). We therefore reared individuals on high, medium and low quality diets from hatching until death, and measured the calling effort of males throughout their entire lifespan. The purpose of our study was to test whether male lifespan and/or calling effort are condition dependent, and measure the correlation between these two male traits. Because male survival and calling effort are both correlated with male fitness (Zuk 1987c, 1988; J. J. Ting, K. A. Judge, and D. T. Gwynne, unpubl. ms), we do not predict a negative relationship between survival and calling effort. Additionally, the pattern of allocation to these two competing life history traits may suggest the optimal way in which males increase their fitness.

3.3 Methods

3.3.1 Study Species

G. pennsylvanicus is univoltine and widely distributed across the eastern half of North America (Capinera et al. 2004). Adults breed in late summer until the autumn's first severe frost, and the eggs overwinter and hatch in the spring (Alexander 1968). All individuals were second-generation offspring of wild animals captured in the summer of 2004 from the grounds of the University of Toronto at Mississauga (43°32'50.51"N, 79°39'37.80"W).

3.3.2 Nymph Husbandry

At hatching, we isolated individuals in separate containers (9 cm diameter) with their own food (one of three diets, see below), cotton-plugged water vial, and egg carton shelter. Over the course of six weeks we isolated 1566 hatchlings and assigned each to one of three diet treatments. All crickets were housed in an environmental chamber that maintained 12 hours light: 12 hours dark, 25ºC and 70% relative humidity. We replaced food and water once a week, and the cardboard shelter and container every four weeks. After six weeks of development, nymphs were checked daily for the presence of newly moulted adults. No cricket moulted to adulthood before six weeks.

3.3.3 Condition Manipulation

We haphazardly assigned hatchlings to one of three experimental diets composed of different proportions of: 1) rabbit food (Martin "Little Friends" Rabbit Food; http://www.martinmills.com/littleRabbit.htm), and 2) white bran, which are known to be of high and low quality respectively (K. A. Judge, unpubl. data). The three diets were as follows: high quality (90% rabbit food: 10% bran), medium quality (50% rabbit food: 50% bran), and low quality (10% rabbit food: 90% bran). Rabbit food and bran were ground up and passed through a 1 mm mesh sieve, combined in the above ratios based on weight, and mixed in a blender. For the first two weeks of life we gave nymphs the ground and blended food that they could easily ingest (presented on a small section of egg carton). After two weeks, all individuals were given the hard pellet form. Pellets were formed by mixing each diet with water until it was a viscous paste, which was then spread onto a Plexiglas sheet into which many uniform 1-cm diameter holes had been drilled (see Hunt et al. 2004). We placed these molds containing the diet pastes into a 55°C drying oven for 24 hours, after which the food pellets could be removed. This technique resulted in food pellets that were highly consistent in size (K. A. Judge, unpubl. data).

3.3.4 Adult Husbandry

Newly moulted adults were weighed and given new food, water, egg carton shelter and container on the day following their moult to adulthood (i.e. one day old) to prevent handling injuries when their exoskeleton had not fully hardened. We changed food and water, and cleaned the containers of all adults every five days thereafter (i.e. 6, 11, 16, etc.) until death. Males were weighed every five days until death to assess the relationship between calling effort and body mass throughout life, whereas females were weighed every five days up to a maximum of 31 days old to investigate the effect of the diet manipulation. We did not mate females unlike the previous experiment on *T. commodus* (see Hunt et al. 2005). Every day, we checked all adults. Date of death was noted and all dead crickets were preserved in 70% ethanol for morphological measurement.

3.3.5 Measurement of Calling Effort

Starting at six days post-adult moult, when most male *G. pennsylvanicus* have reached sexual maturity (Cade and Wyatt 1984), males had their calling effort measured every five days until death. We measured calling effort using microcassette recorders with auto voice record

(AVR) (General Electric model 3-5377, General Electric, Fairfield, CT). Each recorder came with a small lapel microphone that we suspended from the inside lid of a Styrofoam box (interior dimensions: 17 cm wide, 21.5 cm long, 16 cm high). During calling effort measurement, the lid of each male's individual container was replaced with a lid with a mesh screen window to afford better acoustic access to his song. We placed the male's container inside the Styrofoam box so that the lapel microphone was suspended approximately 1 cm above the mesh screen. Males were left for 15 minutes to acclimate to the Styrofoam box before recording was started using the automatic voice record (AVR) function. AVR turned on the recording when the microphone detected sound and shut off the recording following approximately six seconds of silence. Thus, recording occurred only when males called continuously (i.e. in a sample of males recorded in the field, the maximum interchirp interval was less than 0.7 s during bouts of calling song; K. A. Judge, unpubl. data) and stopped when there was a sustained break in singing. Because the maximum duration of our microcassette tapes was 90 min, we halted all recorders after 90 min and then used the tape counter to determine how much time the recorder had been turned on. We then calculated a male's calling effort as a percentage of 90 minutes.

All recorders were checked during the 15-minute acclimation period before each trial to ensure that they did not turn on when the male was not singing. In addition, our recorders were sensitive to an artificial chirp with a carrier frequency of 5000 Hz (mean carrier frequency of lab-reared individuals from this population, K. A. Judge, unpubl. data) broadcast at a sound pressure level of 69.4 ± 0.4 dB (mean ± SE, N = 22, re 20 µPa, measured with a RadioShack [Fort Worth, TX] digital sound level meter, model 33-2055). This is below the average amplitude of male calling song in this population (K. A. Judge, pers. obs.) indicating that the recorders were sufficiently sensitive to male song. However, during the 15-minute acclimation period, if we noticed that a male was singing without the recorder turning on, we increased the gain on his recorder to reduce the threshold sensitivity until the recorder turned on when the male sang. The proportion of males that had the gain adjusted at least once did not differ among the diet treatments (low: 52/170, medium: 46/187, high: 44/190; chi-squared contingency analysis: χ^2 = 2.85, df = 2, p = 0.241).

We had enough recorders to measure the calling effort of 22 males simultaneously. However, due to a combination of high juvenile and adult survival (see below) and large sample sizes, we had more than 22 males to measure on many days. Consequently, we often had to conduct multiple runs of the recorder array in one day. When we did so, we selected a random sample of males for each run of the array. The median start time for calling effort measurements was 9:06 pm (N = 5203, interquartile range = 7:06 - 11:18 pm). Although calling in crickets is known to change with the time of day (Alexander 1968), we did not correct our measurements of calling effort for time of day because Julian time of day explained only 6% of the variation in male calling effort (cubic regression: adjusted $R^2 = 0.059$, $p < 0.001$) and corrected calling effort measurements gave the same results as uncorrected values (K. A. Judge, unpubl. data).

In addition to the condition dependence of calling effort, past experiments have also examined dietary effects on carrier frequency (e.g. Hunt et al. 2004). For logistical reasons we were unable to measure other features of *G. pennsylvanicus* calling song.

3.3.6 Morphological Measurement

Using the program NIH Image (Version 1.62 for Macintosh), we measured pronotum length on all crickets to the nearest 0.01 mm. Pronotum length is a good overall measure of body size in *G. pennsylvanicus* (K. A. Judge, unpubl. data).

3.3.7 Statistical Analysis

Our data for male calling effort were highly right-skewed, with the mode of our 5203 measurements lying at the smallest value. As a result, we calculated a median (as opposed to average) for each male as a measure of his daily investment in calling effort. Although we were able to transform these data to approximate a normal distribution ($[\text{arcsine}(\text{calling effort})^{(1/2)}]^{(1/3)}$), for reasons of clarity we choose to present more conservative non-parametric rather than parametric analyses (in all but one analysis, see below). In all cases the non-parametric and parametric analyses detected the same effects, but interpretation of the response variable is less complicated in the non-parametric analyses.

We conducted all statistical analyses using SPSS 10 for Windows. All tests are two-tailed and have an *a priori* Type I error rate of 5%.

3.4 Results

3.4.1 Survivorship and Body Size

Of the initial 522 hatchlings isolated for each diet, 346 low, 376 medium and 383 high condition individuals survived to reach adulthood (total = 1105). We used Kaplan-Meier survival analysis to test for an effect of diet on survival to adulthood since the Cox regression assumption of proportionality of hazards (effect of the predictor is constant over time) was violated. Although there was a trend for individuals reared on the low quality diet to be more likely to die before reaching adulthood, this was not statistically significant (Log Rank test statistic = 4.96, df = 2, $p = 0.084$).

The adult sex ratio showed a male-biased trend for all three diets, with 178 males: 168 females, 195 males: 181 females and 198 males: 184 females for the low, medium and high quality diets respectively (there was one intersex individual [Johnstone 1975] in the high quality diet). Diet had no significant effect on the sex ratio at the adult moult (Heterogeneity Chi-square [Zar 1996]: $\chi^2 = 0.015$, df = 2, $p = 0.993$), and the pooled sex ratio did not differ significantly from an equal ratio of males to females ($\chi^2 = 1.308$, df = 1, $p = 0.253$).

Both diet quality and sex had significant effects on adult body size (Diet Quality: $F_{2, 1092} = 36.601$, $p < 0.001$; Sex: $F_{1, 1092} = 617.470$, $p < 0.001$; Diet * Sex: $F_{2, 1092} = 0.113$, $p = 0.893$). Crickets reared on the high and medium quality diets did not differ (Tukey HSD: $p = 0.135$), but had larger pronotum lengths than crickets reared on the low quality diet (Tukey HSD: both $p < 0.001$) (Fig. 3.1). Females were larger than males (Tukey HSD: $p < 0.001$) (Fig. 3.1).

There was a significant interaction between diet quality and sex in the amount of mass gained early in life (Diet * Sex: Days 1 to 6 post adult eclosion: $F_{2, 1034} = 16.819$, $p < 0.001$) so we analyzed the sexes separately. Males reared on the different diets did not differ in the amount of mass gained between days 1 and 6 post adult eclosion ($F_{2, 535} = 1.763$, $p = 0.172$), whereas females did ($F_{2, 499} = 24.304$, $p < 0.001$). Females reared on the high quality diet gained more mass than females on both the medium and low quality diets (mean ± SE: high = 113 ± 4 mg, medium = 94 ± 4 mg, low = 73 ± 3 mg; Tukey $\text{HSD}_{\text{high vs medium}}$: $p = 0.001$; Tukey $\text{HSD}_{\text{high vs low}}$: $p < 0.001$), and females reared on the medium quality diet gained more weight than females on the low quality diet (Tukey $\text{HSD}_{\text{medium vs low}}$: $p = 0.001$).

Diet quality, sex and their interaction were entered as categorical covariates in a Cox regression to test for the effect of diet quality and sex on adult survival. The Diet * Sex interaction was not significant (Wald = 4.246, df = 2, $p = 0.120$) so the interaction term was removed and the data reanalyzed. Diet quality had a significant effect on adult survival (Wald = 345.757, df = 2, $p < 0.001$). Crickets raised on the high and medium quality diets lived longer than crickets raised on the low quality diet ($\text{Wald}_{\text{high vs low}} = 276.031$, df = 1, $p < 0.001$; $\text{Wald}_{\text{medium vs low}} = 267.787$, df = 1, $p < 0.001$), but did not differ from each other ($\text{Wald}_{\text{high vs medium}} = 0.032$, df = 1, $p = 0.858$) (Fig. 3.2). And males lived slightly longer than females ($\text{Wald}_{\text{males vs females}} = 5.248$, df = 1, $p = 0.022$) (Fig. 3.2).

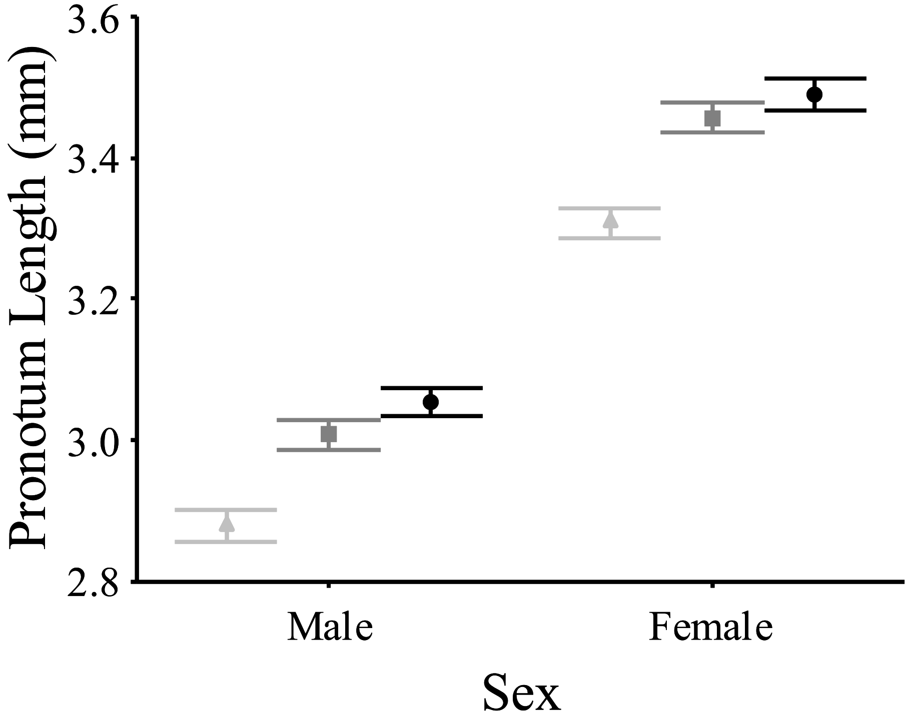
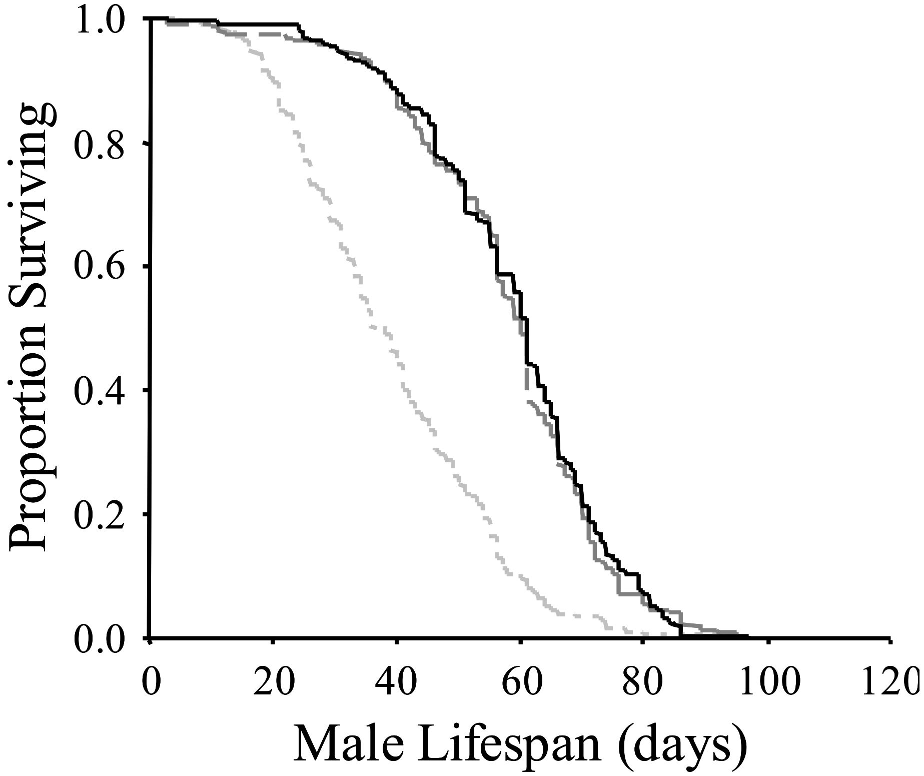


Figure 3.1: Mean (± SE) pronotum length for males and females reared on low (light grey triangles), medium (dark grey squares) and high (black circles) quality diets.

G. pennsylvanicus in our experiment lived longer than the oldest individuals found in a wild population (20 days, Zuk 1987a; see also Murray and Cade 1995). However, this difference likely does not affect the interpretation of our results for three reasons. First, differences among our three diet treatments in adult survival were significant even if we only consider mortality up to 20 days post-adult moult, and longer-lived individuals are treated as right-censored data (results not shown). Second, treatment differences in daily calling effort were significant at 11 days post-adult moult (see Fig. 3.4 below), which is within the age range found in the wild (Zuk 1987a; Murray and Cade 1995). Thirdly, 20 days may be an underestimate for the maximum age of *G. pennsylvanicus* in the wild, due to the error inherent in estimating age using daily growth rings in insect cuticle (see Fig. 1 in Zuk 1987a).



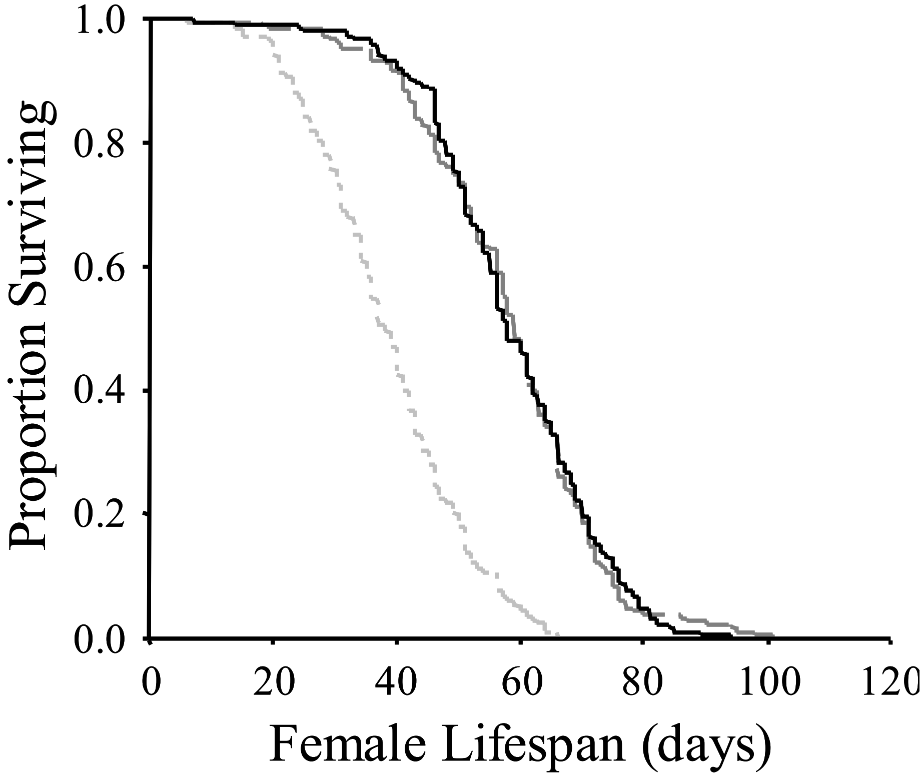


Figure 3.2: Plot of adult male and adult female survival for individuals reared on low (dotted light grey line), medium (dashed dark grey line) and high (black solid line) quality diets.

3.4.2 Calling Effort

Diet affected a male's daily calling effort (Kruskal-Wallis: $\chi^2 = 29.149$, df = 2, $p < 0.001$). High condition males called more per day than both low (Mann-Whitney U = 11016.5, $p < 0.001$) and medium condition males (Mann-Whitney U = 13699.0, $p < 0.001$), which did not differ from one another (Mann-Whitney U = 14634.0, p = 0.195) (median calling effort [interquartile range]: high = 14.5% [2.4 – 40.1%], medium = 6.7% [1.1 – 24.9%], low = 4.8% [1.0 – 16.9%]). Combined with the differences in adult lifespan between the diet treatments (see above), these daily effort differences resulted in a strong effect of diet on lifetime calling effort (lifetime effort = [median daily calling effort] * [lifespan]; Kruskal-Wallis: $\chi^2 = 52.056$, df = 2, $p < 0.001$). High condition males had higher lifetime calling effort than medium (Mann-Whitney U = 13901.5, $p < 0.001$) and low condition males (Mann-Whitney U = 8954.5, $p < 0.001$), and medium condition males had greater lifetime calling effort than low condition males (Mann-Whitney U = 12536.0, $p <0.001$) (median lifetime calling effort [interquartile range]: high = 9.17 days [1.28 – 22.72 days], medium = 3.48 days [0.66 – 13.94 days], low = 1.55 days [0.37 – 6.82 days]).

3.4.3 Age-dependent Calling Effort

Given that diet had an effect on adult lifespan, we tested whether it also had an effect on how males invested in calling effort throughout their lives. To do this, we considered only three calling effort measurements from each male: the first (at six days post-adult eclosion) and last (within 5 days of death) measurements, as well as one measurement from the mid-point of each male's adult lifespan (i.e. the measurement closest [±3 days] in time to the exact halfway point in the male's adult lifespan). These measurements were classified as early, late and middle life calling effort, respectively. This procedure eliminated any differences amongst the three diet treatments in lifespan, and allowed us to examine calling effort differences as the crickets aged, without the complicating factor of dwindling sample sizes. We detected no significant effect of diet on calling effort in early life (Kruskal-Wallis: $\chi^2 = 1.395$, df = 2, p = 0.498) (Fig. 3.3). However, diet had an effect on calling effort at both middle life (Kruskal-Wallis: $\chi^2 = 15.284$, df = 2, $p < 0.001$) and late life (Kruskal-Wallis: $\chi^2 = 14.473$, df = 2, p = 0.001). At both life stages, high condition males called more than both medium (Mann-Whitney $U_{\text{middle life}} = 14772$, p = 0.012; Mann-Whitney $U_{\text{late life}} = 14992$, p = 0.020) and low condition males (Mann-Whitney $U_{\text{middle life}} = 11598.5$, $p < 0.001$; Mann-Whitney $U_{\text{late life}} = 11682$, $p < 0.001$) (Fig. 3.3). Medium condition males did not call for a significantly greater proportion of time than low condition

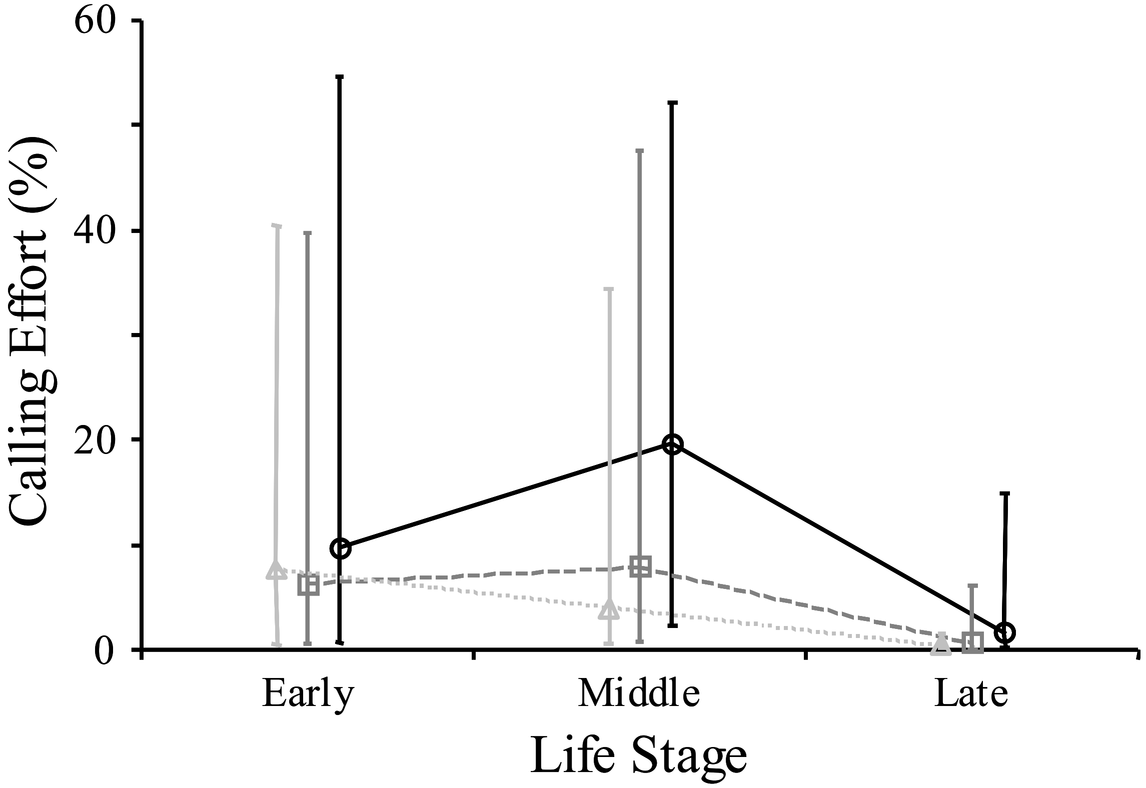


Figure 3.3: Calling effort by life stage. Symbols represent medians and error bars are interquartile ranges. The three diet treatments are represented as follows: low quality – light grey triangles and dotted line, medium quality – dark gray squares and dashed line, and high quality – black circles and solid line.

males at either middle life (Mann-Whitney U = 13541.5, p = 0.169) or late life (Mann-Whitney U = 13470.5, p = 0.143) (Fig. 3.3). We also compared calling effort amongst life stages within diet treatments using nonparametric Friedman tests. Calling effort significantly changed throughout the lives of males on all three diets (low quality: $\chi^2 = 45.138$, df = 2, p < 0.001; medium: $\chi^2 = 45.441$, df = 2, p < 0.001; high: $\chi^2 = 33.959$, df = 2, p < 0.001; Fig. 3.3). For all three diets, early calling effort did not differ from mid-life calling effort (all p > 0.240), but calling effort significantly declined in late life (mid-life vs late life: low: $\chi^2 = 30.716$, df = 1, p < 0.001; medium: $\chi^2 = 41.286$, df = 1, p < 0.001; high: $\chi^2 = 31.077$, df = 1, p < 0.001; Fig. 3.3).

These patterns were echoed in examinations of change in calling effort with age for the three diets. However, due to declining sample sizes with advancing age, we were unable to analyze this dataset using repeated measures. Instead, we tested whether males reared on the three diets differed in their calling effort as they aged by conducting Kruskal-Wallis tests at each age until numbers declined to 90% of the initial sample size for each treatment (after which values fluctuated due to small sample sizes). These were followed by post-hoc Mann-Whitney tests comparing each pair of diets. The overall result of these tests indicate that high condition males were able to sustain greater calling effort for longer periods than medium condition males, who in turn sustained greater calling than low condition males (Fig. 3.4).

Because our calling effort recorders were sometimes triggered by external noise, when noting calling effort measurements we reviewed low effort recordings and found that when measured calling effort was 1% or less, males usually had not called. For this reason we defined a male to have not called when his calling effort was 1% or less. At every age, males were categorized as either calling (>1% calling effort) or not calling (≤1% calling effort) and the proportion of calling males was calculated for each diet for: 1) each life stage, and 2) each age until 90% of males in each treatment had died. We tested whether the diet treatments differed in the proportion of calling males by conducting chi-squared tests at each: 1) life stage or 2) age, and then following each with a series of post-hoc Fisher's exact tests for each pair of diets. The diets differed in the proportion of calling males at middle ($\chi^2 = 9.779$, $p = 0.008$) and late ($\chi^2 = 18.560$, $p < 0.001$) but not early life ($\chi^2 = 1.145$, $p = 0.564$). High condition males were more likely than medium or low condition males to call at middle (high vs low: $\chi^2 = 9.889$, $p = 0.002$; high vs medium: $\chi^2 = 4.816$, $p = 0.037$) and late life (high vs low: $\chi^2 = 18.132$, $p < 0.001$; high vs medium: $\chi^2 = 7.882$, $p = 0.005$), but medium and low condition males did not differ in their probability of calling (middle life: $\chi^2 = 1.031$, $p = 0.351$; late life: $\chi^2 = 2.460$, $p = 0.120$) (Fig. 3.5). We found qualitatively similar results for the proportion of calling males after 11 days post-adult moult (Fig. 3.6).

We also examined diet effects on the time course of investment in calling effort by conducting linear and polynomial regressions of calling effort versus adult age for every male that had more than one calling effort measurement. Due to high variability and low sample sizes for many males, very few of these regressions were statistically significant. However, this procedure (sensu Brooks and Endler 2001) gave us two summary measures that we could compare amongst the three diet treatments. Both the linear and polynomial regression coefficients were normally distributed, so we proceeded to test for an effect of diet on these two measures using a multivariate GLM with diet as a fixed factor and both the standardized linear and nonlinear regression coefficients as response variables. The mean linear and nonlinear regression coefficients were significantly less than zero for males on all three diets (one sample t-tests: all $p < 0.011$). However, diet did not have an effect on the linear or nonlinear relationship between calling effort and age (Pillai's trace = 0.012, $F_{4, 1060} = 1.585$, $p = 0.176$), although there was a

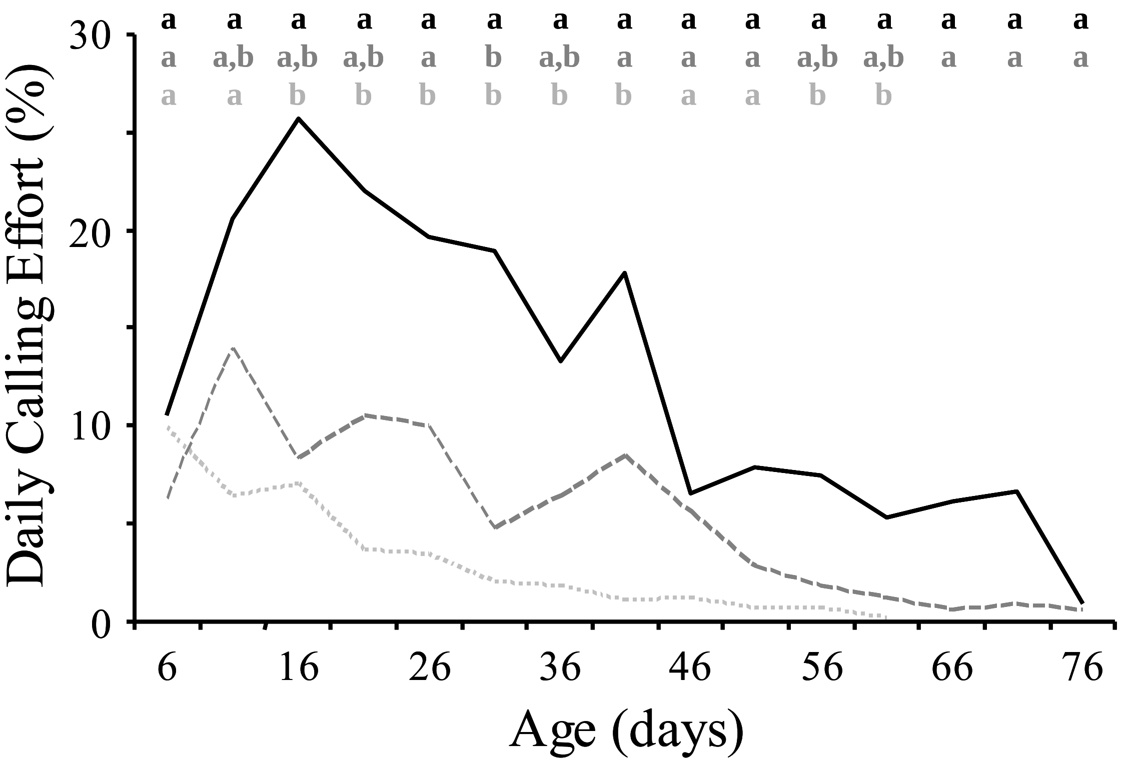


Figure 3.4: Calling effort by age. The three diet treatments are represented as follows: low quality – light grey dotted line, medium quality – dark gray dashed line, and high quality – black solid line. Due to dwindling sample sizes with age, curves extend only to the age at which 90% of the males in a diet treatment had died. Treatments with the same letter on the same day are not statistically different at $\alpha = 0.05$ following sequential Bonferroni correction (Holm 1979).

trend for linear regression coefficients to become less negative as diet quality increased (mean linear coefficient ± SE: low = -0.217 ± 0.038, medium = -0.148 ± 0.029, high = -0.116 ± 0.028).

3.4.4 Lifespan and Daily Calling Effort

We tested for a significant relationship between calling effort and male lifespan. First we tested for homogeneity of slopes for the three diets by conducting an ANCOVA with male lifespan as the response variable, transformed daily calling effort ($[\text{arcsine}(\text{calling effort})^{(1/2)}]^{(1/3)}$) as the covariate, and diet as the independent factor. We interpret a significant covariate by factor interaction as evidence that the relationship between lifespan and daily calling effort varied among the three diets. The interaction between transformed daily calling effort and diet was not

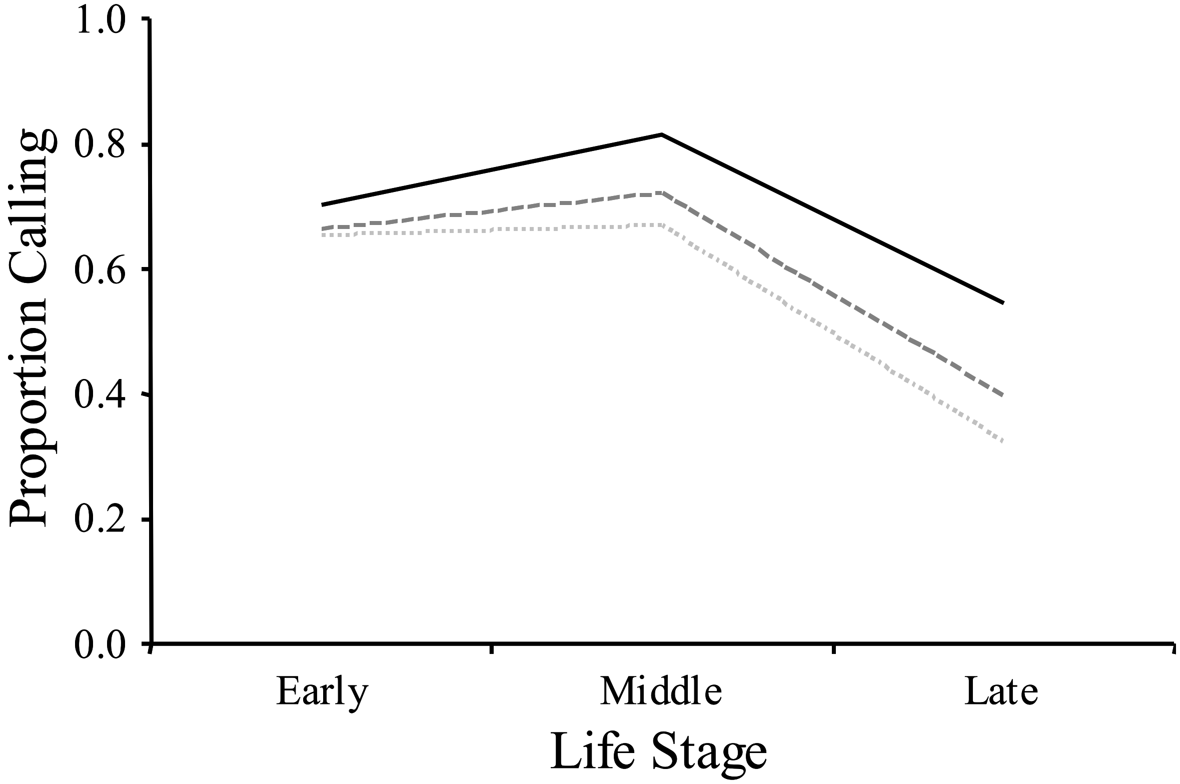


Figure 3.5: Proportion of calling males by life stage. The three diet treatments are represented as follows: low quality – light grey dotted line, medium quality – dark gray dashed line, and high quality – black solid line.

statistically significant, although there was a trend ($F_{2, 541} = 2.637$, df = 2, p = 0.073), so we conducted a regression between lifespan and transformed daily calling effort. There was a statistically significant positive relationship between lifespan and daily calling effort ($F_{1, 545} = 8.034$, p = 0.005), however, calling effort only explained 1.3% of the variation in male lifespan (adjusted R^2) (Fig. 3.7). We also tested for a linear relationship between early calling effort (at six days post-adult moult) and male lifespan. ANCOVA revealed no significant differences among the three diets in the slope of the lifespan versus transformed early calling effort ([arcsine(calling effort)$^{(1/2)}$]$^{(1/3)}$) relationship (interaction: $F_{2, 529} = 0.834$, p = 0.435). The regression of lifespan on transformed early calling effort was marginally non-significant ($F_{1, 533} = 3.234$, p = 0.073).

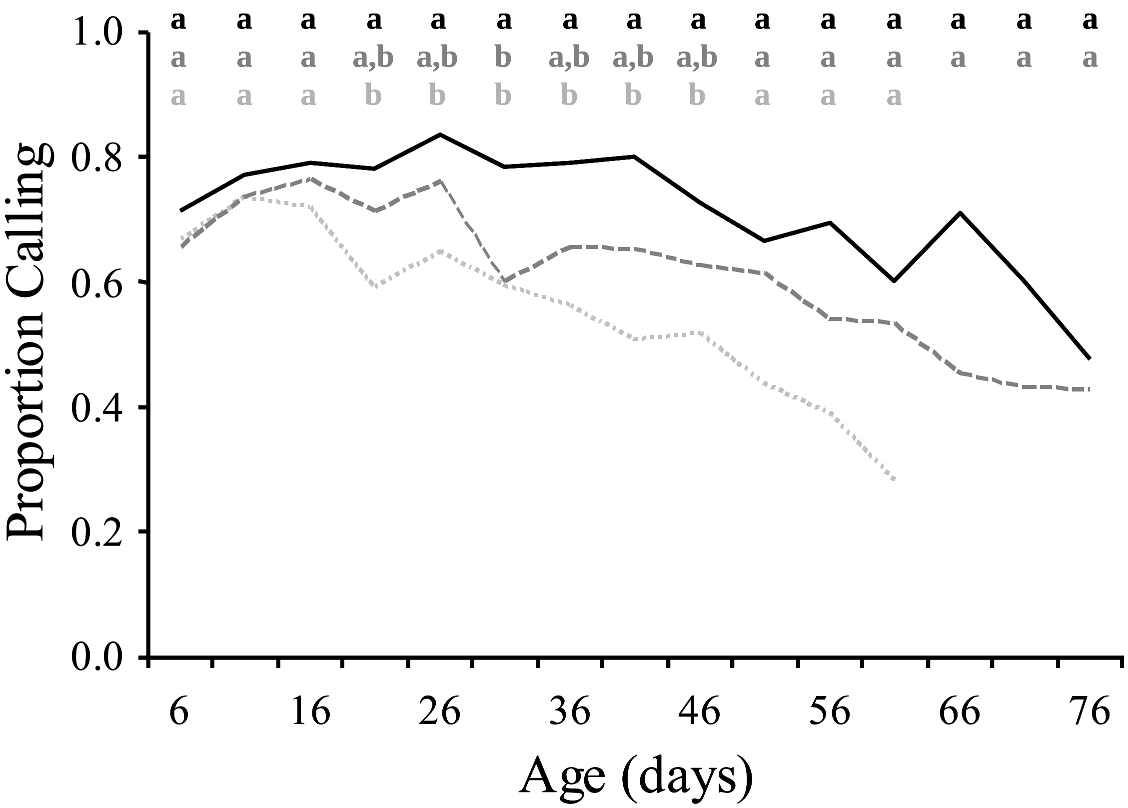


Figure 3.6: Proportion of calling males by age. The three diet treatments are represented as follows: low quality – light grey dotted line, medium quality – dark gray dashed line, and high quality – black solid line. Due to dwindling sample sizes with age, curves extend only to the age at which 90% of the males in a diet treatment had died. Treatments with the same letter on the same day are not statistically different at α = 0.05 following sequential Bonferroni correction (Holm 1979).

3.5 Discussion

We manipulated condition in *G. pennsylvanicus*, where older males have a mating advantage over younger males (Zuk 1987c, 1988). We predicted that as diet quality increased, males would both live longer and invest more in sexual signaling (calling effort). Males reared on high and medium quality diets did not differ in their adult lifespan, but both lived longer than males reared on the low quality diet (Fig. 3.2). And males on a high quality diet invested in daily calling effort more than those on either medium or low quality diets, who did not differ from one another, a result that is broadly consistent with previous work on the condition dependence of grylline calling song (Crnokrak and Roff 1998a; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al.

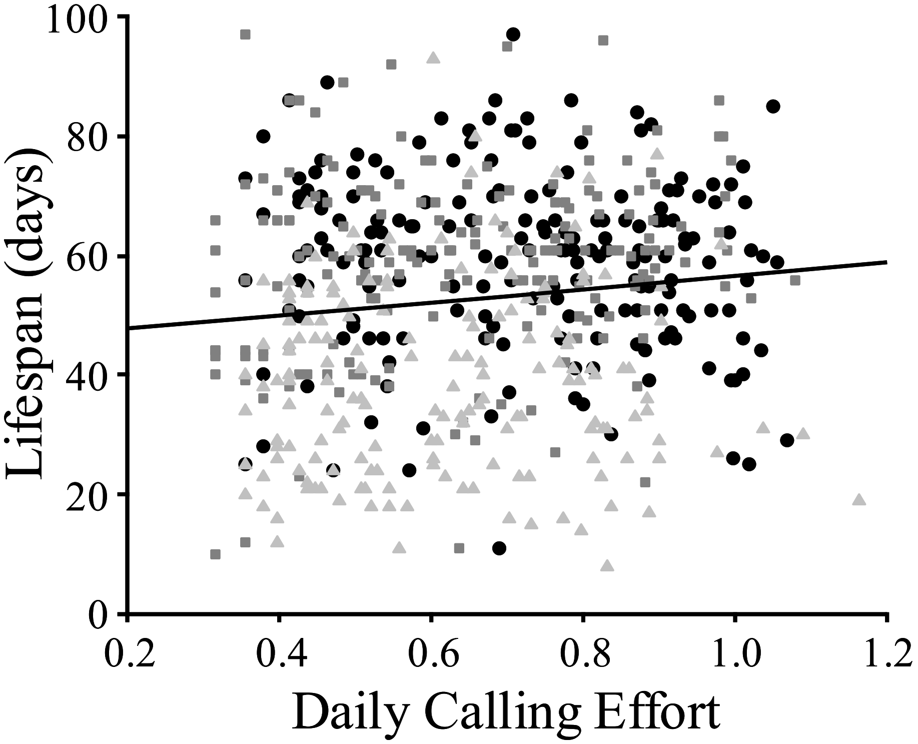


Figure 3.7: Plot of male lifespan versus transformed daily calling effort. The three diet treatments are represented as follows: low quality – light grey triangles, medium quality – dark gray squares, and high quality –black circles. The line represents the common regression slope for all three diets.

2003a,b; Hunt et al. 2004). We found no evidence for age-specific investment in calling varying with diet quality: at sexual maturity (six days post-adult moult) there were no differences among the three diet treatments in either daily calling effort (Figs. 3.3 and 3.4) or the proportion of males that called (Figs. 3.5 and 3.6). Differences among the three treatments grew apparent as males aged (after six days post-adult moult); a greater proportion of high condition males were able to sustain high levels of calling effort longer than medium condition males, both of which by virtue of their longer lifespans maintained calling effort longer than low condition males (Figs. 3.3 to 3.6). Along with a strong effect of diet quality on lifespan and calling effort, there was an indication that longer-lived males called more with the significant (albeit weak) positive correlation between median daily calling effort and male lifespan (Fig. 3.7). There was no significant relationship between calling effort early in life and lifespan.

Sexual selection theory predicts that investment in sexual signaling by high quality males can be sufficiently high that they die younger than low quality males (Darwin 1871; Fisher 1915, 1930; Grafen 1990; Getty 1998; Kokko 1998; Kokko et al. 2002; Getty 2006). Work on a grylline field cricket, *T. commodus* (Hunt et al. 2004), was the first experimental support for this prediction. High condition males signaled more, but died younger than low condition males (Hunt et al. 2004). Furthermore, within diets, males reared on a low quality diet showed a positive correlation between signaling and lifespan; whereas high and medium condition males that signaled more, died younger (Hunt et al. 2004). In contrast, we found in our grylline, *G. pennsylvanicus*, that males reared on a high quality diet both lived longer and invested more effort in sexual signaling than males on a low quality diet. In addition, there was a weak positive correlation between lifespan and daily calling effort that did not differ among treatments (Fig. 3.7). Studies of other taxa have also shown varying support for the basic Darwinian hypothesis. Similar to our *G. pennsylvanicus*, male *H. rubrofasciata* wolf spiders fed at a high level, signaled (drummed their abdomens) more and survived longer than males fed at a low level (Mappes et al. 1996; Kotiaho 2000). There was a weak positive relationship between drumming rate and male survival when females were present, but not when they were absent (Kotiaho 2000). And in three-spined sticklebacks, *Gasterosteus aculeatus*, males fed a diet high in carotenoids had greater red nuptial colouration (which is preferred by females) and survived longer than males fed a diet low in carotenoids (Pike et al. 2007). In contrast, and similar to *T. commodus*, recent work on an ambush bug, *Phymata americana*, found that adult males fed a higher quantity of prey had greater lateral colouration (a sexually selected trait) but tended not to live as long as males fed a lower quantity of prey (D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms). Thus, although the balance of correlative evidence showing that male ornamentation and survival are positively correlated suggests that a negative correlation is not a common occurrence (meta-analysis by Jennions et al. 2001), the question of how often males of other species invest in secondary sexual characters to the detriment of their survival remains open and awaits further testing.

Why do males of some species (e.g. *T. commodus*, *P. americana*) invest in sexual ornaments to the detriment of survival, whereas males of others (e.g. *H. rubrofasciata*, *G. aculeatus*, *G. pennsylvanicus*) do not? A comparison of the two most closely related species (the gryllines) may shed some light on this question. Differences between *T. commodus* and *G. pennsylvanicus* in the relationship between signaling and lifespan may be due to life history differences. First, these two gryllines differ in terms of seasonality: northern hemisphere *G. pennsylvanicus* breed over several weeks from mid-summer to the first severe frost (Alexander 1968) whereas breeding appears to be episodic in Australian *T. commodus* (Jennions et al. 2004). Thus, *G. pennsylvanicus* and *T. commodus* may represent prolonged and explosive breeders respectively. In prolonged breeders, males have the opportunity to monopolize matings over the course of the breeding season. However, female availability is often high and synchronous in explosive breeders, reducing the potential for males to monopolize matings (Wells 1977). In the wild, the peak of female *G. pennsylvanicus* activity-density (an index of abundance) occurs approximately two weeks after the peak in male activity-density (Carmona et al. 1999; see also Ritz and Köhler 2007 for similar data for *G. campestris*). Under these conditions it may be adaptive for male *G. pennsylvanicus* to invest substantially in both survival and signaling. Although comparable data for *T. commodus* are unavailable, males of this grylline may invest primarily in signaling if endurance contributes little to male mating success. Latitudinal variation in the length of the breeding season within a single species (see Walker and Masaki 1989) could provide a model system in which to test this hypothesis.

In *T. commodus*, the allocation of condition altered with increases in condition acquisition (Hunt et al. 2004), whereas in *G. pennsylvanicus* the pattern of allocation did not change drastically with increased acquisition (Fig. 3.7) This could be the result of selection for the ability of male *T. commodus* to adjust allocation of condition depending on local resource availability, whereas *G. pennsylvanicus* may be under selection to maintain a specific allocation pattern. Putatively different selective regimes such as these might be a result of inhabiting unstable versus stable environments. *T. commodus* is recognized as a pest and exists in “large, eruptive populations” (Jennions et al. 2004, p 2475), which suggests high environmental variability and selection for the ability to take advantage of fluctuations in resources. However, we currently do not know how stable the environment of *G. pennsylvanicus* is. If it is stable, then male *G. pennsylvanicus* genetic quality may be reflected in genetic variance for resource acquisition, whereas genetic quality in male *T. commodus* may be based on genetic variance for resource allocation.

Another reason for the difference between *G. pennsylvanicus* and *T. commodus* may be that male *G. pennsylvanicus* are better able to buffer the metabolic demands of calling than male *T. commodus*. Our *G. pennsylvanicus* are micropterous (K. A. Judge, pers. obs.) whereas *T. commodus* are macropterous (Alexander and Otte 1983). Micropterous individuals have reduced or absent flight muscles (Harrison 1980), which not only have lower maintenance costs (Mole and Zera 1993) but can also lead to both the flight muscle space being filled with a fat body (reported in beetles: Jackson 1952) and greater fecundity than with macroptery (reviewed in Roff 1984). Moreover, the energetic costs of calling with short chirps (Alexander 1957) may be lower for *G. pennsylvanicus* males than *T. commodus* males with their longer chirp-trills (Loher and Rence 1978) (Prestwich 1994). Thus, due to microptery, *G. pennsylvanicus* males may be more able to live longer than *T. commodus* males while signaling at a high rate. *G. pennsylvanicus* reared on all three diets attained greater levels of calling effort in our study (high = 25.7%, medium = 14.0%, low = 9.9%; Fig. 3.7) than *T. commodus* reared on the corresponding diets (high = 10.4%, medium = 3.7%, low = 3.0%; proportions calculated from Hunt et al. 2004 Fig. 2b based on a 15 hour [54000 s] observation period). In wing-polymorphic *G. firmus*, micropterous males call more than macropterous males (Crnokrak and Roff 1995, 1998a,b). However, it is unknown whether males of the two wing morphs differ in lifespan (but see Roff 1984 who did not detect a difference between micropterous and macropterous *G. firmus* females). Further progress on the question of the relative importance of investment in sexual signaling versus survival is likely to be made by studying North American gryllines given both a well-supported phylogeny (Huang et al. 2000) and within-species variation in life history parameters (e.g. Harrison 1979; Walker and Masaki 1989; Roff et al. 2003).

Females are generally attracted to greater amounts of sexual signal (reviewed in Ryan and Keddy-Hector 1992; gryllines: e.g. Cade and Cade 1992; Crnokrak and Roff 1995, 1998a,b; Hunt et al. 2004). In *G. pennsylvanicus*, high condition males call more (this study), so females choosing males investing in greater calling effort are likely to pick a high quality mate, but not necessarily an older mate. Zuk (1987c) suggested that female *G. pennsylvanicus* are attracted to older males or to some quality that is correlated with male survival. Our results indicate that females do not prefer old males *per se*, but instead choose high quality males. Furthermore, there is little evidence that male age can be predicted from his song (Ciceran et al. 1994; K. A. Judge, unpubl. data). Previous work showing that female *G. pennsylvanicus* prefer mating with older males (Zuk 1987c, 1988) either did not assess male calling effort (Zuk 1988) or did so using a relatively imprecise measure (see Zuk 1987c p1243). Interestingly, Zuk (1987c) found calling effort to be positively correlated with the number of females attracted, although this relationship was not statistically significant.

Calling effort in six-day-old males did not differ among any of our three diet quality treatments. Treatment differences in calling effort only became apparent in older males as medium condition males reduced their calling effort, and low condition males both reduced their calling effort and died. Thus, low condition males were weeded out of the population and high condition males were distinguishable by their greater signaling effort. These results are consistent with a model by Proulx et al. (2002), in which females prefer older males because they signal their quality more reliably than younger males. However, we found no evidence that males increased their calling effort with age (Figs. 3.3 and 3.4, and see Cade and Wyatt 1984), which is a prediction of reliable signaling by older males in species with multiple reproductive bouts (Proulx et al. 2002). Uncertainty about the timing of the first severe killing frost may enforce maximal signaling by males of all qualities immediately after maturing.

Our finding that male crickets on a high quality diet called more is broadly consistent with previous work on the condition-dependence of grylline calling song (Crnokrak and Roff 1998a; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a,b; Hunt et al. 2004). In studies that manipulated adults, males that received higher quality or more food called more, whereas other call characteristics, such as carrier frequency, were unaffected (Crnokrak and Roff 1998a; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a). However, studies that manipulated juvenile diet have found inconsistent effects on calling. In *G. campestris*, food-stressed late-instar nymphal males moulted with smaller harps (the membranous area of the forewings that radiates song) and consequently produced calls with a higher carrier frequency (Scheuber et al. 2003b). In contrast, *T. commodu*s males that had been reared from hatching on a poor quality diet were small as adults but carrier frequencies (and by implication harp areas [Simmons 1995; Scheuber et al. 2003a]) were unaffected (Hunt et al. 2004). This suggests that carrier frequency is under stabilizing selection in *T. commodus* (see Brooks et al. 2005; Bentsen et al. 2006). In future, it would be highly informative to directly compare the condition-dependence of calling effort with both temporal and spectral characteristics of male *G. pennsylvanicus* calling song.

The pattern of allocation to survival and calling effort in *G. pennsylvanicus* suggests that males gain fitness from initially investing more in survival than calling effort. An increase in diet quality from low to medium was associated with an increase in survival, but a further increase in diet quality brought no change in survival. Instead, high condition males invested significantly more resources into calling effort than medium condition males, who tended to invest more than low condition males. These results suggest a hierarchy to allocation decisions made by *G. pennsylvanicus*. Investment into survival may plateau because uncertainty over the onset of killing frost may weaken selection to increase male lifespan. Latitudinal variation in growing season within many North American gryllines, as well as variation in hatch date (e.g. Harrison 1985; K. A. Judge, pers. obs.) may have important impacts on this selection. Increasing investment in calling effort will have increasingly positive fitness returns due to the multiplicative effects of survival and ornamentation on male fitness (Grafen 1990; Getty 2006). It remains to be seen whether variation in growing season, environmental stability or some other ecological factor can switch the pattern of allocation and cause male *G. pennsylvanicus* to invest in calling effort at the expense of survival.

3.6 Acknowledgements

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CHAPTER 4

Condition dependence of female mating preferences in a field cricket.

Kevin A. Judge, Janice J. Ting and Darryl T. Gwynne

4.1 Abstract

Mate choice is a costly behaviour that is predicted to be condition dependent. Females generally choose mates that produce the loudest, brightest, or most elaborate sexual displays. Male field crickets (Orthoptera, Gryllidae, Gryllinae) produce a conspicuous calling song that attracts females, and males vary widely in the amount of calling. In the fall field cricket, *Gryllus pennsylvanicus*, previous work has shown that males in good condition sing for a greater proportion of time and live longer than males in poor condition. In this study, we tested the condition dependence of both female preferences and their choosiness for male calling effort in *G. pennsylvanicus*. We manipulated female condition by raising crickets from hatching on either a low- or a high quality diet. In a series of two-speaker phonotaxis trials, both low- and high condition females preferred playbacks reflecting greater calling effort, but did not differ from one another. However, high condition females took significantly longer to make a choice and were more likely to fail to choose within the time allotted for a phonotaxis trial. These results are in contrast with previous work that tested the condition dependence of female choosiness and found that high condition females were more responsive than low condition females. We discuss these results with respect to the possibility that female *G. pennsylvanicus* may be foraging for direct benefits when they choose their mates.

4.2 Introduction

Mate choice can be defined as the result of any behaviour that biases an individual's (usually female) pool of potential mates towards certain phenotypes (Halliday 1983); these behaviours are called mating preferences (Heisler et al. 1987; Jennions and Petrie 1997; see also Wiley and Poston 1996). As one of the mechanisms of Darwin's (1871) theory of sexual selection, mate choice was originally quite controversial and not readily accepted by Darwin's contemporaries (Cronin 1991). However, mate choice is now widely recognized as an important selective force and has been the focus of an increasing number of studies (reviewed in Andersson 1994; Birkhead and Pizzari 2002; Andersson and Simmons 2006).

Mating preferences can be summarized by two components: 1) preference functions, or the rank order of favoured mate phenotypes, and 2) level of choosiness, or the effort expended in both time and energy to choose a mate (Jennions and Petrie 1997; see also Gibson and Langen 1996; Widemo and Sæther 1999). Understanding how each of these components varies has important implications for our understanding of how selection varies in space and time (Jennions and Petrie 1997). For example, if females vary in their preference functions (e.g. Wagner et al. 1995; Murphy and Gerhardt 2000; Brooks and Endler 2001; Morris et al. 2003) then the shape of selection on males may change depending on the mix of females in the population. However, if choosiness varies and preference functions are constant among females, the strength of selection on males may fluctuate. Although conceptually distinct, preference functions and choosiness are often quite difficult to measure accurately and independently (Gibson and Langen 1996; Jennions and Petrie 1997; Wagner 1998; Widemo and Sæther 1999).

Finding and choosing a mate is costly, both in terms of energetic demands and predation risk (reviewed in Jennions and Petrie 1997). However, in addition to these costs, there are a host of proposed benefits to exercising mate choice, including direct benefits such as food (e.g. Voigt et al. 2005), oviposition sites (e.g. Howard 1978b) or parental care (e.g. Downhower and Brown 1980), and indirect benefits such as genes for offspring viability (e.g. Welch et al. 1998), genes for offspring attractiveness (e.g. Head et al. 2005) or genes that are compatible with the choosy individual's own genotype (e.g. Tregenza and Wedell 2002). Therefore, given the possibility of both substantial benefits and costs to mate choice, choosy individuals are likely under selection to optimally invest resources (e.g. time and/or energy) in mate choice to minimize the costs and maximize the benefits (Reynolds and Gross 1990). The pool of resources available to an individual for allocating among competing fitness-related traits is defined as condition (Rowe and Houle 1996). Given that females often vary in their quality, i.e. fecundity and/or body size, (Bonduriansky 2001) – demonstrating that condition varies among females – a substantial amount of the variation in female mating preferences may be attributable to variation in female condition (Jennions and Petrie 1997).

An increasing number of studies show that condition affects mate choice (e.g. Morris et al. 1975; Poulin 1994; Simmons 1994; Ortigosa and Rowe 2002; Cratsley and Lewis 2003; Syriatowicz and Brooks 2004; Hunt et al. 2005); however, few studies have distinguished condition effects on preference functions or choosiness. Syriatowicz and Brooks (2004) found that high condition female Trinidadian guppies, *Poecilia reticulata*, were more responsive (interpreted as a component of choosiness) than low condition females, but did not have stronger preference functions. In contrast, high condition female black field crickets, *Teleogryllus commodu*s, were both more responsive and had stronger preference functions for male call parameters than low condition females (Hunt et al. 2005). Given that variation in both preference functions and choosiness may have differing effects on the selection exerted on males, there is a clear need for further study.

We tested the condition dependence of both female preference functions and choosiness in another grylline, the fall field cricket, *Gryllus pennsylvanicus* (Orthoptera, Gryllidae, Gryllinae). As with most gryllines, males of this species inhabit burrows or cracks in the ground, which they aggressively defend from other males, and from which they sing to attract females (Alexander 1961; Loher and Dambach 1989). Female field crickets are attracted to males that sing for a greater proportion of time (Cade and Cade 1992; Crnokrak and Roff 1995, 1998a,b; Hunt et al. 2004). Male investment in calling song (calling effort) is condition dependent in many species of field cricket (Crnokrak and Roff 1998; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a,b; Hunt et al. 2004) including in *G. pennsylvanicus* (Judge et al. 2008, Chapter 3). Furthermore high condition *G. pennsylvanicus* males are larger, develop faster and live longer than low condition males (Judge et al. 2008, Chapter 3). This suggests that females that prefer high calling effort will mate preferentially with high fitness males since large males are able to dominate in aggressive interactions with other males (Souroukis and Cade 1993) and older males are more attractive to females (Zuk 1987c, 1988).

We manipulated female condition by raising females from hatching on diets that are known to have significant effects on *G. pennsylvanicus* development time, body size and weight gain, but not juvenile survival (Judge et al. 2008, Chapter 3). We then measured the effect of this condition manipulation on the female preference function for male calling effort by presenting females with a choice between two speakers broadcasting songs that differed in calling effort. We varied the magnitude of the difference in calling effort between the two speakers and then tested the condition dependence of female ability to discriminate these differences. We also examined the condition dependence of female choosiness, which we estimated in two ways. First, because male grylline calling effort is highly variable (Cade 1991; K. A. Judge, unpubl. data), a male's calling effort over the short-term may be an unreliable estimate of his calling effort over longer timescales. Therefore, the longer a female takes to choose between two alternative calls, the more accurate should be her assessment of his calling effort. We measured female latency to respond to male song and predicted that high condition females would take longer to respond to male calls (i.e. are more choosy) than low condition females. Second, because female gryllines are known to prefer greater calling effort, we presented females with a choice between song models that differed in mean calling effort, but where only the high effort song was variable (i.e. fluctuated between a low and high calling effort phase). Thus the high effort song would become apparent only after an interval of time, which we varied systematically. We predicted that as this interval of time became longer, it would eventually exceed female's attention span at which point females would switch from preferring the higher calling effort song to preferring the lower calling effort song. This approach is based on a study of the assessment time of female grey treefrogs, *Hyla versicolor* (Schwartz et al. 2004). We predicted that high condition females would prefer the higher mean calling effort song for longer time intervals (i.e. are more choosy) than low condition females.

4.3 Methods

4.3.1 Nymph Husbandry

All subjects were third generation offspring of wild animals captured in the summer of 2004 from the University of Toronto at Mississauga grounds (43°32'50.51"N, 79°39'37.80"W). Over five days, we isolated 709 newly hatched crickets in plastic containers (9 cm diameter, 8 cm high) with their own food (one of two different diets, see below), a cotton-plugged water vial for moisture and a piece of egg carton for shelter. We replaced food and water once every seven days and the cardboard shelter and container once every four weeks. After six weeks of development, nymphs were checked daily for the presence of newly moulted adults.

4.3.2 Manipulation of Condition

At hatching we haphazardly assigned all individuals to one of two experimental diets. Both diets were composed of rabbit food (Martin "Little Friends"; http://www.martinmills.com/littleRabbit.htm) and white bran. The low quality diet was composed of 10% rabbit food and 90% bran, whereas the high quality diet was composed of 90% rabbit food and 10% bran. Both rabbit food and bran were ground and passed through a 1 mm mesh sieve, combined in the above ratios based on weight, and mixed in a blender. We gave nymphs the ground food for the first two weeks of life, and after two weeks, we gave all individuals the diets in pellet form (see Judge et al. 2008, Chapter 3 for details on pellet formation). These diets are known to have significantly different effects on a wide variety of life history traits (Judge et al. 2008, Chapter 3, K. A. Judge, unpubl. data). Females raised on the two experimental diets were used in all phonotaxis experiments (see below). Comparisons between high and low condition females allowed us to test for the condition dependence of preferences and choosiness.

4.3.3 Adult Husbandry

Newly moulted adults were weighed and given new food, water, egg carton shelter and a new container on the day following their moult to adulthood (i.e. one day old). We changed food and water and cleaned the containers of all adults every five days thereafter (i.e. 6, 11, 16, etc.) until their death. We weighed all adults on the first, sixth and eleventh day following their adult moult. Day six and day eleven correspond to the ages at which male *G. pennsylvanicus* start calling (Cade and Wyatt 1984) and most female *G. pennsylvanicus* are sexually receptive (K. A. Judge, pers. obs.), respectively. Every day, we checked all adult crickets for dead individuals. Date of death was noted and all dead crickets were preserved in 70% ethanol for subsequent morphological measurement.

4.3.4 Experimental Design

To estimate the preference function for high calling effort, we presented females with a choice between speakers broadcasting alternative calling songs that differed only in their calling effort (proportion of time spent calling, expressed as a percentage). In addition, to estimate females' ability to distinguish differences in calling effort, we varied the magnitude of the difference in calling effort broadcast by alternative speakers to be either: a) large (80%: 10%

versus 90%, see e.g. Fig. 4.1), b) medium (60%: 10% versus 70%, or 30% versus 90%), or c) small (20%: 10% versus 30%, 30% versus 50%, 50% versus 70%, 70% versus 90%) (Table 4.1). Grylline calling effort is highly variable (Cade 1991; K. A. Judge, unpubl. data), and these levels fall within the range of calling effort measured for this species (Judge et al. 2008, Chapter 3; K. A. Judge, unpubl. data).

We estimated choosiness in two ways. First, in all phonotaxis trials we measured the length of time that females took to choose one of the speakers. Second, we presented females with a choice between a higher and a lower calling effort song, where the higher calling effort song (50%) oscillated between low (10%) and high (90%) effort phases and the lower calling effort song was constant (30%). We manipulated the period of oscillation of the higher calling effort song to be: 26 s, 52 s, 104 s, 208 s and 416 s, which corresponded to 2 (see e.g. Fig. 4.1), 4, 8, 16 and 32 bouts of calling song (Table 4.1). In these assessment time phonotaxis experiments, the variable call started with the low effort phase.

Because the high effort songs were also more variable than low effort songs, we conducted a control experiment to test for any preference for variation alone. In this control phonotaxis experiment, the variable and constant calls both had a mean calling effort of 30% and the variable call oscillated between 10% and 50% with a period of 26s (Table 4.1). Again, the variable call started with the low effort phase.

Due to logistical considerations, all females did not take part in all of the phonotaxis experiments. Instead, each female took part in four experiments (on separate but consecutive days): 1) one of either a control or a large difference preference experiment, 2) one of the two medium difference preference experiments, 3) one of the four small difference preference experiments, and 4) one of the five assessment time experiments. The order in which each female took part in these four experiments, as well as the level of each (e.g. medium difference preference experiment: 10% versus 70% or 30% versus 90%), was randomly assigned. We also haphazardly assigned females an age at which they would start their phonotaxis trials (mean ± SD = 21 ± 7 days post adult moult).

4.3.5 Construction of Calling Song Models

We used 114 field-recorded calling songs (collected between 13 August and 14 September 2003 from the grounds of the University of Toronto at Mississauga) to construct the models used in all phonotaxis trials. We used these instead of constructing artificial song models in order to mimic a natural range of acoustic parameters. To collect these songs, we recorded short (2-3

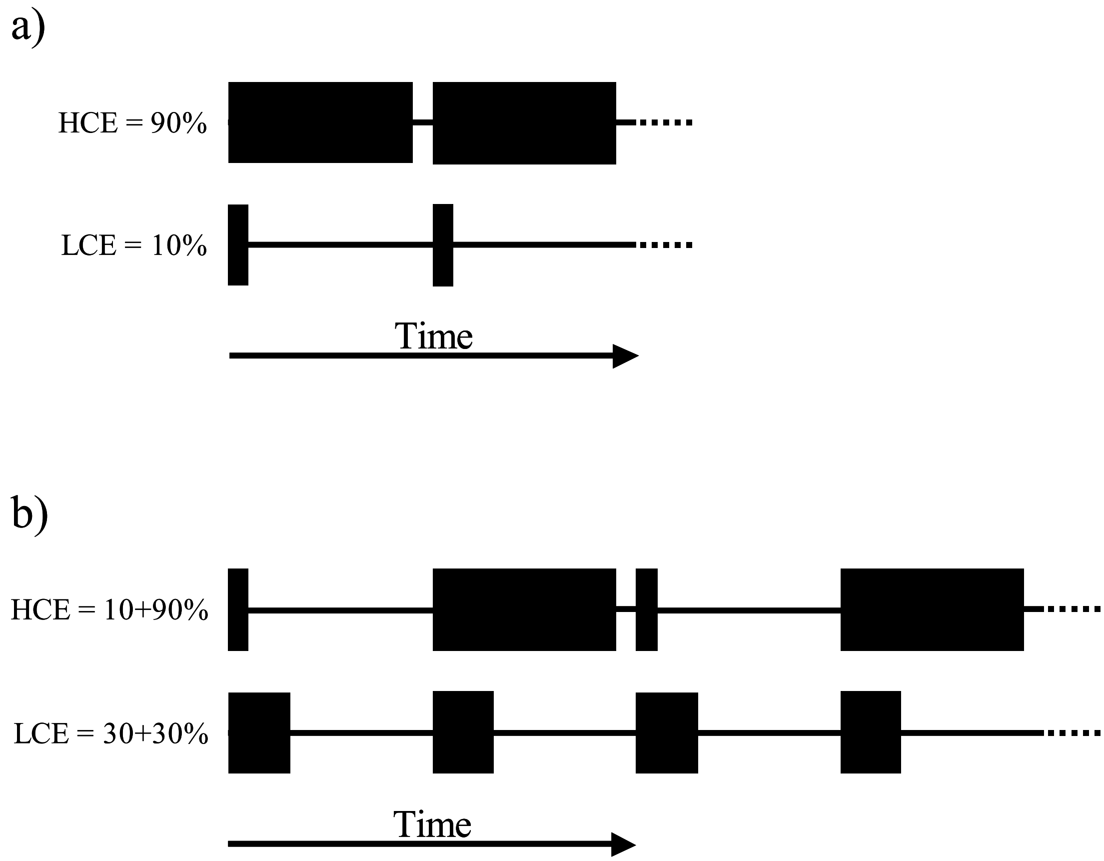

Figure 4.1: Schematic representation of the two general types of phonotaxis trials: a) preference, and b) assessment time. The central black rectangles represent time that is filled with cricket calling song, with the average duty cycle for the higher calling effort (HCE) and lower calling effort (LCE) songs given on the left margin. Dotted lines to the right of the song models indicate that the song model is repeated. Note that the song model example for the assessment time phonotaxis trial (b) is based on a cycle period of two song bouts, and illustrates two cycles.

minute) bouts of calling song using an Audio-Technica AT815b line cardioid/electret condenser microphone (±1 dB SPL [re: 20 μPa] between 3 and 6 kHz, unpubl. data) connected to a Tascam DA-P1 digital audio tape recorder (48 kHz sampling rate). Songs were transferred to computer using custom designed hardware to connect to the digital port on a Soundblaster Audigy soundcard, and saved as 48 kHz, 16-bit mono .wav files using CoolEdit 2000.

We selected one representative chirp from each song (based on bioacoustic analysis of these songs; K. A. Judge, unpubl. data) and used this chirp to construct a series of song models that varied in their calling effort. For example, to construct a 90% calling effort song model, we filled the first 90% of the song model with chirps at a standardized chirp period (see below). Portions of each song model that were not taken up with calling song (e.g. the latter 10% in the previous

Table 4.1: Summary of the song models used in each of the phonotaxis experiments.

Phonotaxis Trials	Song Period (s)	Calling Effort Difference Low vs High (%)	Calling Effort Difference Low vs High (chirps)
Calling Effort Preference			
80% Difference	13	10 vs 90	3 vs 27
60% Difference	13	10 vs 70	3 vs 21
	13	30 vs 90	9 vs 27
20% Difference	13	10 vs 30	3 vs 9
	13	30 vs 50	9 vs 15
	13	50 vs 70	15 vs 21
	13	70 vs 90	21 vs 27
Assessment Time			
13 seconds	26	30 vs 50(10+90)	9 vs 15(3+27)
26 seconds	52	30 vs 50(10+90)	9 vs 15(3+27)
52 seconds	104	30 vs 50(10+90)	9 vs 15(3+27)
104 seconds	208	30 vs 50(10+90)	9 vs 15(3+27)
208 seconds	416	30 vs 50(10+90)	9 vs 15(3+27)
Control	26	30 vs 30(10+50)	9 vs 9(3+15)

example) were filled with background noise from the recording immediately adjacent to the representative chirp (see Fig. 4.2). The temperature of the room in which we planned to conduct the phonotaxis trials was measured to be 24 ºC, so we constructed all song models to have a chirp period of 0.432 s (see Fig. 4.2), which is equal to the mean chirp period measured in the field at that temperature (K. A. Judge, unpubl. data). During the experiment, the temperature averaged 23.6 ºC (SE ± 0.1 ºC). All song models were constructed to be multiples of 13 s, or 30 chirp periods, which allowed for convenient construction of songs of differing calling effort. Thus, for example, a 90% calling effort song model had 27 chirps followed by 3 chirp periods of background noise (27[0.432 s] + 3[0.432 s] = 13 s). Alternative song models within a single trial were offset by 324 milliseconds to ensure that the two songs did not perfectly overlap. This offset was randomly assigned so that in 50% of the trials the low calling effort song model led the high calling effort song model.

We constructed the full set of alternative song models (see Experimental Design above) for all 114 field-recorded chirps, and only song models constructed from the same original recording of a chirp were presented as alternative models in a phonotaxis trial, thus controlling for differences in song parameters other than calling effort within a single phonotaxis trial. Additionally, individual females were only exposed to song models constructed from the same original recording of a chirp in order to control for female preferences based on unique chirp characteristics other than calling effort.

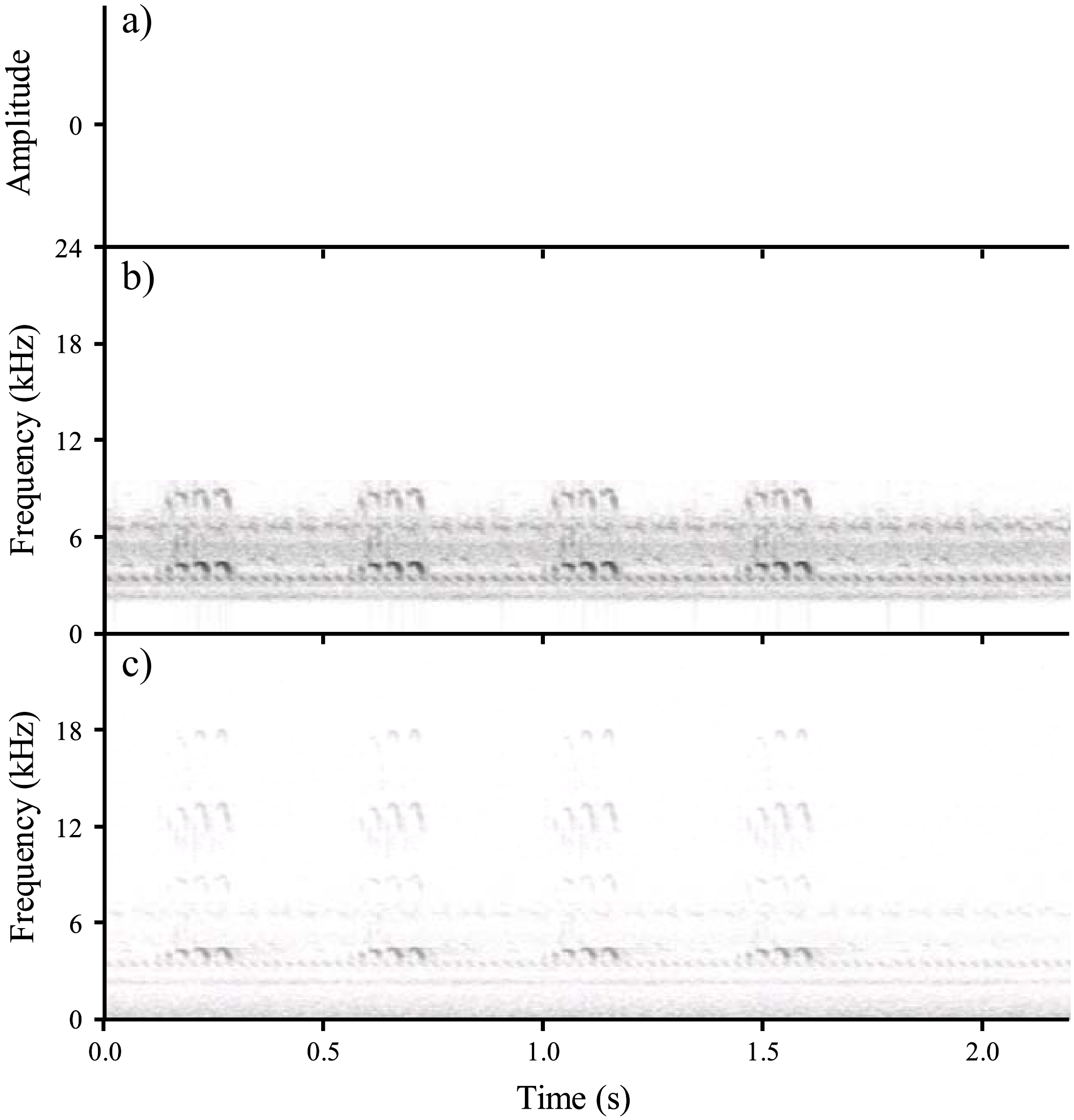

Figure 4.2: A portion of a representative song model showing four chirps followed by a portion without a chirp but still containing background noise. The original song model is shown as: a) a waveform and b) a spectrogram, followed by c) a spectrogram of the song model recorded from the middle of the arena.

4.3.6 Phonotaxis Trials

In all phonotaxis trials, we presented females with a choice between two speakers broadcasting calling songs that differed only in duty cycle. The phonotaxis arena was constructed from a 85 cm long by 47.5 cm wide plastic bin with 12 cm sloped sides (Fig. 4.3). Two 7.5 cm

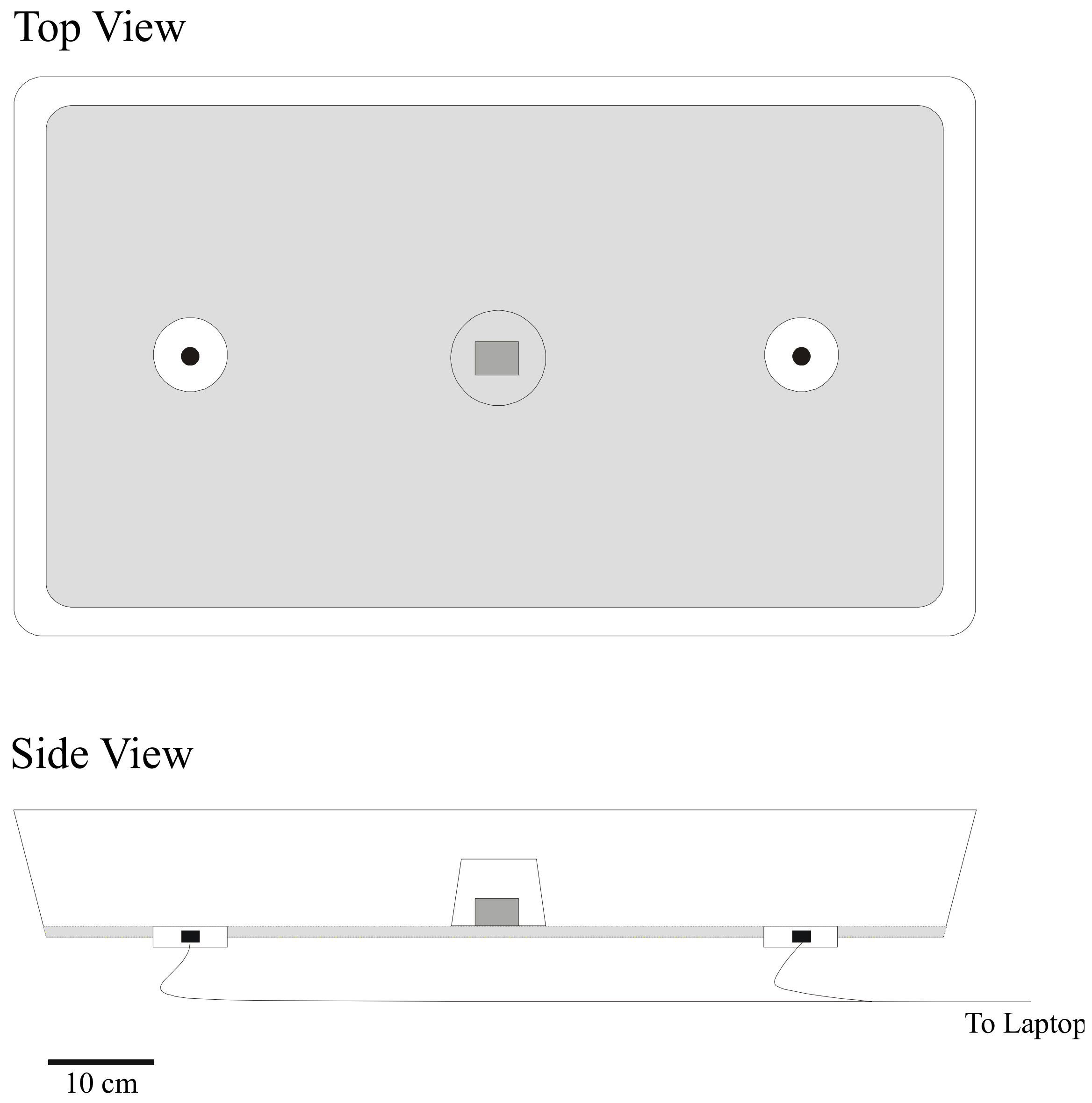

Figure 4.3: Diagram of the arena used in the phonotaxis trials. Light grey areas represent sand covering the floor of the arena. The small black circles surrounded by a wider circle at either ends of the arena represent the headphone speakers surrounded by the choice zone. The central circle represents the plastic container that confined females prior to the start of each trial, and the dark grey box within represents the piece of egg carton provided for shelter.

diameter circles were cut into the floor 10 cm from each end and 50 cm apart. These holes were fitted with a short plastic tube topped with metal screen and coarse sand was spread over the bottom of the arena so the floor of the arena was level with the screen. Songs were broadcast from a pair of iPod® earbud headphones placed 0.5 cm below the center of each screen. These circular screens over each headphone represent the zone of choice (see below), and the position of these zones away from the walls of the arena ensured that females were not guided into choosing a speaker simply by walking around the edge of the arena. The headphones broadcast song at an average (± SE) peak sound pressure level of 72.4 (± 0.1) dB, measured at the center of the arena. Using headphones to broadcast songs allowed us to keep the choice zone small thus improving the distinction between directed phonotaxis and random wandering. Headphones also probably mimicked the size of the sound field from a singing male cricket better than conventional speakers, thus potentially improving the realism of our phonotaxis trials. We recorded all of the broadcast songs from both left and right headphones using an Audio-Technica AT815b shotgun microphone connected to a Tascam DA-P1 digital audio tape recorder. We then digitized the songs, analyzed the carrier frequency of songs broadcast through the left and right speakers and compared them to each other and to the original song files. Songs broadcast through the left and right speakers did not differ significantly from one another (paired t-test: $t = 1.591$, $df = 113$, $p = 0.114$). Although the broadcast songs had a lower carrier frequency than the original song files (paired t-test: $t = 3.610$, $df = 113$, $p < 0.001$), this difference was small in magnitude (mean difference [$\text{broadcast}_{\text{average}}$ – original] ± SE = 27.754 ± 7.689 Hz). Despite the difference between original and broadcast calling songs, repeatability (Lessells and Boag 1987) was 93.6%, indicating that calling song quality was not substantially changed by using headphones in the phonotaxis trials.

Before the start of each trial, we gently enclosed the female, and a small piece of egg carton for shelter, under a plastic container at the centre of the arena (Fig. 4.3). We let the female acclimate for approximately two minutes, after which the plastic container was raised and the playback started. We watched females until they had chosen one of the speakers or until 20 minutes had elapsed. We determined that a choice had been made when a female paused with some part of her body in contact with the screen over a speaker. In the case of a choice, we noted the time (in seconds) from when the female was released to when she chose a speaker. If she failed to make a choice, we retested her approximately (mean ± SE) 5.9 ± 0.2 days after the last of her four scheduled phonotaxis trials (see above). We did not retest her if she failed to make a choice a second time. Following each trial, we wiped the arena walls and the screens above each speaker with 95% ethanol, and raked the sand to eliminate any potential pheromone cues left by females.

4.3.7 Statistical Analysis

We calculated 95% confidence intervals for the proportion of high and low condition females that chose the higher calling effort song (P[HCE]) to test for a significant preference for the higher calling effort song (see Zar 1996 p. 524 for details on calculating 95%CI for a binomial population parameter). For example, if the 95% C.I. for P(HCE) included 50% then we determined that there was no significant preference for higher calling effort. To test the hypothesis that female preference for higher calling effort was condition dependent, we compared P(HCE) between high and low condition females in each phonotaxis experiment using Fisher's exact tests.

We tested the condition dependence of choosiness by comparing latency to choose of high and low condition females for each class of phonotaxis experiment. Kolmogorov-Smirnov normality tests detected that latency to choose (in seconds) was significantly non-normally distributed. However, a log transformation restored normality within each phonotaxis trial (Kolmogorov-Smirnov tests: all $p > 0.054$). Because half of our experimental individuals took part in the large difference preference experiment and the other half took part in the assessment-time control experiment, we could not use repeated measures to test for condition-dependence of latency to choose. We therefore conducted separate analyses for each of the five classes of phonotaxis experiments (i.e. large, medium and small difference preference experiments, assessment time experiments, and the control experiment) and adjust our Type I error rate accordingly using the Bonferroni method (alpha/5 = 0.05/5 = 0.01). For classes of phonotaxis experiment where there was only one level (large difference and control experiments), we conducted a t-test to test for differences in latency to choose between low- and high condition females. For the other three classes of phonotaxis experiment, we conducted univariate GLMs with diet, experiment and their interaction as independent variables. We interpret a significant interaction term as evidence that the difference in latency to choose between low- and high quality females depended on either the average calling effort of the pair of song models (preference experiments) or the cycle period (assessment time experiments). In the case of a nonsignificant interaction, we removed the interaction term and reran the analysis.

Given that all females took part in four phonotaxis trials, we also tested for a change in latency to choose as females progressed through these four phonotaxis trials, and whether high and low condition females differed in this change. We conducted a repeated measures GLM with diet as the between-subjects factor and trial day as the within-subjects factor.

To test for condition dependence of choosiness using the preference for the high calling effort song model in the assessment time phonotaxis trials, we examined whether high and low condition females preferred the high (and variable) or low calling effort song by comparing the 95% C.I. for P(HCE) as described above. If females significantly preferred the low calling effort song, then we noted the cycle period of the trial and interpreted this as an estimate of the upper limit on female assessment time (i.e. if they had taken longer to assess male calling effort then they would have preferred the high calling effort song). If low and high condition females differed in the cycle period at which their preference switched from the high to the low calling effort song, then female assessment time was condition dependent.

All statistical tests were carried out using SPSS 10 (SPSS Inc.).

4.4 Results

4.4.1 Survival

We isolated 356 and 353 hatchlings on the low and high quality diets respectively. Of these, 81.2% (N = 289) and 87.0% (N = 307) survived to moult to adulthood. Diet did not significantly affect survival to adulthood (Kaplan-Meier survival analysis: Log-Rank test statistic = 2.16, df = 1, $p = 0.1418$; Fig. 4.4). There was a trend for the adult sex ratio to be skewed in favour of males in the high quality diet (low condition, 152 males: 136 females (plus one gynandromorph: see Johnstone 1975); high condition, 185 males: 122 females), but this was not statistically significant (Fisher's exact test, $p = 0.069$).

4.4.2 Condition Dependence of Body Mass and Development Time

We tested for condition dependence of: female development time, mass one day after moult to adulthood and mass at maturity (day 11 post-adult moult; K. A. Judge, pers. obs.) using multivariate GLM. Diet had a significant effect on these life history variables (Pillai's trace = 0.193, $F_{3,\,251} = 20.010$, $p < 0.001$). Univariate ANOVAs revealed that females reared on the high quality diet developed faster (mean ± SE: low = 53.9 ± 0.8 days, high = 51.5 ± 0.7 days; $F_{1,\,253} = 4.889$, $p = 0.028$), had a greater mass at the adult moult (mean ± SE: low = 346 ± 7 mg, high = 403 ± 7 mg; $F_{1,\,253} = 32.633$, $p < 0.001$) and gained more weight before sexual maturity (mean ± SE: low = 130 ± 5 mg, high = 174 ± 6 mg; $F_{1,\,253} = 37.147$, $p < 0.001$) than females reared on the low quality diet.

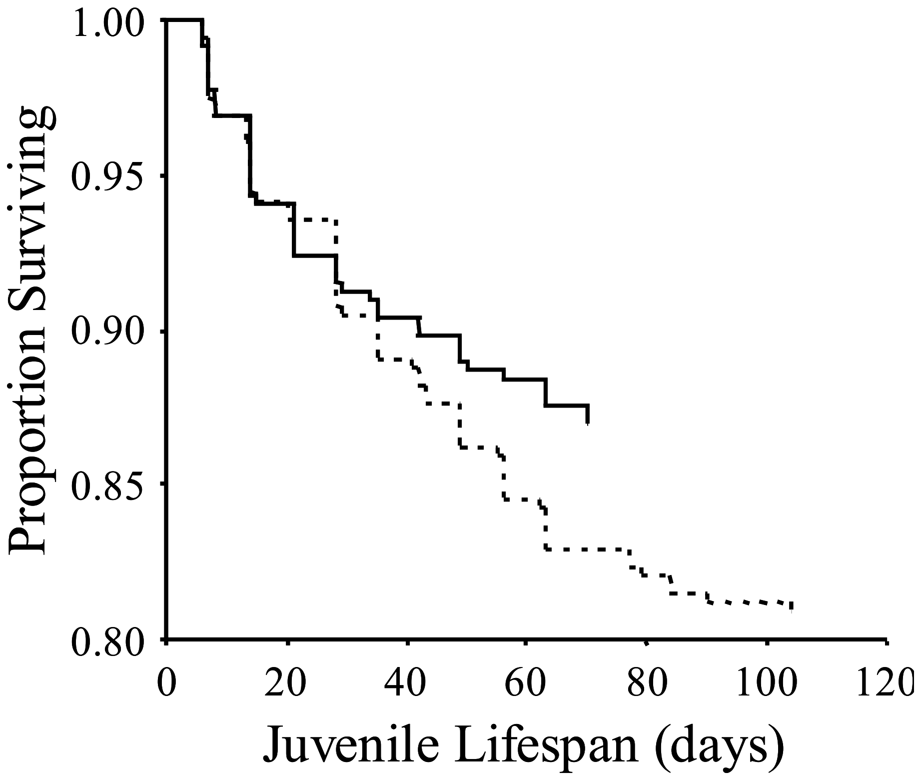

Figure 4.4: Proportion of low- and high condition (dotted and solid lines respectively) crickets surviving to adulthood.

4.4.3 Phonotaxis Trials: General

Of the 258 females that successfully moulted to adulthood (136 low- and 122 high condition), 17 did not participate in any phonotaxis trials because they died before they were scheduled to start the experiment (ten low- and seven high condition females). Also, one low condition female was accidentally mated before completing her trials. This left 125 low- and 115 high condition females that each took part in four phonotaxis trials. On average, females had their first phonotaxis trial at 21 days past their adult moult (mean + SE = 20.5 ± 0.4 days, range = 11 to 31): low- and high condition females did not differ in their age at first trial (mean $\text{difference}_{\text{L-H}}$ ± SE = 0.2 ± 0.9 days; t = 0.272, df = 238, p = 0.785).

More high- than low condition females failed to choose one of the speakers within the allotted 20 minutes during at least one of their four phonotaxis trials (low: 20/125, high: 41/115, Fisher's exact test: $p < 0.001$). After retesting (see above), there was still a significantly greater proportion of high condition females that did not make at least one choice during the allotted time (low: 9/125, high: 19/115, Fisher's exact test: p = 0.028), although every female made a choice in at least one of her phonotaxis trials. In trials where females did not make a choice within the allotted 20 minutes, latency to choose was arbitrarily set at 20 minutes. This procedure did not affect our interpretation of the results as removal of these data had no significant effect on any measured effects (data not shown).

4.4.4 Phonotaxis Trials: Preference for Higher Calling Effort

Both high- and low condition females displayed significant preferences for the speaker broadcasting the higher calling effort song (i.e. 95% C.I. for P[HCE] excluded 50%) in each of the three classes of preference phonotaxis experiments: 80%, 60% and 20% differences (Fig. 4.5). However, condition did not affect female preference for higher calling effort (Fisher's exact tests: all $p > 0.454$). These patterns were consistent when the 60% and 20% preference experiments were broken up into their constituent phonotaxis experiments (Fig. 4.6), although female preference was no longer statistically significant at 20% (except low condition females choosing between 50% and 30% calling effort; see Fig. 4.6). Condition did not affect the probability that a female would choose the higher duty cycle call in any of the preference phonotaxis experiments (Fisher's exact tests: all $p > 0.274$, Fig. 4.6).

4.4.5 Phonotaxis Trials: Assessment Time

In the assessment time phonotaxis experiments, we predicted that as the cycle period of the higher calling effort song (i.e. time that it took to switch from the low [10%] to the high [90%] calling effort phase) increased, it would exceed the average time that females spend assessing male calling effort. Therefore, we predicted that the average female preference would switch from the higher calling effort song (average = 50%) to the lower calling effort song (constantly 30%) as the higher calling effort cycle period increased. We failed to detect this switch in preference for either low- or high condition females (i.e. 95% C.I. for P[HCE] never fell completely below 0.50, see Fig. 4.7). In addition, an ordered heterogeneity test (Rice and Gaines 1994), which combines a non-directional heterogeneity test (in this case a chi-square test) with a Spearman rank correlation between the observed and predicted category values, failed to support the hypothesis that the proportion of females choosing the high duty cycle call declined with increasing cycle period (test value: $r_sP_c = 0.00$, $p = 1.00$). This is likely due to the fact that females displayed some of the weakest preferences when the cycle period was shortest – the

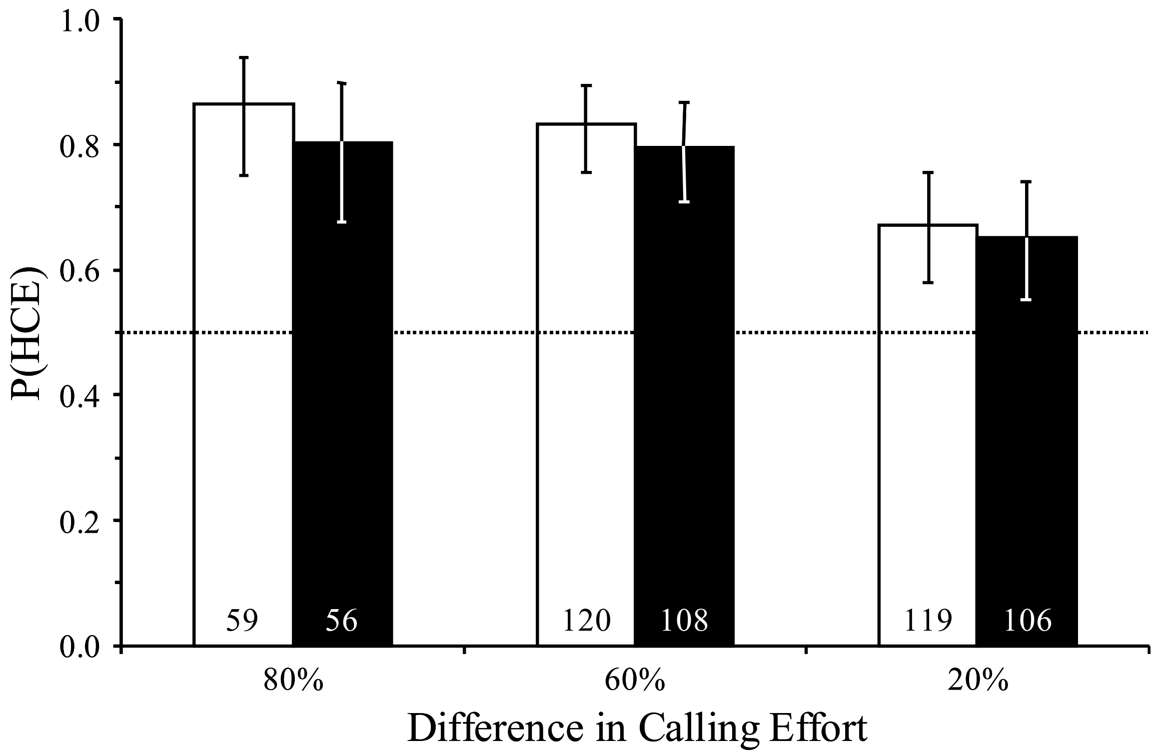

Figure 4.5: Proportion of low- and high condition (open and filled columns respectively) female crickets that chose the speaker broadcasting the higher calling effort song (P[HCE]) for each class of preference phonotaxis experiment (80%, 60% and 20% difference in calling effort). Bars represent 95% confidence limits on P(HCE), and sample sizes are given at the base of each column. The horizontal dotted line is at P(HCE) = 0.50 to allow for rapid assessment of which preferences are statistically significant.

experiment where we predicted the strongest preference for the higher calling effort song (Fig. 4.7).

As with all of the preference experiments, low- and high condition females did not differ in their preference for the higher calling effort song in the assessment time phonotaxis experiments (Fisher's exact tests: all $p > 0.544$) or the control phonotaxis experiment ($p = 0.708$) (Fig. 4.7).

4.4.6 Phonotaxis Trials: Latency to Choose

We tested for an effect of condition on latency to choose within each class of phonotaxis experiment. There were no significant interactions between condition and experiment for any of the classes of phonotaxis experiments (60% difference: $F_{1, 236} = 0.007$, $p = 0.932$; 20% difference: $F_{3, 232} = 1.735$, $p = 0.161$; assessment time: $F_{4, 230} = 1.021$, $p = 0.397$; Note: interactions were only

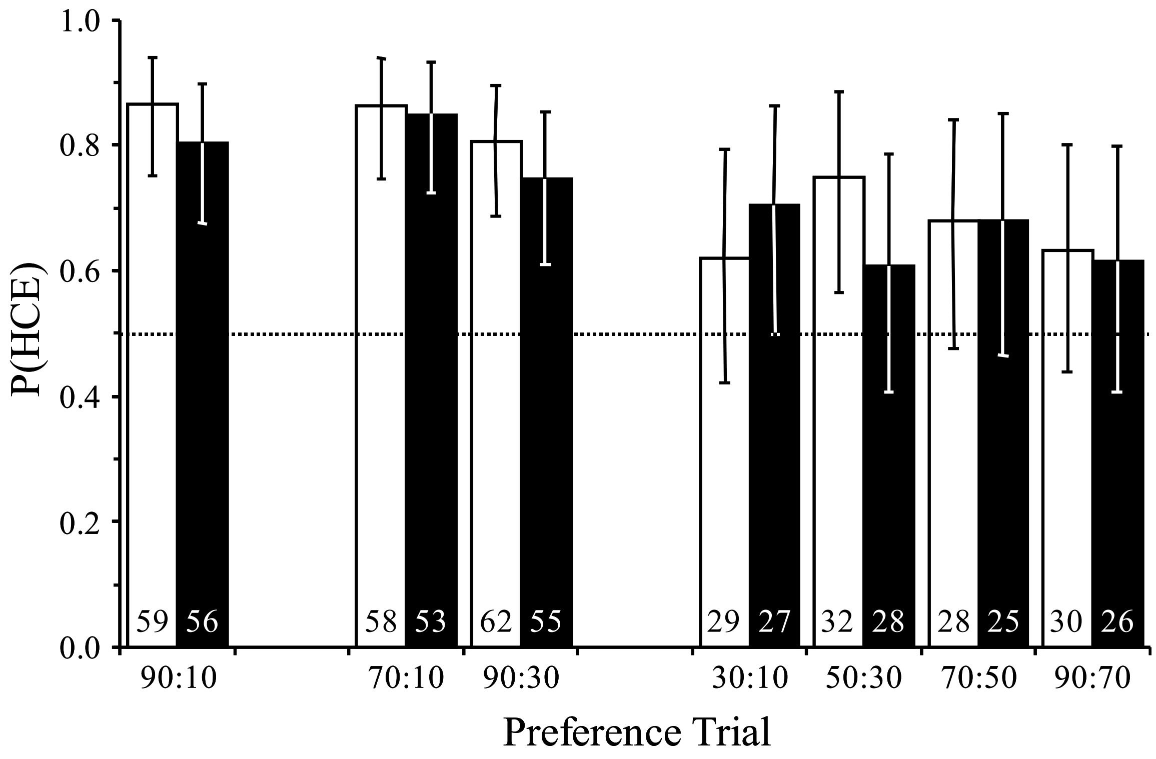

Figure 4.6: Proportion of low- and high condition (open and filled columns respectively) female crickets that chose the speaker broadcasting the higher calling effort song (P[HCE]) for each preference phonotaxis experiment. Bars represent 95% confidence limits on P(HCE), and sample sizes are given at the base of each column. The horizontal dotted line is at P(HCE) = 0.50 to allow for rapid assessment of which preferences are statistically significant.

tested when there was more than one level in a class of phonotaxis experiments). In general, high condition females took longer to choose than low condition females (Figs. 4.8 and 4.9), and this difference was statistically significant in the 80% difference (t-test: $t = 2.220$, $df = 116$, $p = 0.028$), 60% difference ($F_{1,237} = 6.193$, $p = 0.014$), 20% difference ($F_{1,235} = 8.133$, $p = 0.005$), and assessment time ($F_{1,234} = 5.322$, $p = 0.022$) phonotaxis experiments, but not in the control experiments (t-test: $t = 1.182$, $df = 120$, $p = 0.240$). There was a significant effect of average duty cycle on female latency to choose in the 20% difference phonotaxis experiments ($F_{3,2350} = 5.130$, $p = 0.002$), but not in the 60% difference ($F_{1,237} = 0.300$, $p = 0.584$) or assessment time ($F_{4,234} = 1.731$, $p = 0.144$) phonotaxis experiments. Within the 20% difference phonotaxis experiments, there was a significant decrease in latency to choose with increasing average duty cycle (Tukey's post hoc tests; see Fig. 4.8 for significant differences).

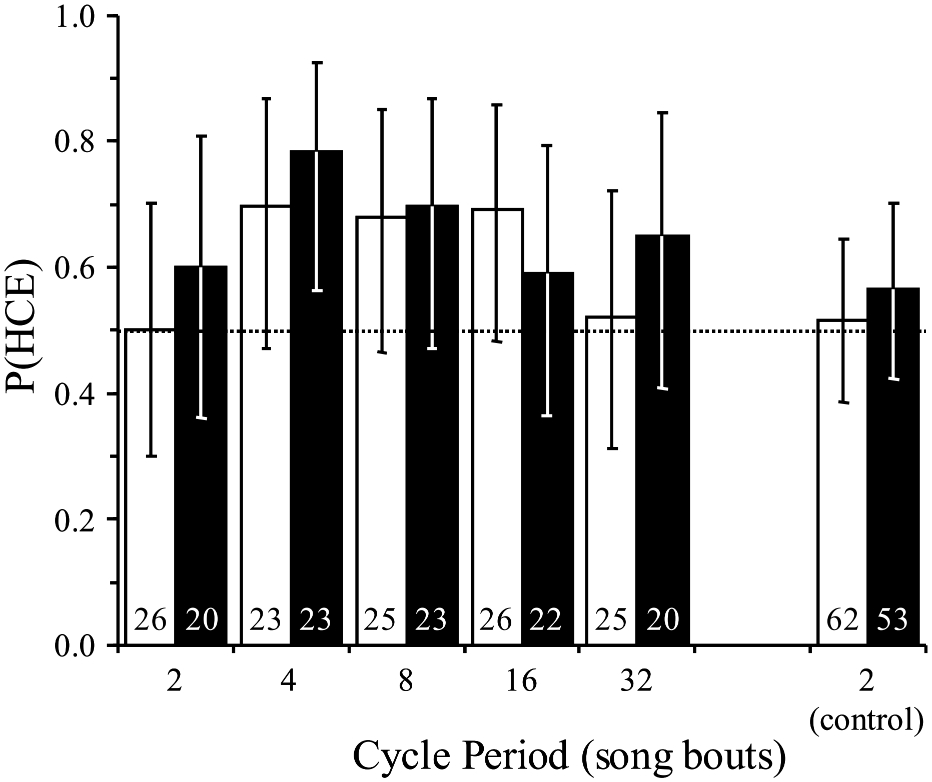

Figure 4.7: Proportion of low- and high condition (open and filled columns respectively) female crickets that chose the speaker broadcasting the higher calling effort song (P[HCE]) for each assessment time phonotaxis experiment and the control experiment. Bars represent 95% confidence limits on P(HCE), and sample sizes are given at the base of each column. The horizontal dotted line is at P(HCE) = 0.50 to allow for rapid assessment of which preferences are statistically significant.

We also tested for an effect of experience on individual females' latency to choose. There was no significant condition by order (First Trial to Fourth Trial) interaction (Pillai's Trace = 0.015, $F_{3, 236}$ = 1.184, p = 0.317). Both low- and high condition females began their phonotaxis trials with the same latency to choose (First Trial: t = 1.385, df = 238, p = 0.167). However, after the first trial high condition females took significantly longer to choose than low condition females (Second Trial: t = 2.637, df = 238, p = 0.009; Third Trial: t = 2.117, df = 223.9, p = 0.035; Fourth Trial: t = 3.756, df = 238, p < 0.001) (Fig. 4.10).

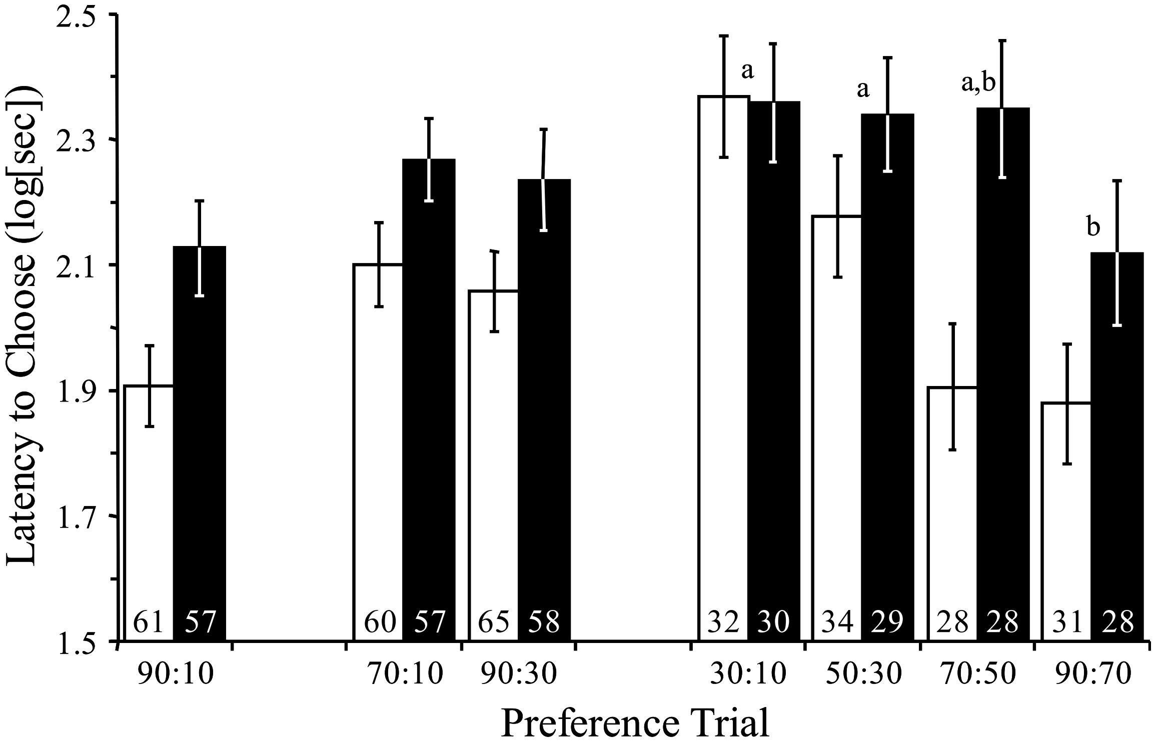

Figure 4.8: Latency to choose for low- and high condition (open and filled columns respectively) female crickets in the preference phonotaxis trials. Bars represent ± one standard error of the mean, and sample sizes are given at the base of each column. Statistically significant differences (between phonotaxis experiments) are indicated by different letters.

4.5 Discussion

Male calling effort (the proportion of time spent calling) in field crickets is condition-dependent (Crnokrak and Roff 1995, 1998a,b; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a,b; Hunt et al. 2004; Judge et al. 2008, Chapter 3). We tested the hypothesis that two components of female mating preferences for male calling effort, both preference functions and choosiness (Jennions and Petrie 1997), were condition dependent. Similar to our previous study (Judge et al. 2008, Chapter 3), our experimental diets were successful in producing groups of adult females that differed in condition – as reflected in the higher mass and more rapid growth rates of individuals of the high diet – without imposing differential mortality during development (and thus voiding issues relating to mortality selection). Similarly to many other taxa (Ryan and Keddy-Hector 1992), females displayed preferences for greater amounts of

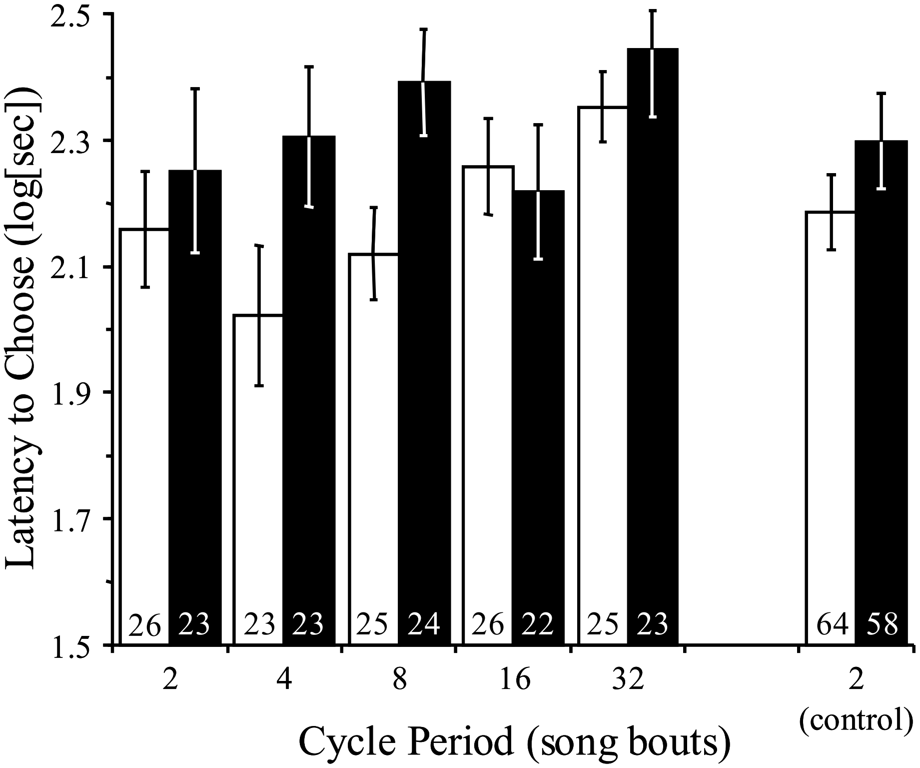

Figure 4.9: Latency to choose for low- and high condition (open and filled columns respectively) female crickets in the assessment time phonotaxis trials. Bars represent ± one standard error of the mean, and sample sizes are given at the base of each column.

male display (i.e. higher calling effort), although condition had no detectable effect on preference functions (Figs. 4.5 and 4.6). We were unable to measure female assessment time using a predicted switch in phonotaxis (Fig. 4.7), even following pooling of low- and high condition females (data not shown); and thus this measure of choosiness was uninformative with respect to condition. However, as predicted high condition females were more choosy in that they were significantly more likely to: a) fail to choose within the time allotted for phonotaxis (20 minutes), and b) take longer to choose than low condition females (Figs. 4.8 and 4.9). Moreover, differences between low- and high condition females in latency to choose became apparent only in later phonotaxis trials with the same females (Fig. 4.10). Based on these results, we conclude that in *G. pennsylvanicus*, choosiness but not female preference for higher calling effort is condition-dependent or at least that choosiness is more condition-dependent than preferences.

Hunt et al. (2005) studied the condition dependence of female choice in the black field cricket, *Teleogryllus commodus*. They found that females raised from hatching on a high quality

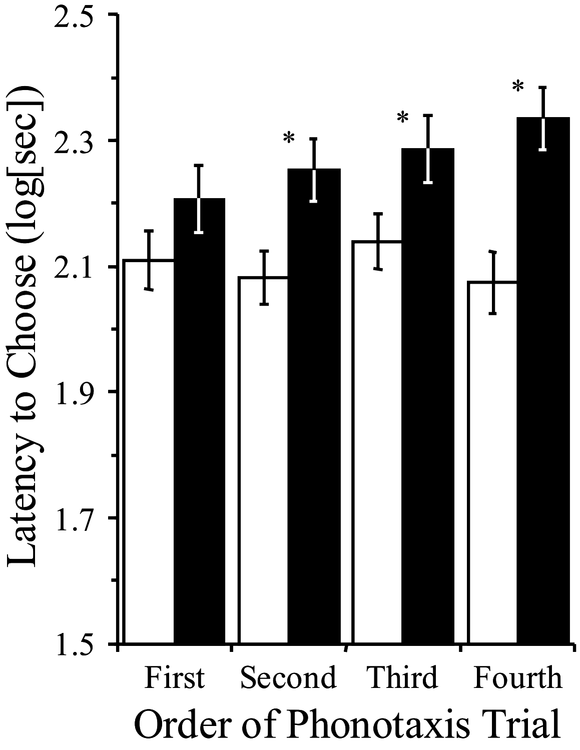

Figure 4.10: Latency to choose for low- and high condition (open [N = 125] and filled [N = 115] columns respectively) female crickets for the order in which they took part in phonotaxis trials. Bars represent ± one standard error of the mean.

diet expressed stronger preference functions for aspects of male song (dominant frequency and call rate), and responded to male song faster than females raised on the low quality diet (Hunt et al. 2005). In contrast, we did not detect any effect of condition on the female *G. pennsylvanicus* preference function for male calling effort, but high condition females took longer to choose than low condition females. There are a number of differences between the two grylline studies that make it difficult to compare the results. First, our arena design forced female *G. pennsylvanicus* to take a relatively greater risk when they chose a song by moving away from the sides, whereas female *T. commodus* could make a choice simply by sticking to the edge of the arena (Hunt et al. 2005). Second, we presented female *G. pennsylvanicus* with alternative songs that differed by a fixed amount; however, Hunt et al. (2005) presented female *T. commodus* with a choice between a test stimulus and a reference song that did not change. As a result, the magnitude of the difference between alternative stimuli changed depending on test stimulus being presented. Third, the average calling efforts used in the two studies differed: calling songs were broadcast continuously to female *T. commodus* (Hunt et al. 2005), which may have had the effect of increasing female responsiveness. In our study, when comparing the latency to choose of females exposed to different average duty cycles in the 20% difference phonotaxis experiments, there was a statistically significant trend for a decline in latency to choose with increasing average duty cycle. Finally, whereas we tested the condition dependence of female mating preferences for a male song parameter (calling effort) that was itself condition-dependent (Judge et al. 2008, Chapter 3), Hunt et al. (2005) tested song parameters (dominant frequency and call rate) that were not found to be condition-dependent (Hunt et al. 2004). We did not detect condition dependence of female preference function for calling effort, but Hunt et al. (2005) found that high condition females displayed stronger preference functions for both dominant frequency and call rate. One possible explanation for this difference is that high condition female *T. commodus* were simply better able to discriminate between male songs than low condition females, whereas differences in calling effort may be relatively easy to detect even by female *G. pennsylvanicus* in low condition. Manipulating condition during the nymphal stage seems to have divergent effects on preference functions for different components of male calling song.

Previous work has supported the prediction that mate choice is condition-dependent (Morris et al. 1975; Poulin 1994; Simmons 1994; Brown 1997; Clark et al. 1997; Ortigosa and Rowe 2002; Cratsley and Lewis 2003; Syriatowicz and Brooks 2004; Hunt et al. 2005). However, the effect of condition on mate choice behaviour is not consistent. For example, parasitized (and presumably low condition) female katydids, *Requena verticalis*, attempted to mate more often than unparasitized (high condition) females (Simmons 1994). However, starved (low condition) female water striders, *Gerris buenoi*, were more likely than fed (high condition) females to resist mating attempts by males (Ortigosa and Rowe 2002). In the katydid, males provide females with a nutritious nuptial gift during mating and females gain direct benefits from eating male nuptial gifts (Gwynne 1988), whereas mating is costly for female water striders (Rowe 1994) and directly conflicts with foraging (Rowe 1992). As Hunt et al. (2005) point out, the effect of condition manipulation on mate choice seems to fall into two categories: a) females of species in which males provide a nutritious food gift during mating often mate more readily (i.e. less choosy) when in poor condition (Simmons 1994; Brown 1997; Cratsley and Lewis 2003; but see Morris et al. 1975), and b) females of species where males are not known to provide any nuptial gift usually become less responsive to male mating attempts or mating signals (i.e. more choosy) when in poor condition (e.g. Poulin 1994; Clark et al. 1997; Ortigosa and Rowe 2002; Syriatowicz and Brooks 2004; for additional references see Hunt et al. 2005). Field crickets are not known to produce large nutritious nuptial gifts during mating (but see Simmons 1988; Wagner et al. 2001; Wagner and Harper 2003 for potential material benefits of polyandry): why then are low condition female *G. pennsylvanicus* apparently more motivated to mate than high condition females (this study; D. Chui, J. Vaughan, and K. A. Judge, unpubl. data)? In the following paragraphs, we discuss several alternative hypotheses to explain this result and propose a novel hypothesis to explain the evolution of condition-dependent phonotaxis in female field crickets.

One possible explanation for the increased responsiveness of low condition female *G. pennsylvanicus* to male calling song is that they receive direct benefits through either ingestion of the spermatophore or genitally-absorbed substances from the male ejaculate as shown in other gryllines (e.g. Simmons 1988; Wagner et al. 2001). Thus low condition females may be more motivated to mate in order to acquire these direct benefits. The small size of the typical grylline spermatophore (a few exceptional species have a spermatophylax gift: Alexander and Otte 1967) suggests that they are unlikely to deliver direct nutritious benefits to females. However, starved female *Plebeiogryllus guttiventris* survived longer when artificially fed male spermatophores (Bentur and Mathad 1975). Also, female *G. bimaculatus* laid heavier eggs when they were allowed to consume many male spermatophores (Simmons 1988). Additional ejaculates of male *G. lineaticeps* increased female lifespan (Wagner et al. 2001) and the ejaculates of males with preferred song characteristics increased both female lifespan and fertility (Wagner and Harper 2003). Male *G. pennsylvanicus* that were infected with gregarine gut parasites took longer to replace spermatophores following mating than uninfected males (Zuk 1987b), indicating that spermatophore production is costly. If some of the costliness of spermatophore production is a result of males including nutrients in their ejaculate or in the sperm ampulla (which females often eat following copulation; K. A. Judge, pers. obs.), then low condition females may mate more readily in order to obtain these nutrients.

Alternatively, high condition females may have been less likely to display phonotaxis during our experiments because they were more resistant to male song. Sexual differences in motivation to mate should result in selection for, “skilled salesmanship among the males and an equally well-developed sales resistance and discrimination among the females.” (Williams 1966, p. 184). This idea has flourished with the recognition of conflict between the sexes over any stage in the mating process in which the interests of both males and females are at stake (Parker 1979, 1983; Arnqvist and Rowe 2005). Under sexual conflict, calling by male crickets may well be a “sales pitch”, and thus females could be under selection to resist the attractiveness of this male display. Although calling song is energetically costly to produce (Prestwich and Walker 1981; Prestwich 1994; Hoback and Wagner 1997) and calling effort is condition-dependent (Crnokrack and Roff 1998a; Holzer et al. 2003; Hunt et al. 2004; Wagner and Hoback 1999; Judge et al. 2008, Chapter 3), low condition males are still able to sing at a high level of calling effort for at least a short period of time (Judge et al. 2008, Chapter 3). Thus, a female who is less resistant to male calling song has a greater chance of mating with a low condition (low calling effort) male. Although, low condition females in our study chose male calling song more quickly (suggesting that they were less resistant) than high condition females, they were not more likely to choose the low effort calling song. This may have been due to the relatively low amount of variation in calling effort within song models in our experiment (see Assessment Time phonotaxis trials, Table 4.1). Future work should: a) test more realistic variation in calling effort, and b) examine whether low condition females also expose themselves to a greater risk of predation (e.g. Sakaluk and Belwood 1984), for example, by walking over open ground to get to singing males.

A third hypothesis (not exclusive of others) for the decreased choosiness of low condition female *G. pennsylvanicus* is that higher calling effort guides females to areas where non-mating food resources are available. More or better quality food causes male gryllines call more (Crnokrak and Roff 1998a; Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a,b; Hunt et al. 2004; Judge et al. 2008; Chapter 3). And females are attracted to higher calling effort (Cade and Cade 1992; Crnokrack and Roff 1995, 1998a,b; Hunt et al. 2004; this study) and thus to areas in which males are likely to be well fed. The territories (Alexander 1961; Loher and Dambach 1989) of males investing in high effort calling may thus contain food resources available to females attracted to song. The fact that males calling at a high rate are likely to defend territories higher in food resources might explain why low condition and likely hungry females did not display weaker preferences for calling effort. Males would be under strong selection to prevent females from visiting his territory without mating. However, the behaviour of territorial male field crickets before mating and/or during female phonotaxis (e.g. searching for females within territory) is largely unknown (but see Alexander 1961; French and Cade 1987, 1989; Souroukis and Cade 1993 for studies of males in artificial enclosures). All three of the above hypotheses place a much stronger emphasis on direct selection on female mating preferences than has recently been suggested for gryllines (e.g. Tregenza and Wedell 1998, 2002; Simmons et al. 2006).

Our study is the first attempt to measure the time that female field crickets take in assessing male calling effort, and is based on the experimental design of Schwartz et al. (2004). We predicted that females would switch from preferring a high effort (but variable) call to preferring a lower effort (and constant) call as we increased the period over which the variable call cycled. We controlled how much song female *G. pennsylvanicus* were exposed to by initiating playback of alternative male songs at the same time as we started to record female behaviour. Although there was a trend for the proportion of females attracted to the high effort call to decline with increasing cycle periods (greater than two song bouts), we were unable to reverse the preference even at cycle periods of 32 bouts (corresponding to a minimum assessment time of 16 bouts or 208 seconds) (Fig. 4.7). This result suggests that, on average, female crickets listen to male song for longer than approximately 3.5 minutes, which is longer than the approximately 2 minutes that female grey treefrogs take to assess male songs (Schwartz et al. 2004). This may relate to the relative intra-male variability of male signals in treefrogs versus field crickets. Female treefrogs prefer longer calls (Klump and Gerhardt 1987) and call duration has an average within-individual coefficient of variation of 16.4% (Gerhardt 1991). If male *G. pennsylvanicus* display greater within-individual variation in calling effort, then this could explain why female *G. pennsylvanicus* appear to listen for longer. Although male *G. pennsylvanicus* vary greatly in nightly calling effort (K. A. Judge, unpubl. data), we do not yet have an estimate of the variability of individual males over shorter time scales.

4.6 Acknowledgements

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CHAPTER 5

Experienced (but not virgin and socially-naive) female field crickets exert directional selection on males.

Kevin A. Judge

5.1 Abstract

A large and increasing number of factors have been shown to affect female mating behaviour, and thus to affect the strength and/or direction of selection that females exert on males. One of these factors is female social experience (including mating history), which has received relatively little attention. I used multivariate selection analysis to estimate the linear and nonlinear selection gradients exerted by female field crickets (*Gryllus pennsylvanicus*) with different social backgrounds. Females were either: 1) virgins with no experience of conspecifics as adults or 2) experienced females from a large, mixed-sex population. I assessed relative fitness through either mating success (mated or not) or attractiveness (inverse of latency to copulate) and calculated selection gradients based on each fitness measure for four male traits: age, body size, residual head width and residual mass. Based on previous research, I predicted that older males would have higher relative fitness than younger males. I also tested the prediction that, virgin females would exert weaker selection than experienced females. Overall, this prediction was supported, and experienced females exerted significant positive directional selection on male age and residual head width. I also detected significant nonlinear selection by both virgins and experienced females, and these gradients differed between the two classes of female. I discuss these results in light of the prediction that virgin females should be less choosy than mated females. And, in the context of previous work on *G. pennsylvanicus*, the present study replicates an important earlier result that older males have a mating advantage.

5.2 Introduction

Female mating behaviour is affected by a variety of factors, for example parasite infection (e.g. Morris et al. 1975), predation risk (e.g. Hedrick and Dill 1993), age (e.g. Gray 1999), condition (Hunt et al. 2005), ambient light spectrum (e.g. Gamble et al. 2003), ambient acoustic environment (Gerhardt and Klump 1988) juvenile experience (e.g. Hebets 2003) among many others (reviewed in Jennions and Petrie 1997). In fact, mating itself may induce a change in a female's behaviour, and thus the selection that she exerts on males. Bateman (1948) noted that because of the diminishing returns to mating more than once - observed in his studies of female *Drosophila melanogaster* - "there would be selection in favour of obtaining only one mate after which they would become relatively indifferent [to mating]" (p. 365, text in square brackets added). This loss of receptivity has been documented in dipterans (Leopold et al. 1971, and references therein), ensiferan orthopterans (Loher et al. 1993; Lickman et al. 1998; Bateman 2001), poecilid fish (Pitcher et al. 2003), salamanders (Gabor and Halliday 1997), and spiders (Uetz and Norton 2007). However, multiple mating by females is much more common than theory (Bateman 1948) would suggest (e.g. Arnqvist and Nilsson 2000), which poses the question of what effect this variation in female behaviour could have on sexual selection on males.

The trading up hypothesis (Halliday 1983) states that polyandrous females should mate relatively indiscriminately with conspecifics when they are virgin, but should be more choosy for subsequent matings. Thus, the intensity, direction and form of selection exerted by females on males may change within individual female's lifespan and across the breeding season as the proportion of mated (and experienced) females increases. Several studies have tested the effect of mating status on female mating behaviour (Loher et al. 1993; Gabor and Halliday 1997; Bateman 2001, Bateman et al. 2001; Kodric-Brown and Nicoletto 2001; Ortigosa and Rowe 2003, Ivy et al. 2005; Peretti and Carerra 2005; K. A. Judge and K.-C. Tran, unpubl. ms; present study). Virgin female smooth newts, *Triturus vulgaris*, did not discriminate among males on the basis of male crest height, but exerted substantial selection for higher-crested males in subsequent pairings (Gabor and Halliday 1997). For grylline crickets (Orthoptera, Gryllidae, Gryllinae) Ivy et al. (2005) found that both virgin and mated female decorated crickets, *Gryllodes sigillatus*, preferred novel males over familiar males, indicating that the direction and apparent strength of selection exerted by females did not depend on mating status. However, although virgin female *Gryllus bimaculatus* did not discriminate against small males, with their second mates the same females allowed longer insemination (spermatophore attachment time: a way of biasing paternity in gryllines) by large males relative to small males (Bateman et al. 2001).

Studies that document differences in, for example, virgin and mated females' preferences for a single male trait are relatively weak tests of the hypothesis that virgins are generally less choosy than non-virgin females. For example, virgins may simply prefer different traits than mated females. A robust test of this hypothesis should measure the intensity of selection as well as the direction and shape of selection on a variety of male traits. However, to my knowledge, no studies have done this. In this paper, I quantify net selection, and both linear and nonlinear selection exerted on multiple male traits by female fall field crickets *Gryllus pennsylvanicus* (Burmeister) with two different backgrounds: 1) virgin females that had no experience as adults of conspecifics of either sex, and 2) experienced females that were housed in a large, mixed-sex population. Although this design does not allow me to isolate the specific cause(s) of changes in selection with female experience (e.g. mating *per se*), the purpose of this experiment was simply to demonstrate a change in selection with female experience.

The fall field cricket is a common, univoltine species found in grassy, disturbed areas (Alexander 1968; K. A. Judge, pers. obs.) across North America (Capinera et al. 2004). Eggs hatch in the spring and adults are mating by mid-July. Breeding continues throughout the fall – hence the common name – until the first severe frost (Alexander 1968). Female *G. pennsylvanicus* have a mating bias for older males (Zuk 1987c, 1988), although this may be because males in good condition both live longer and call more than males in poor condition (Judge et al. 2008). Male gryllines use their heads and mouthparts during violent male-male combat (Alexander 1961), and male *G. pennsylvanicus* have larger heads and more widely-spaced maxillae (pointed, sclerotized mouthparts used in male-male fights) than females for a given body size (Appendix A). Moreover, male *G. pennsylvanicus* with more widely-spaced maxillae are more likely to win in aggressive contests with males with less widely-spaced maxillae (Appendix A).

I measured selection on male: age (days past adult moult), body size (pronotum length), relative head size (residual head width) and condition index (residual body mass). I predicted that there would be positive linear selection on all four male characteristics because females show a mating bias towards older males (Zuk 1987c, 1988), and males in good condition invest more in calling effort (Judge et al. 2008) and are larger and have proportionately wider heads (K. A. Judge, unpubl. data) than males in poor condition. Further, because experienced females have likely mated and definitely experienced a wider variance in mate quality than virgins, experienced females should exert stronger selection than virgin females (mating: e.g. Gabor and Halliday 1997; variance in mate quality: e.g. Wagner et al. 2001). However, experienced females may be older and in poorer condition than virgin females, characteristics that would tend to lessen the choosiness of experienced females (age: e.g. Gray 1999; condition: e.g. Hunt et al. 2005).

5.3 Methods

5.3.1 Animal Rearing

All experimental individuals were first generation offspring of approximately 150 male and 150 female adult *G. pennsylvanicus* collected from around the campus of the University of Toronto at Mississauga campus (43°32'50.51"N, 79°39'37.80"W) in August and September of 2004.

Juveniles were held in large plastic bins (48 cm long, 35 cm wide, 31 cm high). I fed all juvenile crickets rabbit chow (Martin “Little Friends”, ground pellets for the first two to three weeks after hatching and whole pellets later on). Water was provided in cotton-plugged plastic vials, and shelter consisted of layers of egg cartons. Every three to four days new food was added and water vials changed as needed. To reduce cannibalism of smaller individuals, I moved mid- to late-instar nymphs to a separate bin. I isolated penultimate-instar nymphs in individual containers (9 cm in diameter, 8 cm high) with two pieces of food, a cotton-plugged water vial and a small piece of egg carton. Food in individual containers was changed weekly and water was changed at least bi-weekly or more frequently if needed.

Experienced females came from a large mixed-sex bin of adults (approximately 150 males and 150 females) in which individuals likely acquired at least one mating, and were individually isolated at least 24 hours prior to the experiment. Both virgin females and males were housed individually from the last nymphal instar (see above) and so had no adult experience of conspecifics.

5.3.2 Morphological Measurements

On the day following his moult to adulthood, each male was weighed using a Mettler AE 50 balance to the nearest milligram. To measure head width and pronotum length (using NIH Image 1.62), I restrained crickets on the surface of a petri dish with a small piece of plastic wrap weighed down by a plastic ring. This allowed me to position the cricket so that the frontal plane was perpendicular to the line of sight under a dissecting microscope which had a camera mounted on it to transmit live images to a computer. Focal height of the microscope was fixed for each measurement, which ensured a high degree of repeatability (>99%) for each of these measurements (K. A. Judge, unpubl. data).

5.3.3 Mating Trials

Each male (n = 94) was paired with a virgin female and an experienced female on consecutive days. I randomly assigned the order in which each male encountered the two classes of female, so that half of the males encountered the virgin on day one and the other half on day two. For each mating trial, I moved males and females to clean containers; the female's container had a paper towel circle for substrate. Approximately two minutes later, I introduced the male into the female's container. I recorded the time until the male initiated courtship (latency to court; to the nearest minute) and the time from courtship initiation until copulation (latency to copulate; to the nearest second). If a male failed to initiate courtship within 30 minutes, I separated the pair and attempted the mating trial again at the end of the day with a new female. If the male did not court the new female, he was discarded. After the male initiated courtship, I scored him as successful if the female started copulating within 20 minutes, and unsuccessful if she did not. After 20 minutes the pair was separated and each insect placed back in their original individual containers.

5.3.4 Statistical Analyses

I determined four traits for each male for inclusion in the selection analysis: adult age (days past adult moult), body size (pronotum length), residual head width and residual mass. Residual head width and residual mass were the y-axis residuals from two separate linear regressions: of head width on pronotum length and of mass on pronotum length respectively. Residual head width is sexually dimorphic in this species with males having wider heads than females for a given body size (Chapter 4). Residual mass is an index of condition and is hypothesized to represent energy reserves (Jakob et al. 1996; Schulte-Hostedde et al. 2005). I standardized each trait to a mean of zero and standard deviation of one for all selection analyses.

I calculated selection differentials, univariate linear selection gradients, univariate nonlinear selection gradients and bivariate nonlinear selection gradients (Lande and Arnold 1983) on: age, body size, residual head width and residual mass. I estimated selection coefficients for selection exerted by virgin females and experienced females separately. For both classes of female, I estimated selection exerted through both mating success (mated or not mated) and attractiveness

(attractiveness = [1/latency to copulate]; therefore shorter latency = greater attractiveness). For males that failed to successfully mate, I arbitrarily set their latency to copulate to the maximum trial duration (20 min), which gave them the lowest attractiveness score. Although gradients were estimated using ordinary least squares regression, multiple logistic regression was used to test the significance of gradients whenever the response variable was binary (selection exerted through mating success, Fairbairn and Preziosi 1996). To further examine nonlinear selection, I also conducted canonical rotation (Philips and Arnold 1989; Blows and Brooks 2003) of each of the four matrices of nonlinear selection gradients (i.e. two classes of female X two measures of relative fitness) using PopTools (Hood 2005).

To compare selection exerted by virgins to selection exerted by experienced females, I conducted a repeated measures general linear model (GLM) with Female Experience (virgin or experienced) as the within-subjects factor and the four male traits both: 1) alone (for comparisons of the linear gradients), and 2) together with the squared and crossproduct terms (for comparisons of the nonlinear gradients) as between-subjects covariates; the dependent variable was relative fitness. I interpret a significant interaction between Female Experience and any of the covariates as an indication that virgin and experienced females differ in the selection gradient that they exert on males.

To investigate the possibility that males might adjust their courtship based on the class of female they encounter, I analyzed male's latency to court using repeated measures GLM with Female Experience as the within-subjects factor and Female Order (virgin female first or experienced female first) as the between-subjects factor; the dependent variable was latency to court. I interpret a significant interaction between Female Experience and Female Order as indicating male choice behaviour, particularly if all males courted the first female equally and there was a drop in males' willingness to court the second female depending on her background.

All statistical analyses were carried out using SPSS 10 for Windows, all tests are two-tailed and carried out with an a priori Type I error rate of 5%.

5.3.5 Visualization of Selection

I used nonparametric cubic splines (Schluter 1988) to visualize univariate fitness functions. Cubic splines were estimated using the program glmsWIN 1.0 (available at: http://www.zoology.ubc.ca/~schluter/software.html). To visualize multivariate fitness surfaces, I used thin-plate splines, which were estimated using the Tsp function in the *fields* package of R (available at: http://www.r-project.org). In both univariate and multivariate fitness functions, splines were fitted that used a value of the smoothing parameter (λ) that minimized the generalized cross-validation (GCV) score. Whenever the programs failed to find λ that minimized GCV, I used the value of λ where the GCV score appeared to plateau.

5.4 Results

All four male traits were normally distributed and for the most part phenotypically uncorrelated with one another (Table 5.1). The exceptions to this were residual head width and residual mass, which were strongly positively intercorrelated ($r = 0.626$, $p < 0.001$, Table 5.1).

Seventeen of the 94 males failed to court either the virgin female (five males), the experienced female (four males), or both (eight males), and were removed from all subsequent analyses. Experienced females were significantly more likely to reject males as mates (33/77 = 42.9%) than virgin females (10/77 = 13.0%; $\chi^2 = 17.07$, $p < 0.001$).

Table 5.1: Phenotypic correlations between the four male traits in the initial sample of male field crickets.

Trait	N	Mean	SD	PL	RM	AGE
Residual Head Width (mm) (RHW)	94	0.000	0.176	0.000	0.612*	0.063
Pronotum Length (mm) (PL)	94	2.953	0.268		0.000	-0.007
Residual Mass (mm) (RM)	94	0.000	0.037			0.034
Mean Age (days) (AGE)	94	17.330	5.931			

* $p < 0.001$

<u>5.4.1 Selection on male traits exerted through mating success</u>

There was a lower opportunity for sexual selection (I = variance in relative fitness; Arnold and Wade 1984) on males exerted through mating success with virgin females compared to the experienced female treatment ($I_{\text{virgin}} = 0.151$ and $I_{\text{experienced}} = 0.760$; variance ratio test [Zar 1996]: $F_{76,76} = 5.03$, $p < 0.001$). I detected no significant net selection on male traits exerted through virgin females (s = standardized selection differential: all $|s| < 0.058$, all $p > 0.203$, Table 5.2). However experienced females exerted significant net selection on age (i.e. older males favoured over younger males; $s = 0.210$, $p = 0.034$), but not on any of the other male traits (all $|s| < 0.151$, all $p > 0.134$, Table 5.2).

Table 5.2: Standardized linear selection differentials (s), linear selection gradients (ß) and the matrix of nonlinear selection gradients (γ) exerted on four male characters by females of two different experience classes (virgin or experienced) through two measures of relative male fitness (mating success or attractiveness). Abbreviations for male characters are as follows: RHW = residual head width; PL = pronotum length; RM = residual mass; AGE = mean age. Bolded values are statistically significant at $p < 0.05$.

Fitness Measure Class of Female					γ			
Male Character	s	p	ß(SE)	p	RHW	PL	RM	AGE
Mating Success								
Virgin Females								
RHW	0.016	0.717	0.028(0.058)	0.630	0.007			
PL	0.057	0.204	0.054(0.045)	0.233	0.025	-0.026		
RM	-0.006	0.890	-0.020(0.058)	0.730	0.049	-0.037	-0.060	
AGE	-0.046	0.309	-0.046(0.045)	0.312	-0.109^{+}	**0.121**	0.071	-0.019
Experienced Females								
RHW	0.150	0.135	**0.271(0.123)**	**0.031**	-0.016			
PL	-0.096	0.339	-0.115(0.097)	0.238	0.120	0.099		
RM	-0.015	0.880	-0.188(0.124)	0.134	0.120	0.167	-0.255^{+}	
AGE	**0.210**	**0.034**	**0.206(0.096)**	**0.035**	-0.017	-0.021	0.079	0.076
Attractiveness								
Virgin Females								
RHW	0.053	0.484	0.087(0.098)	0.379	-0.102			
PL	-0.004	0.956	-0.010(0.077)	0.896	0.166	-0.015		
RM	0.001	0.988	-0.055(0.099)	0.582	**0.367**	-0.194^{+}	-0.116	
AGE	-0.008	0.916	-0.009(0.076)	0.907	-0.060	**0.237**	0.142	0.061
Experienced Females								
RHW	0.173	0.176	0.311(0.163)	0.060	-0.040			
PL	-0.035	0.784	-0.058(0.128)	0.653	0.100	0.081		
RM	-0.022	0.865	-0.216(0.164)	0.191	0.185	0.096	-0.333^{+}	
AGE	0.169	0.189	0.164(0.127)	0.199	-0.095	-0.269^{+}	-0.116	0.100

$^{+}$ $p < 0.10$

No significant linear selection by virgin females was detected (ß = standardized linear selection gradient: all |ß| < 0.055, all p > 0.232, Table 5.2). However, experienced females exerted positive linear selection on both residual head width and age (ß = 0.271, p = 0.031; and ß = 0.206, p = 0.035 respectively; Table 5.2). There was negative linear selection on body size and residual mass in the experienced female treatment, although neither of these two selection gradients was statistically significant (Table 5.2).

Virgin and experienced females differed in the linear gradients that they exerted on age (Female Experience * Age: $F_{1,72} = 6.085$, p = 0.016; Fig. 5.1), but not on residual head width although this interaction between Female Experience and residual head width was only marginally non-significant ($F_{1,72} = 3.432$, p = 0.068).

In contrast to the linear selection gradients, I detected significant nonlinear selection on males by virgin females (Table 5.2). There was significant positive correlational selection between male body size and age ($\gamma = 0.121$, p = 0.014) and negative correlational selection between residual head width and age ($\gamma = -0.109$, p = 0.080) although the latter was only significant at $p < 0.1$ (Table 5.2). One nonlinear selection gradient for the experienced females was marginally significant: the quadratic gradient for male residual mass ($\gamma = -0.255$, p = 0.054, Table 5.2). Canonical rotation of the matrices of nonlinear selection gradients (gamma) revealed one statistically significant vector of nonlinear selection exerted by virgins (Table 5.3, Fig. 5.2), and one statistically significant vector of linear selection by experienced females (Table 5.3, Fig. 5.3).

Virgin and experienced females did not differ in any of the nonlinear gradients that they exerted on males (all p > 0.160).

5.4.2 Selection on male traits exerted through attractiveness

As with the mating success data the opportunity for sexual selection exerted through attractiveness by virgin females was lower than for experienced females ($I = 0.266$ and I = 0.552 respectively). None of the selection differentials nor the linear selection gradients were statistically significant for selection by either virgin or experienced females (Table 5.2). However, virgin females exerted significant nonlinear selection. There was positive correlational selection on residual head width and residual mass ($\gamma = 0.367$, p = 0.037, Table 5.2), and positive correlational selection on body size and age ($\gamma = 0.237$, p = 0.003, Table 5.2). I detected two marginally significant nonlinear selection gradients exerted by experienced females: negative

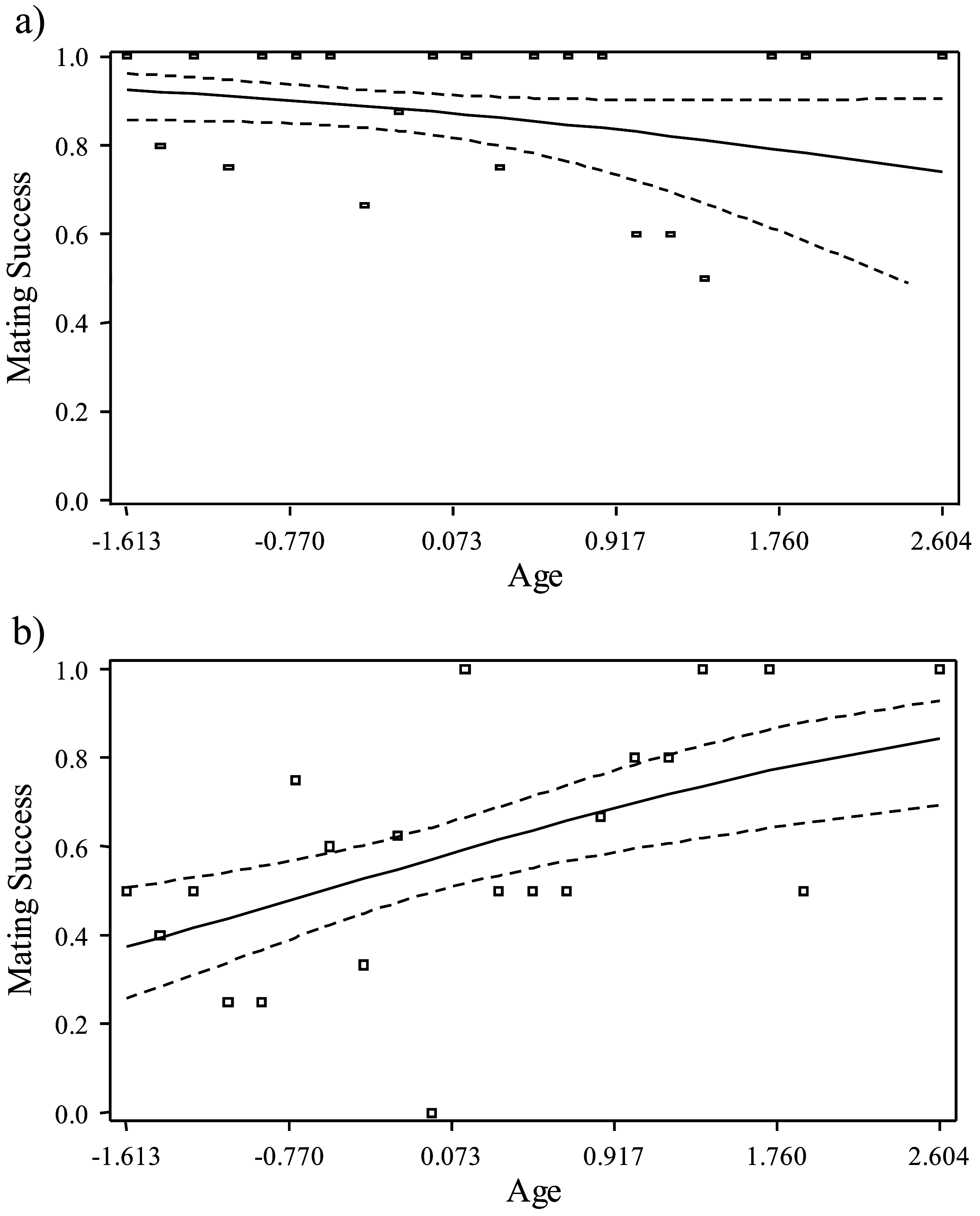

Figure 5.1: Univariate cubic splines representing selection exerted by: a) virgin females, and b) experienced females on male age. Mating success is the proportion of males who mated successfully. Dotted lines represent ± 1 SEM of the fitness prediction line, and were derived from 50 bootstrap replicates.

Table 5.3: M matrices of eigenvectors (m_i) from the canonical rotation of the γ matrices given in Table 5.2. Linear and quadratic selection gradients along each eigenvector are given by θ_i and λ_i respectively. Abbreviations for male characters are as follows: RHW = residual head width; PL = pronotum length; RM = residual mass; AGE = mean age. Values that are statistically significant at $p < 0.05$ are bolded for visual clarity.

Fitness Measure Class of Female	M				Selection			
m_i	RHW	PL	RM	AGE	θ_i	p	λ_i	p
Mating Success								
Virgin Females								
m_1	0.595	-0.390	-0.027	-0.702	0.028	0.560	**0.063**	**0.016**
m_2	0.711	0.617	0.223	0.252	0.038	0.365	0.006	0.454
m_3	0.068	-0.495	0.813	0.301	-0.055	0.281	-0.034	0.524
m_4	-0.368	0.472	0.538	-0.594	0.032	0.628	-0.133	0.124
Experienced Females								
m_1	0.407	0.881	0.237	-0.042	-0.117	0.633	0.149	0.073
m_2	-0.001	0.015	0.120	0.993	0.486	0.064	0.081	0.173
m_3	0.896	-0.436	0.081	-0.002	**0.737**	**0.020**	-0.040	0.765
m_4	-0.176	-0.183	0.961	-0.114	-0.590	0.090	-0.287	0.062
Attractiveness								
Virgin Females								
m_1	0.187	0.576	0.128	0.786	-0.004	0.963	**0.152**	**0.017**
m_2	0.628	-0.281	0.723	-0.061	0.018	0.762	0.075	0.093
m_3	0.467	0.656	-0.198	-0.559	0.050	0.565	-0.028	0.726
m_4	0.594	-0.400	-0.649	0.257	0.089	0.459	**-0.372**	**0.019**
Experienced Females								
m_1	0.270	0.648	0.163	-0.693	-0.102	0.414	**0.257**	**0.024**
m_2	0.816	0.171	0.182	0.521	**0.290**	**0.034**	-0.039	0.732
m_3	-0.443	0.740	-0.119	0.492	-0.074	0.577	-0.046	0.694
m_4	-0.255	-0.051	0.962	0.080	-0.271	0.150	-0.364	0.110

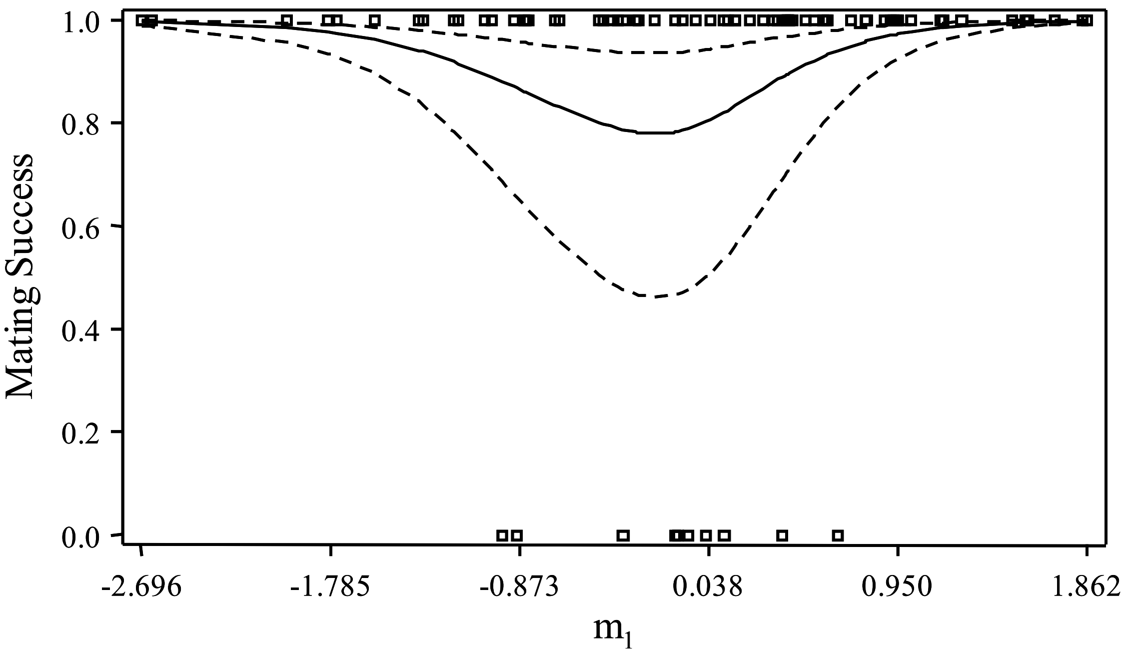

Figure 5.2: Univariate cubic spline representing selection exerted along the only statistically significant axis of nonlinear selection by virgin females when relative fitness was measured by mating success (m_1: see Table 5.3). Dotted lines represent ± 1 SEM of the fitness prediction line, and were derived from 50 bootstrap replicates.

correlational selection on the relationship between body size and age ($\gamma = -0.269$, $p = 0.051$) and negative quadratic selection on residual mass ($\gamma = -0.333$, $p = 0.059$, Table 5.2).

Virgin and experienced females did not differ in any of the linear selection gradients that they exerted on males through attractiveness (all $p > 0.276$). However, virgins and experienced females differed in the gradient for correlational selection on body size and age (Female Experience * [Pronotum Length * Mean Age]: $F_{1,62} = 9.750$, $p = 0.003$; Fig. 5.4).

Canonical rotation revealed two statistically significant vectors of nonlinear selection exerted by virgins (Table 5.3, Fig. 5.5) and one vector of linear and one vector of nonlinear selection exerted by experienced females (Table 5.3, Fig. 5.6).

5.4.3 Latency to court

There was no significant *Female Experience**Order interaction ($F_{1,75} = 0.015$ $p = 0.904$) indicating that males' latency to court either virgins or experienced females did not depend on the order in which they were encountered. However, there was a significant effect of *Female*

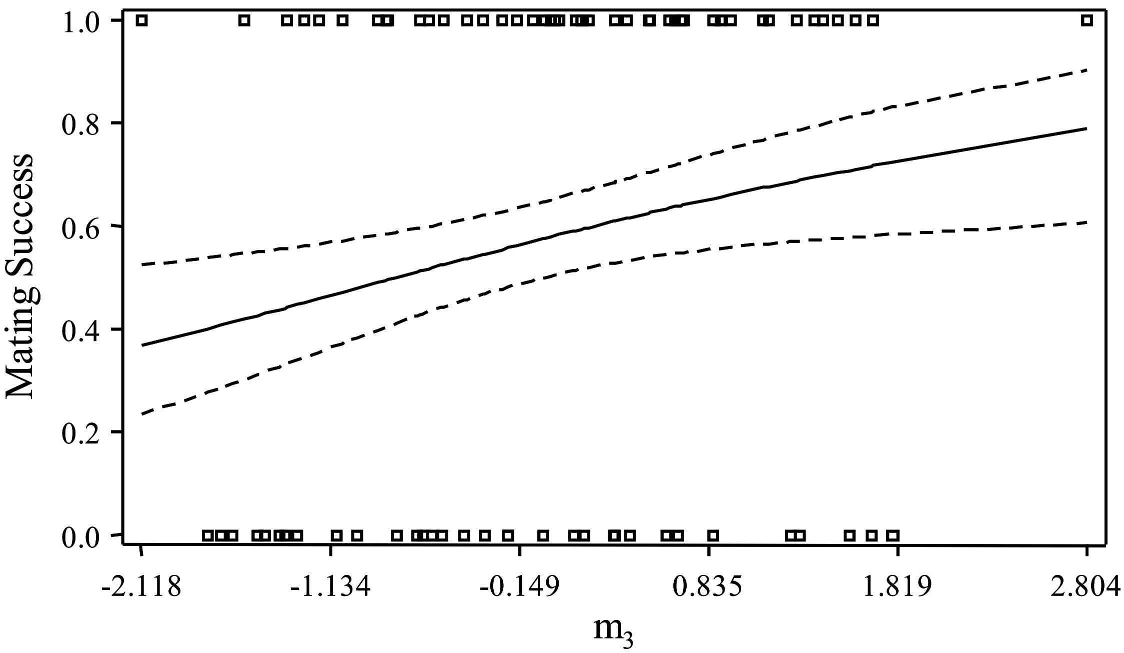

Figure 5.3: Univariate cubic spline representing selection exerted along the major axis of linear selection by experienced females when relative fitness was measured by mating success (m_3: see Table 5.3). Dotted lines represent ± 1 SEM of the fitness prediction line, and were derived from 50 bootstrap replicates.

Experience ($F_{1,75} = 6.953$, $p = 0.010$): males took significantly longer to court experienced females than virgin females (mean difference in latency to court: experienced – virgins; mean ± SE = 1.18 ± 0.45minutes).

5.5 Discussion

In general, the results of this study show that virgin female field crickets are less choosy than experienced females, and that experienced females exerted significant net selection on male age or some unmeasured trait that was correlated with male lifespan (Table 5.2). Also, I found that virgin and experienced females differed in the form of selection (i.e. linear or nonlinear) that they exerted on males: experienced females exerted mainly linear selection whereas virgin females exerted only nonlinear selection on males (Tables 5.2 and 5.3). Comparison of the linear and nonlinear selection gradients exerted by the different classes of female revealed significant differences in the direction and shape of selection exerted on males. Experienced females

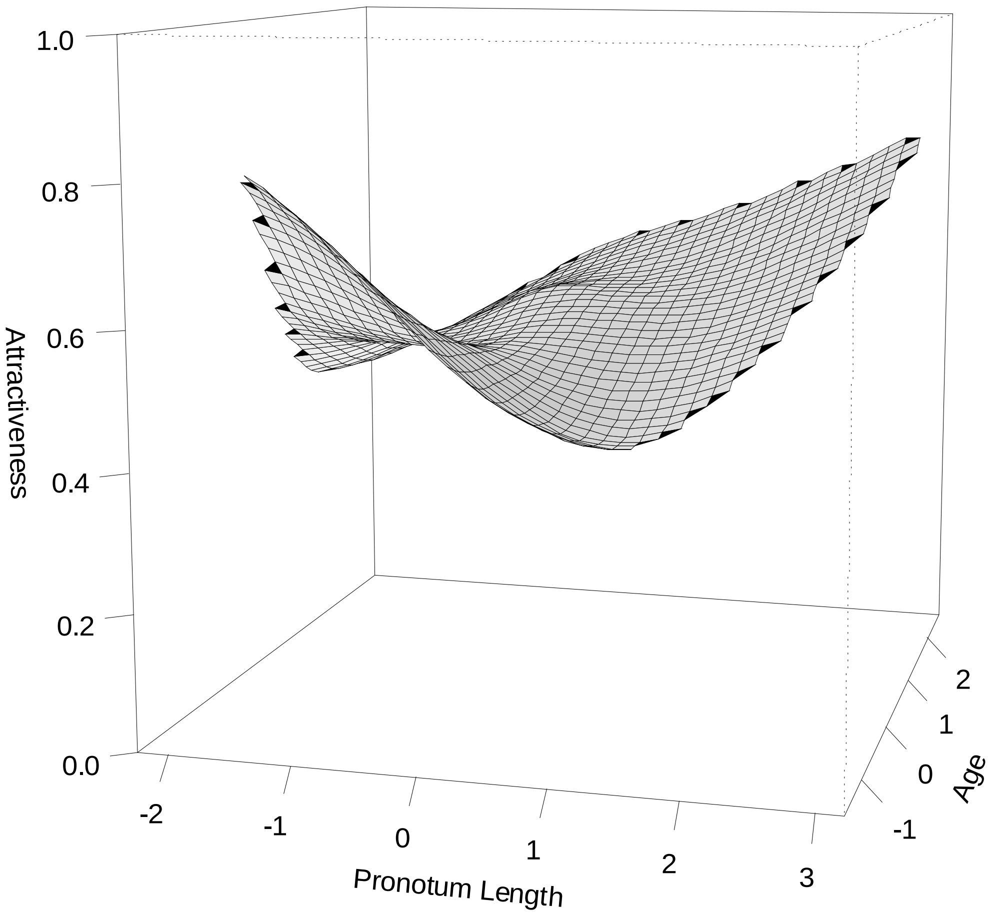

Figure 5.4a: Thin-plate splines representing selection exerted by virgin females on male age and pronotum length. Attractiveness is the inverse of latency to copulate (see text).

appeared to select males that were older (Table 5.2, Fig. 5.1b) and had relatively larger heads (Table 5.2), whereas virgin females tended to select combinations of male traits (correlational selection), for example, smaller/younger or larger/older males were favoured (Fig. 5.4a).

The results of the current study provide support for the prediction that virgin, inexperienced females should be less reluctant to mate than mated, experienced females. The intensity of sexual selection was weaker for virgin than experienced female field crickets. Furthermore, to my knowledge, this study is the first to measure the direction and shape of multivariate selection

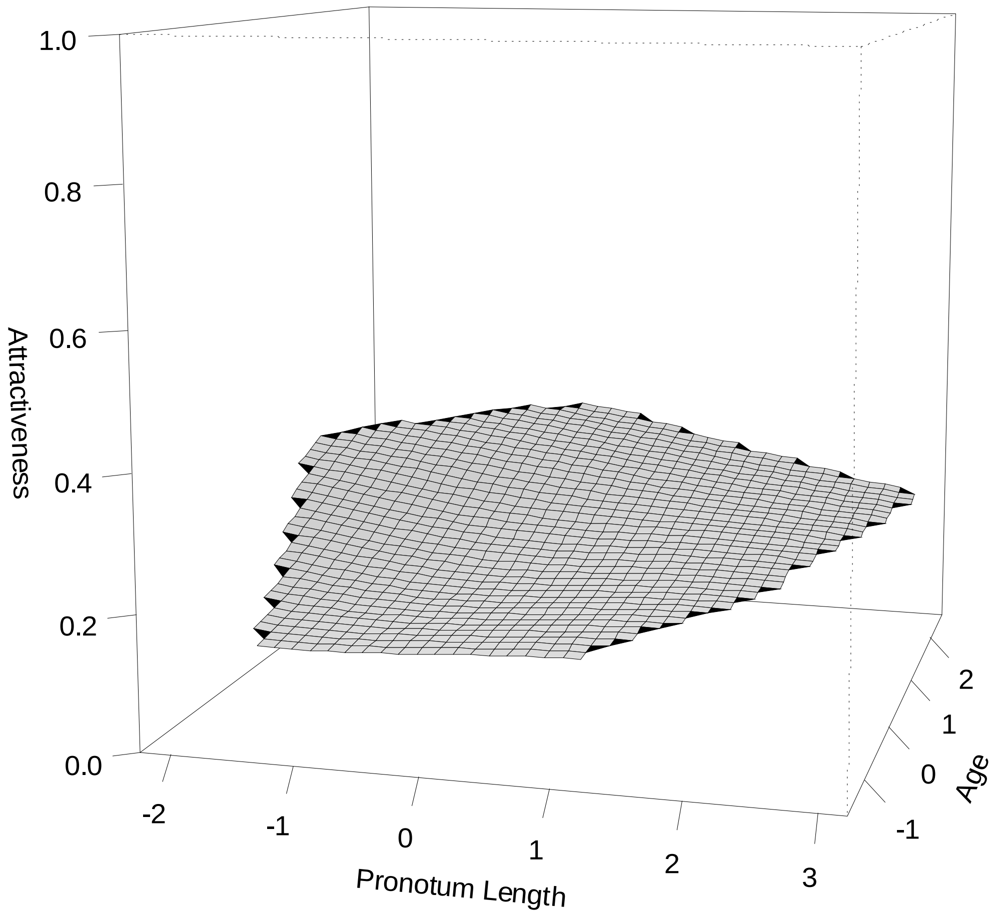

Figure 5.4b: Thin-plate splines representing selection exerted by experienced females on male age and pronotum length. Attractiveness is the inverse of latency to copulate (see text).

exerted by two classes of female differing in their mating status and/or experience of potential mates. For example, regardless of the measure of relative male fitness used, I failed to detect linear selection by virgin females along axes representing either: the original four male traits predicted to be under sexual selection (Table 5.2; Figs. 5.1a, 5.4a), or linear combinations of the four traits resulting from canonical analysis (Table 5.3; Figs. 5.2, 5.5). In contrast, selection by experienced females was primarily linear in form, regardless of whether it was measured along

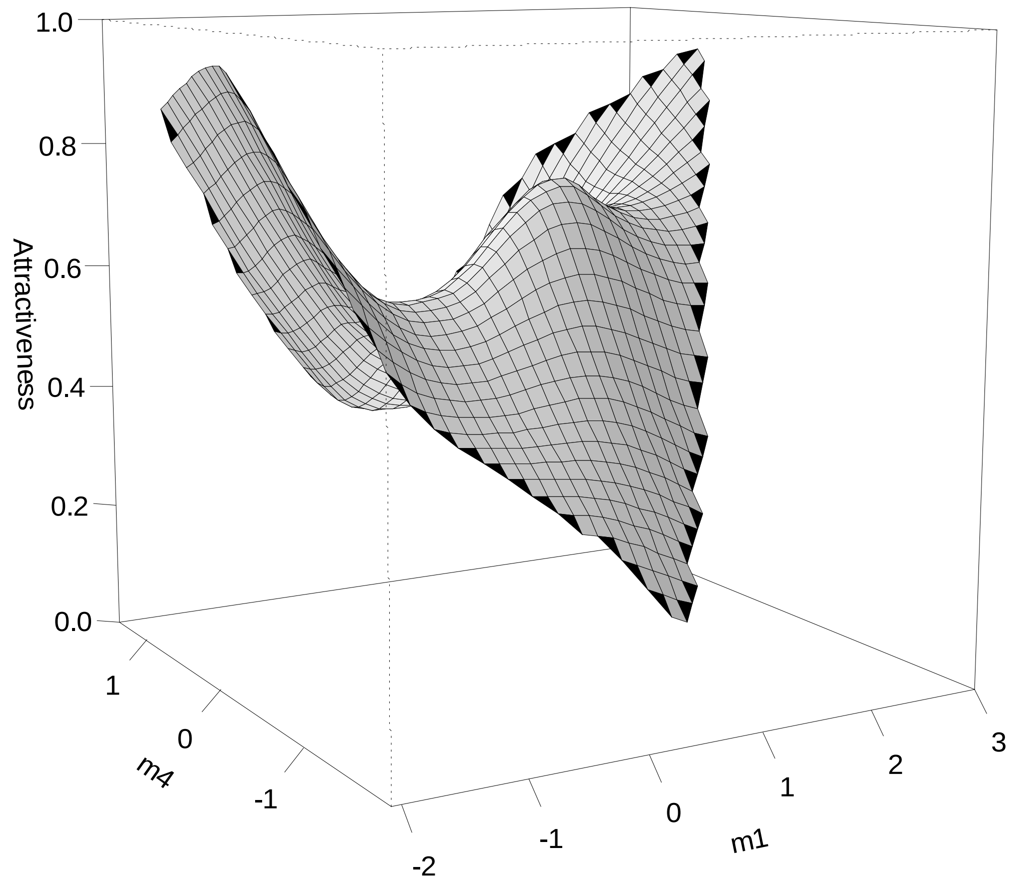

Figure 5.5: Thin-plate spline representing selection along the two major axes of nonlinear selection, m_1 and m_4, exerted by virgin females when relative fitness was measured by attractiveness (see Table 5.3).

the original male trait axes (Table 5.2; Figs. 5.1b, 5.4b) or the canonical axes (Table 5.3; Figs. 5.3, 5.6). These results are consistent with previous studies that have shown that mated, but not virgin females, exert directional selection on a male trait of interest (e.g. Gabor and Halliday 1997, Bateman et al. 2001). However, by considering both multiple male traits and selection along axes other than the original male traits, the present study broadens that result by showing that virgin females did not simply exert directional selection on alternative male traits or trait combinations.

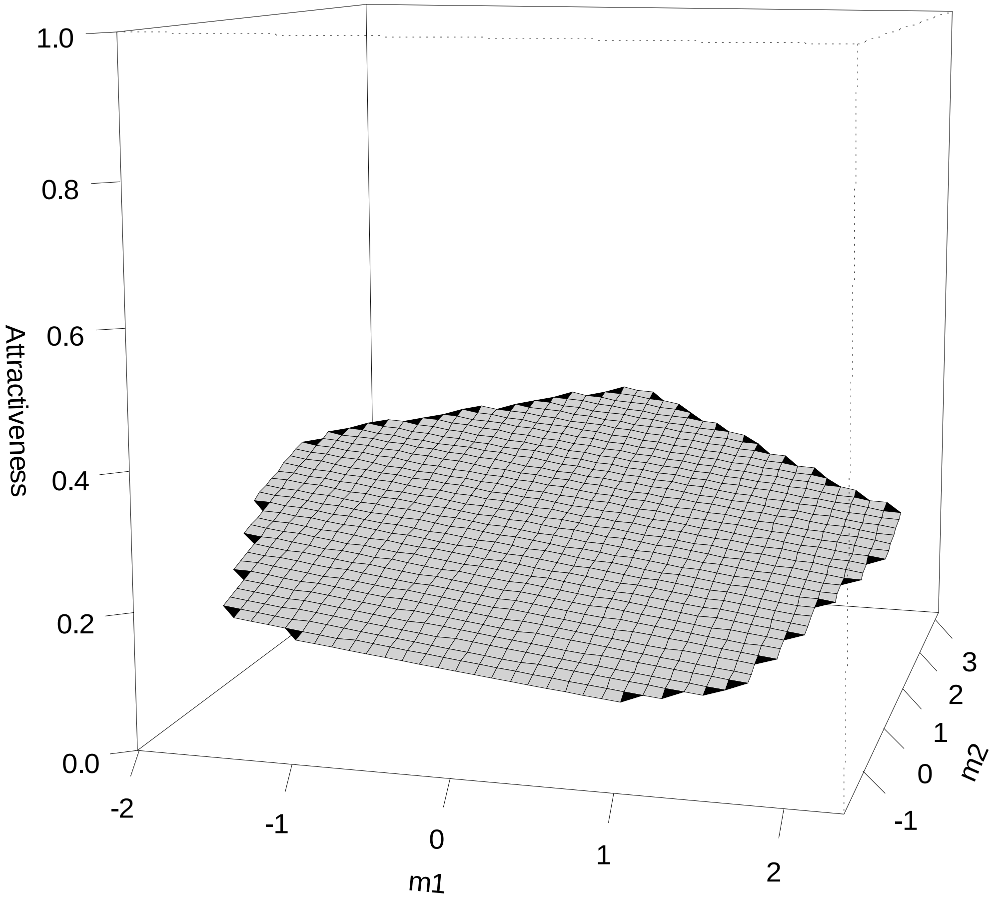

Figure 5.6: Thin-plate spline representing selection along the major axis of nonlinear selection (m_1) and linear selection (m_2) exerted by experienced females when relative fitness was measured by attractiveness (see Table 5.3).

Although other studies have compared the choosiness of different classes of females (e.g. Poulin 1994; Simmons 1994) or different populations of females (e.g. Gwynne 1984), this is only the second study to find that the shape of sexual selection differed between two classes of females. The first was Head et al. (2006) who compared the selection on males exerted by daughters of attractive fathers in *Acheta domesticus*, to that exerted by daughters of unattractive fathers. They found that only large daughters of both classes were selective, but that the large daughters of attractive males mated preferentially to the largest males, whereas the large daughters of unattractive males mated preferentially with intermediate-sized males (Head et al. 2006). The differences in selection exerted by virgin and experienced females seen in the current study (nonlinear and primarily linear, respectively) were more marked than the genetic differences detected by Head et al. (2006). Together, these two studies highlight the importance of variation in female phenotype (this study) and genotype (Head et al. 2006) to variation in selection on males.

Studies that estimate selection often use different measures of relative fitness depending on the experimental design, and few studies estimate selection using more than one measure of relative fitness (Kingsolver et al. 2001). In the current study, I measured selection on males using both mating success (mated or not) and attractiveness (inverse of latency to copulate) as measures of relative male fitness. To assess the relative consistency of estimates of selection gradients using these two measures, I conducted pairwise correlations between each category of female class/fitness measure, using the four linear, and 10 nonlinear selection gradients as data (i.e. a 4 category by 14 gradient data matrix). Although mating success and attractiveness gave slightly different estimates of selection gradients, estimates were correlated within female class. In other words, selection by experienced females as estimated by male mating success was significantly positively correlated with selection by experienced females as estimated by male attractiveness (Table 5.4). This suggests that the effect of female experience on the direction and shape of selection is not an artifact. Brooks et al. (2005) mention that their finding of multivariate stabilizing selection on calling song exerted by female *Teleogryllus commodus* field crickets was similar whether they used absolute, or time-weighted preference scores, and thus present only the results using the absolute preference scores. Although these two studies suggest that estimates of selection may not be sensitive to the measure of relative fitness that is used, this may be unsurprising given that the measures were not radically different in either case. It is still an open question as to how representative any individual measure of fitness is of total fitness, however, the similarity of the two fitness measures in the current study is an encouraging result.

Previous work on *G. pennsylvanicus* found that older males had a mating advantage over younger males (Zuk 1987c, 1988). In a field enclosure experiment, the number of lab-reared virgin females that a male attracted was significantly positively related to his age. This finding was supported when males of two age classes: old (>14 days) and young (7-9 days) were placed in an open field and allowed to attract wild females: older males attracted more wild females than younger males (Zuk 1987c). In another study, Zuk (1988) found that wild males taken paired with

Table 5.4: Matrix of Pearson correlations between selection gradients (linear and nonlinear) exerted by different classes of female (virgin and experienced) using different measures of relative male fitness (mating success and attractiveness).

Fitness Measure	Mating Success		Attractiveness
Class of Female	Virgin Females	Experienced Females	Virgin Females
Mating Success			
Experienced Females			
Linear (N = 4)	-0.162		
Nonlinear (N = 10)	0.246		
Attractiveness			
Virgin Females			
Linear (N = 4)	0.413	0.796	
Nonlinear (N = 10)	0.736^{*}	0.289	
Experienced Females			
Linear (N = 4)	0.049	0.978^{*}	0.892
Nonlinear (N = 10)	-0.058	0.848^{**}	0.192

* $p < 0.05$; ** $p < 0.01$

a female in the field, were significantly older than a wild, unpaired singing male from close by. In the present study, experienced females exerted significant net positive selection on male age, i.e. older males were more likely to mate than younger males (Table 5.2). The current study differs from Zuk's (1987c, 1988) work in a host of ways including: time period (early 1980s vs 2004), geographic location and latitude (Michigan vs Ontario) and experimental method (field choice trials vs lab no-choice trials), among many others (i.e. conceptual replication; Kelly 2006). In light of the many differences between our two studies, the common finding that male age (or some correlate thereof) is under significant positive selection in *G. pennsylvanicus* takes on added significance. There is weak evidence to suggest that male age is signaled directly through his calling song (Ciceran et al. 1994; Chapter 2), making selection on male age per se unlikely. However, recent work on the condition dependence of male lifespan and calling effort found that high condition males both lived longer and called more than low condition males (Judge et al. 2008). Thus, female selection for "older" males may in fact represent selection for males in good condition and/or males who invest more in calling effort. I was unable to measure males' calling effort in this study, so I am unable to directly evaluate this hypothesis. However, subsequent research has shown that females are attracted to greater investment in calling effort (K. A. Judge, unpubl. data).

G. pennsylvanicus is univoltine and breeds in the late summer and early fall (Alexander 1968). Given that the adult population is killed by the first severe frost, and there is substantial phenotypic variance in hatch date (Harrison 1985; K. A. Judge, pers. obs.) females are likely under strong selection to breed early. However, strong seasonality such as that found in southern

Ontario is not experienced by all gryllines in North America and perhaps not even by all populations of *G. pennsylvanicus*, as the species' range extends south into northern Georgia (Capinera et al. 2004). It would be interesting to test the hypothesis that females from different populations are more or less selective as virgins depending on the severity and unpredictability of the onset of winter (greater severity and/or unpredictability should lead to females being less selective as virgins). Variation in the level of predation risk should have the same effect on females' choosiness as virgins (Kokko and Mappes 2005).

Males initiated courtship faster with virgin females than with experienced females, but latency to court did not depend on the order in which males encountered the virgin or experienced female. The question arises whether this is male mate choice behaviour, or a consequence of differences in the behaviour of virgin and experienced females. Males of many insect species display a preference for virgin over mated females (reviewed in Bonduriansky 2001), and the relatively greater alacrity with which males of *G. pennsylvanicus* courted virgin females may represent a male preference for reduced risk of sperm competition. An interaction between Female Experience and Female Order on latency to court might indicate male choice behaviour, particularly if all males courted the first female equally and there was a drop in males' willingness to court the second female if she was an experienced female, however, this was not the case. The alternative hypothesis to male mate choice is that experienced females behaved in such a way as to inhibit males from initiating courtship. This seemed to be the case as experienced females appeared much more active while in the mating arena (K. A. Judge, pers. obs.). Further research is needed to distinguish between alternative hypotheses for the apparent trend of males to court virgin females more rapidly than experienced females.

Although I did not directly manipulate the mating history of the experienced females used in this experiment, the expected variation in these females' mating history should have introduced variation in the results and make selection differentials and gradients harder to detect (i.e. a more conservative test). Experienced females are also likely older than the virgin females, however mating experience has been shown to have a much greater effect on female choosiness in this species than female age (K. A. Judge and K.-C. Tran, unpubl. ms). In addition, experienced females may have expended more energy than virgins while living in the communal bin. But if experienced females had lower energy reserves, then one would expect them to be less choosy, which was not the case. Experienced females differed from virgins in their housing conditions and other social experience besides mating (e.g. experience of male-male interactions, male calling song, etc.). However, this experiment was designed to test for differences between two classes of female in the direction and shape of selection that they exert on multiple male traits, and not just whether mating or any one particular factor affects selection on males. Future research could identify what specific factors (e.g. mating history, experience of conspecific males) are most important in causing the differences observed in the present study.

5.6 Acknowledgements

Many thanks to Michelle den Hollander, Mark Fitzpatrick and Kim-Chi Tran for their invaluable help with no-choice trials. David Punzalan was very generous with his time in discussions about selection analyses. Mark Fitzpatrick and Clint Kelly raised important issues with statistical analyses, Luc Bussière, Mark Fitzpatrick, Darryl Gwynne, Glenn Morris, David Punzalan and Locke Rowe made helpful comments on earlier drafts of the manuscript. Special thanks to Darryl Gwynne for his constant support and encouragement to attempt to replicate earlier work on this species. This work was funded by an NSERC Postgraduate Scholarship to the author and an NSERC Discovery grant to Darryl Gwynne.

CHAPTER 6

General Discussion.

Kevin A. Judge

> *"Eighty percent of success is showing up."*
>
> \- Woody Allen

> *"All in all it's just another brick in the wall."*
>
> \- Pink Floyd

6.1 Summary of the Thesis

A negative correlation between male ornamentation and survival lies at the heart of Darwin's theory of sexual selection (Darwin 1871), but was disputed by his contemporaries (Cronin 1991; see quotes at the beginning of Chapter 1). Although validated in theoretical work by Fisher (1915, 1930), a negative correlation is not supported by the balance of correlative empirical data, where the overall pattern is a positive correlation (Jennions et al. 2001). Recent experimental studies have found both a negative (Hunt et al. 2004; D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms) and a positive (Kotiaho 2000; Pike et al. 2007) relationship between male survival and sexually selected traits. In this thesis I investigated the relationships between a male's investment in ornamentation, survival, and mating success. In drawing comparisons with other published research (particularly the grylline work by Hunt et al. 2004, 2005) I sought to explain the possible selective pressures causing either a positive or negative correlation between male ornament and survival.

I studied the fall field cricket, *Gryllus pennsylvanicus*, a species in which older males have a mating success advantage over younger males (Zuk 1987c, 1988) suggesting that male survival is positively correlated with a sexually selected trait. The design of Zuk's (1987c) experiment excluded all stimuli available to females but male song, meaning that there had to have been some characteristic of male calling song that differed between the old and young males. In Chapter 2, I found that, indeed, several calling song characters changed as males aged, and some of these characters were correlated with male age in a sample of males recorded at varying ages. This result is consistent with the hypothesis that females are attracted to older males – termed the age indicator mechanism (AIM). The AIM suggests that older males are more attractive because they have demonstrated their survivability and thus females who mate with them will pass on good genes for survival to their offspring (Trivers 1972; Halliday 1978). This hypothesis has come under general criticism because researchers realized that older males may not always be the most fit (Hansen and Price 1995; Kokko 1998). Also, the generality of a preference based on age rather than a more direct indicator of fitness has been criticized (Brooks and Kemp 2001). However, to the extent that male quality is positively linked with survival, the AIM is a plausible mechanism for mate choice since: a) age-related changes in male phenotype are widespread (e.g. plumage changes in birds; Chapter 2), and b) female phonotactic preferences are related to song parameters that differ with age (Jeffery et al. 2005; D. Chui, J. Vaughan, and K. A. Judge, unpubl. data).

An alternative hypothesis for the mating success advantage of older males is that females are attracted to high quality males who also survive well. Although recent work in field crickets (Hunt et al. 2004) and ambush bugs (D. Punzalan, M. Cooray, F. H. Rodd, and L. Rowe, unpubl. ms) suggests that male survival and sexually selected traits may be negatively correlated, the balance of correlative evidence and two other experiments suggests otherwise (meta-analysis: Jennions et al. 2001; see also Kotiaho 2000; Pike 2007). In Chapter 3, I examined the condition dependence of male song and survival. I found that high condition males both called more and lived longer than low condition males. However, male investment in survival levelled off whereas investment in calling effort increased monotonically: medium and high condition males did not differ in survival whereas high condition males called more than medium condition males.

The results of Chapter 3 suggest that there is strong directional selection on calling effort in *G. pennsylvanicus*. In addition to testing this hypothesis in Chapter 4, I also tested the condition dependence of mate choice. Mate choice is costly and there are also important benefits, so there is likely strong selection to minimize the costs and maximize the benefits of choosing a mate. I detected condition dependence in the amount of time that females spend before choosing (female choosiness) but not in their optimum mate phenotype (female preference function). Interestingly, high condition females took longer to choose than low condition females, which is suggestive either of sexual conflict over mating rate (i.e. low condition females may be less “resistant” to male calling song) or that females gain direct benefits from mating or visiting the territories of males who call more (i.e. well-fed males call more and thus greater calling effort may indicate the presence of greater food resources or will provide more spermatophores from which females can obtain material benefits).

Condition dependent traits are thought to be under directional selection. Although previous work on *G. pennsylvanicus* found that male survival is under directional selection (Zuk 1987c, 1988) the possibility of nonlinear stabilizing selection was not tested. This is important because the leveling-off of male investment in survival with increasing condition (Chapter 3) suggests that selection on male survival should be directional up to some threshold beyond which it should level off. Chapter 5 examined the shape of selection on male age exerted by both virgin (and naive) and experienced (and likely mated) females. I found that experienced but not virgin females exerted positive linear selection on male age. However, no nonlinear selection on male age by either class of females was detected. These results lead to the question as to why males' investment in survival plateaus with increasing condition. Adult maturation of males before females (protandry; e.g. Murray and Cade 1995; Carmona et al. 1999) as well as uncertainty about the onset time of killing frosts may mean that there is strong selection on males to survive until the majority of females have matured sexually, but that selection on survival weakens with the onset of cold weather. The wide geographic distribution of *G. pennsylvanicus* (Capinera et al. 2004), spanning areas with contrasting environmental conditions, make it a very useful study system in which to test the effect of climate on allocation patterns.

6.2 Implications and Future Directions

Like many scientific endeavours, this thesis has lead me in directions that I had not expected, and I'm excited by the prospects for research that it raises. The bioacoustic analysis of male song (Chapter 2) raises the possibility that a female bias for older males may represent a pleiotropic effect of selection on females to avoid heterospecific matings, since, with increasing age, male *G. pennsylvanicus* song becomes less like the calling song of the closely related *G. firmus* with which it hybridizes. The reverse might be true also: selection on females to acquire good genes from older males (AIM hypothesis) may have allowed *G. pennsylvanicus* and *G. firmus* to coexist in areas of sympatry. However, the observation of hybrids between these two species indicates that the preference for older male *G. pennsylvanicus* is an imperfect isolating mechanism. Little work has been done on the phonotactic preferences of *G. pennsylvanicus* and *G. firmus* (but see Doherty and Storz 1992; Jeffery et al. 2005). The hypothesis that a female preference for the songs of older male *G. pennsylvanicus* is a side effect of selection on females to avoid mismatings makes several predictions: 1) female *G. pennsylvanicus* should exhibit stronger preferences for “older” male song within the hybrid zone relative to outside, and 2) if age-related changes in male *G. firmus* song parallel those in *G. pennsylvanicus*, then female *G. firmus* should exhibit a preference for “younger” male songs.

Rowe and Houle (1996) hypothesized that genetic variance in sexually selected traits (and indeed any fitness enhancing trait) is maintained because they are condition-dependent, that is, their size or elaboration is related to the amount of resources (condition) that an individual can acquire during its lifetime. Most studies, including mine, simply manipulate resource acquisition and measure the response of sexual trait development (reviewed in Cotton et al. 2004). Few studies control for potential flexibility in resource allocation, and as such cannot rule out phenotypically plastic changes in allocation decisions. Recent work suggests that there is substantial genetic variance in the allocation of resources to survival and calling effort in male *Teleogryllus commodus* field crickets. In an artificial selection experiment, Hunt et al. (2006) found that when high male survival is selected, calling effort declines, and vice versa. The production of calling song can be both induced and inhibited pharmacologically (Wenzel et al. 1998), thus allowing experimenters the flexibility to control allocation to calling. This raises the intriguing possibility of manipulating both resource acquisition and allocation in a fully factorial manner, thus enabling researchers to assess the relative importance of resource acquisition and allocation to male fitness.

Hunt et al. (2005) pointed out that when males provide a nutritious nuptial gift to females during mating, low condition females tend to mate more readily than high condition females. Low condition female *G. pennsylvanicus* spend less time to choose preferred male song (Chapter 4), however, males of this species are not known to provide nutritional benefits to females. Although direct benefits through male ejaculates are known to accrue to females of other *Gryllus* species (Simmons 1988; Wagner et al. 2001) the benefits of female mate choice are often thought to be genetic in nature (Tregenza and Wedell 1998, 2002; Head et al. 2005). The results of Chapter 4 raise the possibility that either direct benefits or direct costs are important to female *G. pennsylvanicus* when they choose their mates. Given that males sing more when they are well fed (Chapter 3), males with greater singing effort may have more food resources in their territories. Thus, females may use high effort song to guide them to food resources. If this is true then low condition females may display phonotaxis but may not mate more readily than high condition females when subsequently presented with a mate. On the other hand, low condition females may both walk toward male song and mate more readily if: a) they are mating to acquire direct benefits through male ejaculates, or b) they are less able to resist coercive male mating signals. In the future, I intend to investigate these possibilities. In particular, given that *G. pennsylvanicus* is omnivorous (Criddle 1925), areas close to males calling at a high rate are predicted to contain more insect larvae (e.g. Monteith 1971) or plant seeds (e.g. O'Rourke et al. 2006) or both. Also, there may be substantial predation costs for females moving towards singing males (e.g. Sakaluk and Belwood 1984). If there is sexual conflict over mating rate, and female resistance to male calling song is condition-dependent as suggested by Chapter 4, then low condition females are predicted to expose themselves to a higher risk of predation than high condition females.

Another direct benefit of mate choice may be to find good oviposition substrate as has been suggested for another orthopteran, the southern mole cricket, *Scapteriscus acletus* (Forrest 1980). From laboratory studies we know that moisture is an important feature for female oviposition and egg survival (Rakshpal 1962; Harris and Svec 1964). Thus, males may defend areas of optimal moisture content (Forrest 1980). This could be assessed indirectly by measuring the moisture content of the soil in territories of singing males and comparing high calling effort to low calling effort male territories as well as to nearby areas without singing males. Interestingly, female choice of good oviposition sites may explain the mating success advantage of older males if older and presumably more experienced males are better able to detect optimal sites for egg laying (perhaps through detecting soil drying rate or variability in soil moisture content).

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APPENDIX A

Male weaponry in a fighting cricket.

Kevin A. Judge and Vanessa L. Bonanno

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A.1 Abstract

Sexually selected male weaponry is widespread in nature. Despite being model systems for the scientific study of male aggression and for cricket fights in Chinese culture, field crickets (Orthoptera, Gryllidae, Gryllinae) are not known to possess sexually dimorphic weaponry. In wild populations of the fall field cricket, *Gryllus pennsylvanicus*, we report sexual dimorphism in the span between the maxillae – mouthparts used in grappling when aggressive contests between males escalate. Male *G. pennsylvanicus* have greater maxillae spans than females when controlling for pronotum length. We conducted two experiments to test the hypothesis that a relatively greater maxillae span conveys an advantage to males in aggressive contests. Pairs of males were selected for asymmetry in maxillae span. In the first experiment, males were closely matched for body size (pronotum length), and in the second, they were matched for body mass. Males with proportionately greater maxillae spans won more fights and increasing maxillae span asymmetry increased the fighting success of the male with the greater maxillae span. However, contest duration was not related to either winner or loser maxillae span. These results are the first evidence that male maxillae function as weapons and are under selection via male-male competition.

A.2 Introduction

Darwin (1871) hypothesized that male-male competition should favour the evolution of male weaponry, the greater relative size or development of which would give an individual male an advantage in aggressive contests with other males. Male weaponry, and its role in determining male mating success, has been studied in a variety of species, from male antlers in red deer, *Cervus elaphus* (Lincoln 1972; Kruuk et al. 2002), to the greatly-enlarged male mandibles and maxillae in a group of ensiferan orthopteran insects, tree weta, *Hemideina* spp. (Maskell 1927; Field and Sandlant 1983; Gwynne and Jamieson 1998; Kelly 2006). Field crickets (Orthoptera, Gryllidae, Gryllinae) are model organisms for the study of male-male aggression (e.g. Alexander 1961; Dixon and Cade 1986; Hack 1997; Hofmann and Schildberger 2001; Shackleton et al. 2005; Briffa 2008). However, aside from the sexual dimorphism in specialized sound producing structures on the forewings, used by males in part for an aggressive function (Alexander 1961), few studies have reported any sexually dimorphic morphological trait that might influence fight outcome (but see below).

The long Chinese tradition of fighting grylline crickets, and betting on the participants (Chia 1260; Laufer 1927; Hsu 1928-29; Arlington 1929; Hofmann 1996; Jin and Yen 1998; Suga 2006) has resulted in an anecdotal list of traits thought to be possessed by superior fighters (Chia 1260; Hsu 1928-29; Laufer 1927). An early work on cricket fighting by Chia Szu-Tao in the thirteenth century states that the best fighters have large heads (Chia 1260; transl. I. S. Chan). More recently, Berthold Laufer, the noted American sinologist of the early twentieth century (Latourette 1936), wrote, “The good fighters, according to Chinese experts, are recognized by their loud chirping, their *big heads and necks*…” (p. 18, Laufer 1927, emphasis added; see also Hsu 1928-29). Even today, males with big heads are valued as good fighters (Eckholm 2000; Schiller 2007).

In nature, male field crickets defend burrows from which they call to attract females (Loher and Dambach 1989). Males are extremely aggressive towards each other (Darwin 1871; Alexander 1961), and vigorously defend their territories from intruding males (Loher and Dambach 1989). Contests between males proceed through a highly stereotyped series of aggressive behaviours, with the most escalated contests ending with males grappling with their mouthparts (Alexander 1961). Female field crickets can also be aggressive, however they rarely grapple with their mouthparts (Adamo and Hoy 1995). Thus, male-male competition may select for larger heads and mouthparts in male gryllines.

Alexander (1957) noted that the head capsules of both *Gryllus pennsylvanicus* (then *Acheta pennsylvanica*) and *G. firmus* (*A. firma*) were wider than the pronotum, especially in males.

Walker et al. (2008) showed that for *Acheta domesticus*, a commercially-raised species, males had larger heads (and presumably also larger mouthparts) than females. However, mandible size was not related to a male's ability to win in an aggressive contest (Briffa 2008). In other ensiferan Orthoptera – the weta (Anostostomatidae) – the size of enlarged mandibles (e.g. Field and Sandlant 1983; Gwynne and Jamieson 1998; Kelly 2005) and mandiblular tusks (e.g. Bateman 2000) have been implicated in male fighting success (Bateman 2000; Kelly 2006). Male field crickets use both their mandibles and their maxillae in aggressive contests (Rillich et al. 2007; K. A. Judge, pers. obs.). Although researchers consistently refer only to the mandibles when describing mouthparts used in cricket fights (e.g. Alexander 1961; Adamo and Hoy 1995; Hofmann and Schildberger 2001; Briffa 2008; but see Rillich et al. 2007), the paired maxillae of field crickets terminate in scimitar-like (pointed and sclerotized) laciniae (see Fig. A.1; also Gangwere 1965) and males must open their maxillae in addition to their mandibles in order to grapple (K. A. Judge, pers. obs.). Because the maxillae are posterior and somewhat lateral to the mandibles, the maximum gape of a male field cricket is, in part determined by the distance between his maxillae.

In this paper we describe sexual dimorphism in the transverse distance between maxillae (hereafter maxillae span, Fig. A.1) in the fall field cricket, *G. pennsylvanicus*. We then test the hypothesis that maxillae span is important to male fighting success and predict that: 1) males with greater maxillae spans, relative to body size, will win more fights than males with narrower maxillae spans, and 2) an increase in the difference in proportional maxillae span between males will increase the likelihood that the male with the greater maxillae span will win. We also tested whether maxillae span affected the duration of fights.

A.3 Methods

A.3.1 Study Species

G. pennsylvanicus is a univoltine, egg-diapausing field cricket widespread across much of eastern North America (Alexander 1968; Capinera et al. 2004). To analyse sexual dimorphism, we collected individuals from the grounds of the University of Toronto at Mississauga (43°32'50.51"N, 79°39'37.80"W) from 13 August to 21 September 2003. All animals used in the aggressive contests were third generation offspring of adult *G. pennsylvanicus* caught from the same location during August and September of 2002.

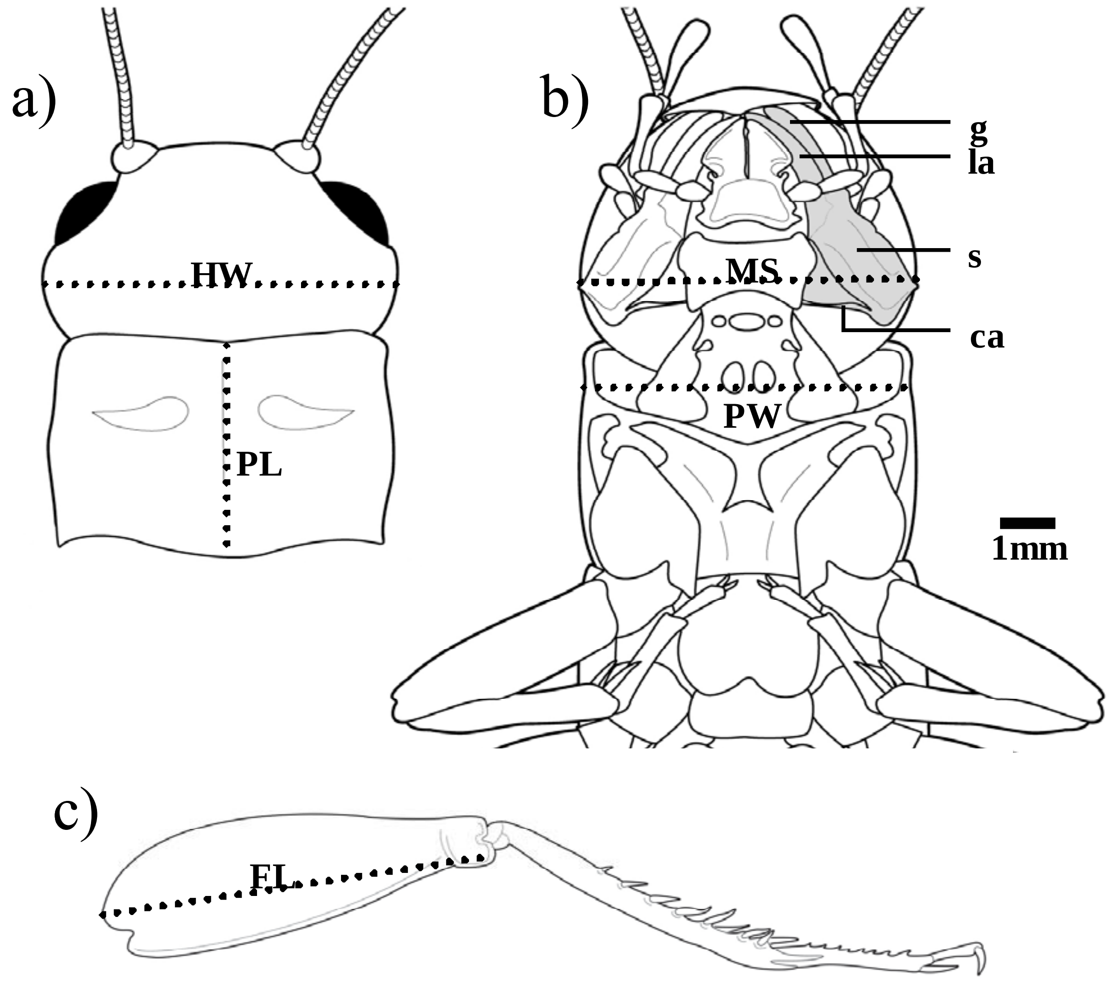

Figure A.1: Drawings of a male cricket showing the: a) dorsal view of the head capsule and pronotum, b) ventral view of the head capsule and pronotum (head has been tilted dorsally to expose ventral mouthparts), and c) left hind leg. Morphological measurements are shown as dotted lines and abbreviations are as follows: HW = head width, PL = pronotum length, MS = maxillae span, and PW = pronotum width, and FL = femur length. The left maxilla, excluding the palp, is shaded grey and labeled with abbreviations indicating the various parts: la = lacinia, g = galea, s = stipes, and ca = cardo (after Gangwere 1965). Drawings by Janice J. Ting.

A.3.2 Animal Rearing

Juvenile crickets were kept in large plastic containers (48 cm long, 35 cm wide, 31 cm high) at 25˚C, 70% relative humidity and a light cycle of 12 hr light: 12 hr dark. All crickets were fed Purina® Cat Chow (ground pellets for the first two to three weeks of life and whole pellets later on) and provided with water in cotton-plugged vials. We added new food every three to four days and changed water vials as needed. Layers of egg cartons provided shelter. To reduce cannibalism of smaller individuals, we moved larger nymphs to a separate bin. We isolated penultimate-instar nymphs in individual containers (9 cm diameter, 8 cm high) with two pieces of food, a cotton-plugged water vial and a small piece of egg carton. Food in individual containers was changed weekly and water was changed at least bi-weekly or more frequently if needed. Every day we checked individually housed nymphs for newly moulted adults. This allowed us to assign ages that were accurate to within one day, to all individuals.

A.3.3 Sexual Dimorphism in Morphology

We collected 151 males and 75 females in 2003 and euthanized them by freezing. These individuals were stored frozen until we measured: left femur length, right femur length, pronotum length, pronotum width (spanning the ventral margins across the neck membrane and cervical sclerites), head width, and maxillae span (the transverse distance between the cardo-stipes articulation of the right and left maxillae viewed ventrally) (Fig. A.1). Measurements were taken using NIH Image 1.62 on images captured from a camera mounted dissecting microscope. The focal height of the microscope was fixed for each measurement, which ensured a high degree of repeatability (> 99%) for each (K. A. Judge, unpubl. data).

A.3.4 Experiment 1: Aggressive Contests Controlling for Pronotum Length

To test whether relative maxillae span has an effect on the outcome of male-male aggressive contests, we matched pairs of males for age and body size (pronotum length), but differed in maxillae span, and staged a series of male-male contests. Although we could not measure maxillae span on live male *G. pennsylvanicus*, head width (which we could measure on live males) positively correlates with maxillae span (see Results). Therefore, we used head width as a proxy for maxillae span.

One to two days following the adult moult, we measured each male’s head width and pronotum length. We did this by first restraining them on the surface of a petri dish under a small piece of plastic wrap weighed down by a plastic ring. This allowed us to position the cricket so that the frontal plane was perpendicular to the line of sight under a dissecting microscope. As above, all measurements were taken using NIH Image 1.62. Subsequent measurement of each individual following preservation confirmed that pairs of males that were different in head width were also different in maxillae span (see Results).

Within each pair of males, individuals were randomly assigned one of two identification colours, and a small dot of nail polish of the appropriate colour was applied to the pronotum 24 hours before the aggressive contest (to allow time to recover from handling). All males were weighed to the nearest 0.1 milligram using a Mettler AE 50 balance approximately one hour before the contests were conducted.

The contest arena was a square glass box with an open top (12.5 cm long, 12.5 cm wide, 18.0 cm high). Coarse sand covered the bottom, and brown paper covered the outside surface to minimize visual disturbances. A removable opaque plastic wall divided the box into two equal triangles. Before each trial, the interior walls and divider were rubbed with an ethanol-soaked cotton ball, and the sand base was shaken and tossed to minimize and disperse any pheromonal cues left by the previous contest. We recorded all trials from directly above the arena with a SONY® digital video camera (model # DCR-TRV740). For each contest, we introduced each male of a pair into opposite sides of the box, and after a two-minute acclimatization period, we started the video recording and withdrew the divider. Recording was halted after 10 min and the males were returned to their individual containers. Males were weighed after the contest, euthanized by freezing and then placed in 95% ethanol for subsequent measurement of maxillae span.

A.3.5 Experiment 2: Aggressive Contests Controlling for Body Mass

As body mass differences are known to affect the outcome of aggressive contests in field crickets (e.g. Hack 1997) and were not experimentally controlled in experiment 1, we conducted a second experiment in which males were matched for body mass instead of pronotum length. All males were handled and marked as in experiment 1, except that we weighed males several hours before the aggressive contests and then matched pairs of males for similar body mass but different head width.

A.3.6 Video Analysis

We transferred the video of each contest to DVD on a Macintosh G5 using the software iMovie® (Apple Computer, Inc.). For each trial we recorded the duration of the aggressive contest – from first contact until the contest ended (one male retreated from its opponent following a break in the aggressive contest) – and the contest victor, determined as the individual that tremulated, or stridulated and chased its opponent. In most cases, the identity of contest winners is determined with little difficulty (e.g. Shackleton et al. 2005).

Although we did not explicitly design the video analysis to be truly blind to differences in head width, in practice, the experimenter watching the video was unaware of this information.

A.3.7 Statistical Analysis

We tested whether male and female *G. pennsylvanicus* could be distinguished on the basis of morphology by conducting a discriminant function analysis using mean femur length, pronotum length, pronotum width, head width and maxillae span as predictors of membership in either sex.

We tested the prediction that males with greater maxillae spans would win contests more often than males with narrower maxillae spans by using a binomial test (Zar 1996) to compare the proportion of fights won by males with greater maxillae. To test for the effect of the degree of maxillae span asymmetry on contest outcome, we first randomly selected one focal male from each pair and then conducted a logistic regression with contest outcome (focal male won = 1, focal male lost = 0) as the dependent variable, and maxillae span asymmetry (maxillae span asymmetry = $\ln(ms_{focal}/ms_{rival})$, see Enquist and Leimar 1983) as the independent variable. We also conducted a linear regression to test for an effect of the maxillae span on contest duration, with both winner and loser maxillae span as independent variables (following Taylor and Elwood 2003).

Statistical tests were carried out at alpha = 0.05 using SPSS 10 (SPSS Inc.). Because there is a clear directional prediction that males with greater maxillae spans are better fighters, all tests involving maxillae span were one-tailed.

A.4 Results

A.4.1 Sexual Dimorphism in Morphology

Discriminant function analysis resulted in a linear combination of the five morphological traits (i.e. the discriminant function) that accurately distinguished wild-caught male and female *G. pennsylvanicus* 100% of the time (Wilks' Lambda = 0.071, $\chi^2 = 584.437$, df = 5, $p < 0.001$). Maxillae span and pronotum length loaded most heavily on the discriminant function (Table A.1); males had greater maxillae spans (mean ± SE = 5.68 ± 0.05 mm) but smaller pronotum lengths

Table A.1: Summary of sexual differences in five homologous morphological measurements in *G. pennsylvanicus*. Loadings give the correlations between each measurement and the discriminant function separating males and females.

	Males				Females					
Measurement	N	Mean (SE)	Range	CV (%)	N	Mean (SE)	Range	CV (%)	$t^{\dagger}$	Loading
Maxillae Span (mm)	151	5.68 (0.05)	4.16-7.03	9.82	75	4.68 (0.03)	4.07-5.46	5.83	18.044^{***}	0.271
Head Width (mm)	151	5.71 (0.04)	4.43-6.95	8.75	75	5.38 (0.03)	4.91-6.21	5.07	6.403^{***}	0.099
Pronotum Width (mm)	151	5.41 (0.04)	4.25-6.42	8.30	75	5.30 (0.04)	4.65-6.17	5.76	2.069^{*}	0.034
Pronotum Length (mm)	151	3.48 (0.02)	2.83-4.20	8.22	75	3.84 (0.02)	3.42-4.42	5.52	10.570^{***}	-0.177
Mean Femur Length (mm)	151	10.45 (0.06)	8.47-12.83	6.96	75	10.97 (0.07)	10.02-13.13	5.52	5.692^{***}	-0.099

† All differences (except for mean femur length) were tested using Student's t-tests for unequal variances after Levene's tests for equality of variances detected significant heteroscedasticity.

* $p < 0.05$; *** $p < 0.001$

(3.48 ± 0.02 mm) than females (maxillae span: 4.68 ± 0.02 mm; pronotum length: 3.84 ± 0.02 mm) (Table A.1, Fig. A.2).

A.4.2 Experiment 1: Aggressive Contests Controlling for Pronotum Length

Differences in head width were highly correlated with post-hoc measurements of maxillae span differences (Pearson $r = 0.856$, $N = 52$, $p < 0.001$). We could unambiguously determine a winner within the ten-minute time limit in 47 contests. In five contests without a clear winner, there was no overt aggression and the males either courted each other or failed to make contact.

Males with greater maxillae spans won significantly more aggressive contests (31 of 47 = 66.0%, one-tailed binomial $p = 0.020$; Fig. A.3). And as the asymmetry in maxillae span increased (i.e. focal male became bigger), so too did the likelihood that the focal male would win the aggressive contest ($\chi^2 = 4.208$, df = 1, $N = 47$, one-tailed $p = 0.020$, Nagelkerke $R^2 = 0.114$; Fig. A.4a).

Inclusion of age, pronotum length and body mass asymmetries (see Table A.2; asymmetry = $\ln[\text{value}_{\text{focal}}/\text{value}_{\text{rival}}]$) in the above logistic regression did not greatly affect its significance ($\chi^2 = 8.802$, df = 4, $N = 47$, two-tailed $p = 0.066$, Nagelkerke $R^2 = 0.228$). Asymmetry in maxillae span was still a significant predictor of fight outcome ($B = 10.898$, $SE = 5.775$, Wald = 3.561, one-tailed $p = 0.030$), and age asymmetry was marginally insignificant ($B = 3.290$, $SE = 1.836$, Wald = 3.213, $p = 0.073$) with increasing asymmetry in both variables increasing the focal male's likelihood of winning (odds $\text{ratio}_{\text{maxillae span asymmetry}} = 54075.372$; odds $\text{ratio}_{\text{age asymmetry}} = 26.841$).

We included maxillae span, age, pronotum length and mass for both winners and losers as predictors in a multiple regression to test for the effect of asymmetries in these variables on contest duration (Taylor and Elwood 2003). Contest duration was not normally distributed (Kolmogorov-Smirnov test statistic = 0.185, $p < 0.001$), however, a square-root transformation restored normality (K-S test statistic = 0.104, $p > 0.200$). Multiple regression failed to detect a significant effect of any of the predictors on contest duration ($F_{8,38} = 1.642$, $p = 0.145$, adj.-$R^2 = 0.100$).

A.4.3 Experiment 2: Aggressive Contests Controlling for Body Mass

As in experiment 1, head width differences were highly correlated with differences in

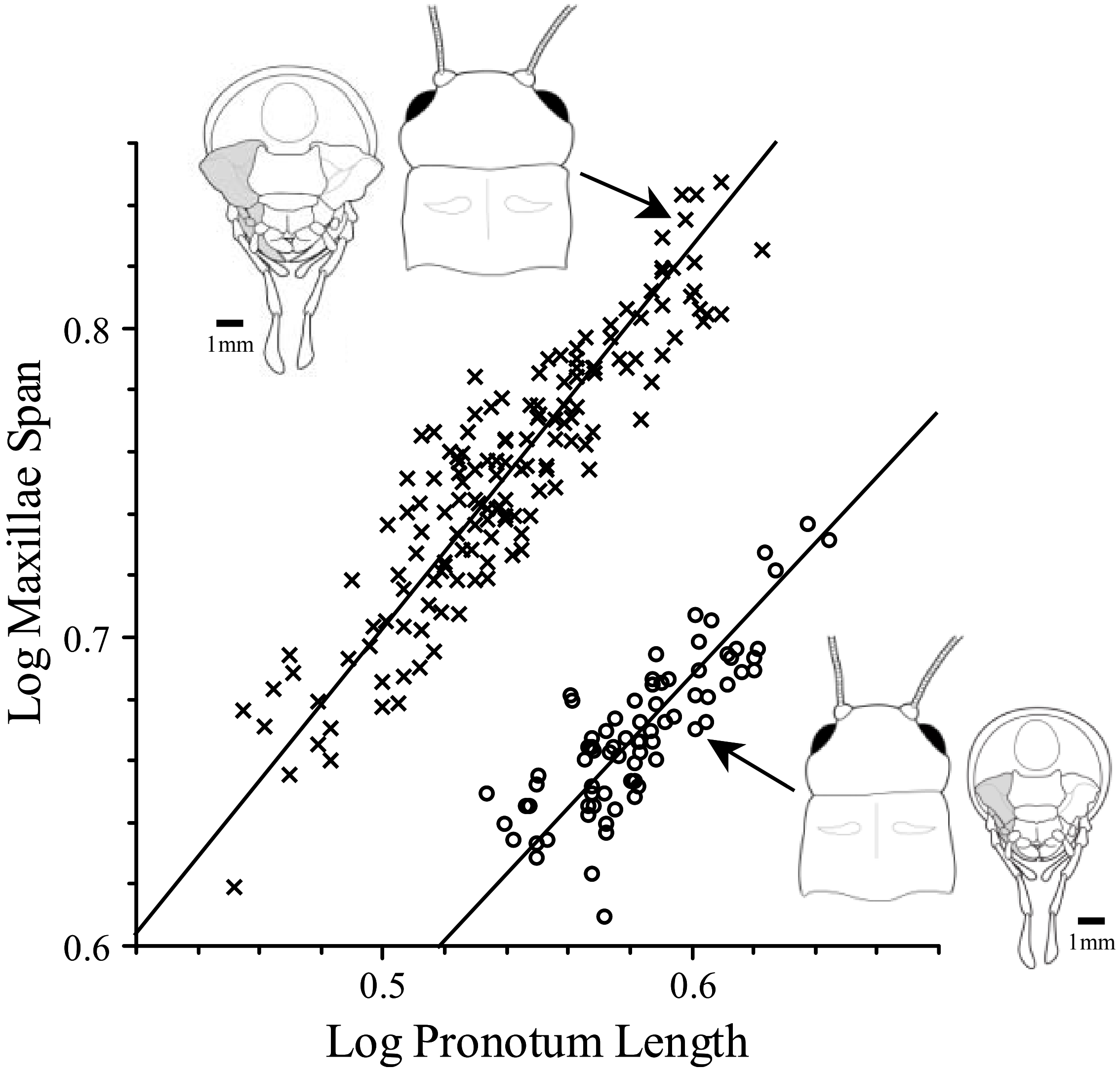

Figure A.2: Scatterplot of log maxillae span versus log pronotum length showing the sexual dimorphism in maxillae span in a sample of 151 males (X) and 75 females (O). Lines are major axis regressions (see Table A.3 for slopes). Drawings are of a representative male (upper left) and female (lower right) showing the dorsal view as in Fig. A.1 as well as the posterior view of the ventral surface of the head (left maxilla is shaded grey as in Fig. A.1). Arrows point to the individual whose measurements are depicted. Drawings by Janice J. Ting.

maxillae span (Pearson r = 0.859, N = 43, $p < 0.001$). There was a clear fight winner in 40 contests (as in experiment 1, three contests with mutual male courtship were excluded). Males with greater maxillae spans won 62.5% (25/40; Fig. A.3) of all aggressive contests, although this was marginally statistically insignificant (one-tailed binomial p = 0.077). Increasing asymmetry in maxillae span significantly increased the focal male's likelihood of winning an

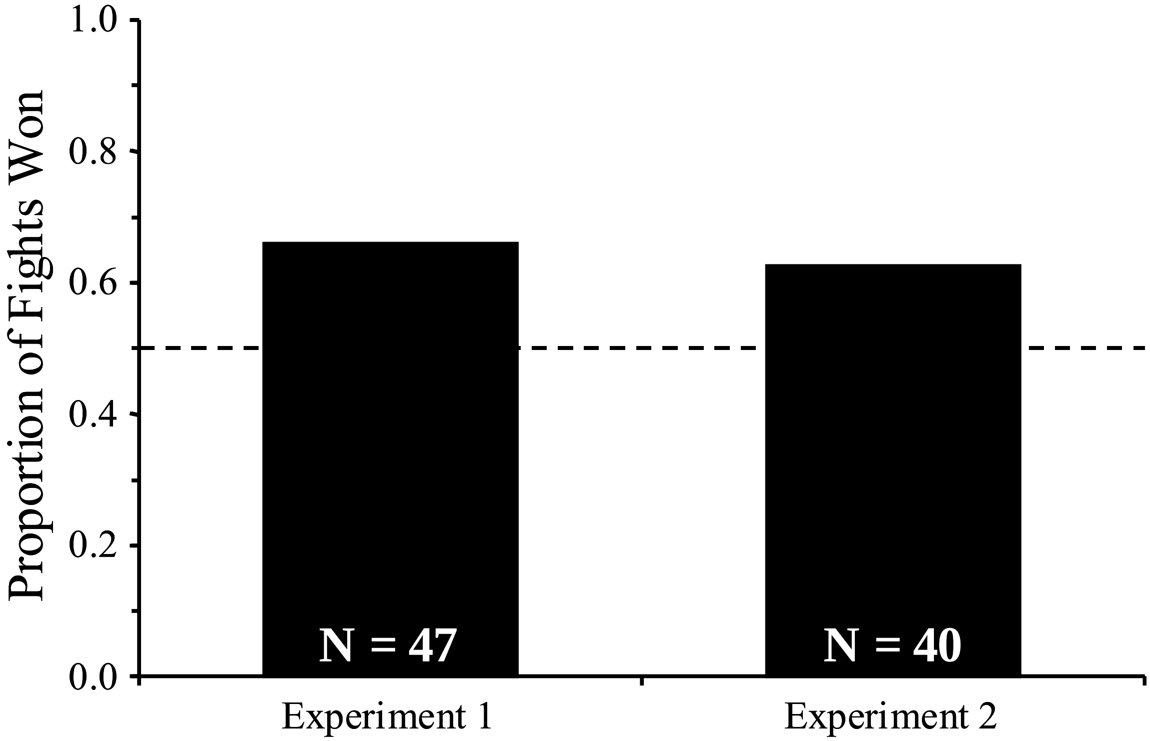

Figure A.3: Proportion of fights won by the male with the greater maxillae span in experiment 1 (pronotum length controlled) and experiment 2 (body mass controlled). The dashed line represents the null hypothesis of no advantage conferred by having a greater maxillae span.

aggressive contest over his opponent as the only predictor in a logistic regression (χ^2 = 3.528, df = 1, N = 40, one-tailed p = 0.030, Nagelkerke R^2 = 0.113; Fig. A.4b), but not when asymmetries in age, pronotum length and body mass were included (χ^2 = 5.200, df = 4, N = 40, two-tailed p = 0.267, Nagelkerke R^2 = 0.163).

Contest duration was again not normally distributed (K-S test statistic = 0.139, p = 0.049), however, a square-root transformation restored normality (K-S test statistic = 0.074, p > 0.200). The magnitude of asymmetry in the four predictors did not significantly affect contest duration ($F_{8,31}$ = 0.866, p = 0.555, adj.-R^2 = 0.028).

A.5 Discussion

Our study is the first to provide evidence that the sexual dimorphism in weaponry found in grylline crickets (*A. domesticus*: Walker et al. 2008; *G. pennsylvanicus*: this study) is likely due to male-male competition selecting for larger weaponry. When controlling experimentally for body

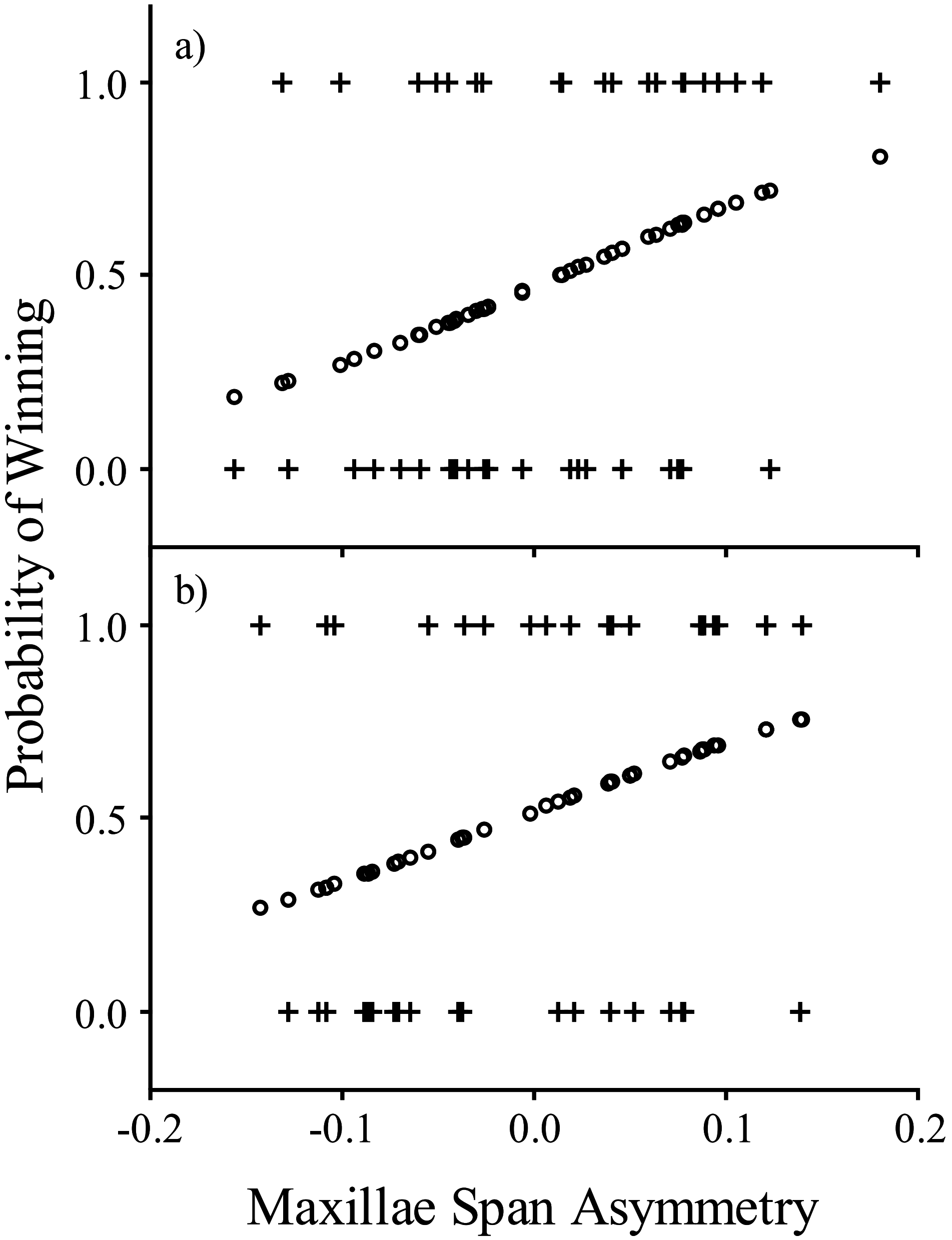

Figure A.4: Probability that the focal male would win a fight versus the asymmetry in maxillae span between the focal male and his rival for: a) experiment 1, and b) experiment 2. Positive values of maxillae span asymmetry mean that the focal male had a greater maxillae span than his rival, whereas negative values indicate that the focal male had a narrower maxillae span than his rival. Observed values are represented by pluses (+) and predicted values from the logistic equations are represented by circles (O).

Table A.2: Differences (larger – smaller) and correlations within pairs of males for four variables in each of the aggression experiments. Standard errors of the mean followed by minimum and maximum values are given in parentheses. All correlations are significant at $p < 0.001$.

	Experiment 1		Experiment 2	
Variable	Difference	Correlation	Difference	Correlation
Maxillae Span (mm)	0.28 (0.02; 0.03-0.79)	0.756	0.32 (0.03; 0.01-0.64)	0.671
Age (days)	1.8 (0.2; 0.0-5.0)	0.903	1.4 (0.2; 0.0-5.0)	0.827
Pronotum Length (mm)	0.04 (0.01; 0.00-0.21)	0.966	0.18 (0.02; 0.00-0.67)	0.614
Body Mass (mg)	49 (5; 0-146)	0.658	10 (10; 0-42)	0.981

size (pronotum length) or body mass, male *G. pennsylvanicus* with greater maxillae spans won more fights than males with narrower maxillae spans. Furthermore, as the asymmetry in maxillae span increased, so too did the likelihood that the male with the greater maxillae span would win the fight when we controlled for body size – supporting our two *a priori* predictions. However, contest duration was not affected by the maxillae span of either winning or losing males.

Other studies have found that males with larger weapons win more fights (reviewed in Andersson 1994; see also Kelly 2006; Pomfret and Knell 2006, but see Briffa 2008), but have usually controlled statistically for the correlation between weapon size and body size. Statistical correction for a body size difference between contestants is at best weak support for the hypothesis that weaponry asymmetry between contestants is responsible for success in fights; however, few studies have matched contestants for similar body size but different weapon size. In a notable exception, Brown and Bartalon (1986) found that, controlling for body size of forked fungus beetles, *Bolitotherus cornutus*, males with relatively larger horns were more successful in competition with relatively smaller-horned males. However, this study did not examine the effect of magnitude of horn size asymmetry on success in competition. Thus, it is unknown whether increasing horn size asymmetry confers greater advantage in male-male competition. Like Brown and Bartalon (1986), we experimentally controlled for differences in male body size when pairing males of different weapon sizes (maxillae span). When we controlled for pronotum length, we found that the male with the greater maxillae span was more likely to win, and there was a trend for this same effect when we controlled for body mass. Furthermore, we found that in both experiments, the male with the greater maxillae span had an increasing advantage as the difference in maxillae span increased. Together these results are strong support for the hypothesis that greater maxillae span is under selection through male-male competition in *G. pennsylvanicus*.

Although asymmetry in maxillae span affected the outcome of fights between male *G. pennsylvanicus*, it did not affect contest duration, a relationship predicted by many models of animal conflict (reviewed in Taylor and Elwood 2003). This may have been the result of our measure of contest duration not reflecting the contest duration from the participants' perspective. In many of our contests (38/87), one or both males performed courtship song before the contest escalated and backed toward their opponent in a posture that is more typical of male-female interactions. In these cases it is not clear whether the courting individual recognized the sex of the other cricket in the arena, even when the opponent reacted aggressively. Furthermore, other unmeasured asymmetries may have influenced contest duration. For example, Hofmann and Schildberger (2001) suggested that male *G. bimaculatus* who display more rapid antennal lashing are more willing to fight. And although mandible length was not related to contest outcome or duration in *A. domesticus*, males with more asymmetric mandibles (left – right) were more likely to win contests and contest duration decreased with the loser's mandible asymmetry (Briffa 2008). Inclusion of more traits relevant to male resource holding potential (RHP, Parker 1974) will allow a more accurate assessment of the relationships between RHP and both resource value and reproductive success (Kelly 2008).

The relatively weak effect of asymmetry in maxillae span, and thus low power to detect effects on contest outcome (Fig. A.3), may be partially due to maxillae span not varying widely among male *G. pennsylvanicus* (Table A.1, Fig. A.2); pairings between males markedly different in maxillae span were rare, and the mean maxillae span difference was 6.0% (SE = 0.5; range = 0.6 to 17.9)) and 7.2% (SE = 0.6; range = 0.3 to 14.3) in experiment one and two respectively. In contrast the minimum difference in horn length between paired male *B. cornutus* was approximately 12.5% (Brown and Bartalon 1986). Despite this, we were able to detect a significant effect when maxillae span was the only variable explaining contest outcome.

Wild-caught male and female *G. pennsylvanicus* could be reliably distinguished using linear dimensions of homologous structures (also found in lab-reared populations; K. A. Judge, unpubl. data). Although they are about 9% smaller in body size (pronotum length) than females, males have maxillae spans that average 21% larger (Table A.1, Fig. A.2). Larger ensiferan orthopteran species (Anostostomatidae) show even more striking sexual dimorphisms in head size and mouthpart morphology (Field and Sandlant 1993; Gwynne and Jamieson 1998; Bateman 2000; Gibbs 2001; Kelly 2005). For example, in the Wellington tree weta, *Hemideina crassidens*, there is hypertrophy in male mandible and maxilla length (on average almost twice the size of female mouthparts), despite males being more than 11% smaller than females in body size (femur length) (Maskell 1927; Kelly 2006). Such sexual dimorphism in mouthparts is likely to be widespread in grylline crickets in which males are known to fight. Alexander's (1957) observations on North American gryllines suggest that this dimorphism is present in *G. firmus* and *G. veletis*. Preliminary analysis shows a similar pattern (head width versus pronotum length) in a sample of

G. texensis (K. A. Judge, unpubl. data). And a recent study reported sexual dimorphism in head size in the commercially raised grylline *A. domesticus* (Walker et al. 2008). Interestingly, *G. bimaculatus* has been a model system for the study of male-male aggression (e.g. Simmons 1986; Adamo and Hoy 1995; Tachon et al. 1999; Hofmann and Schildberger 2001; Rillich et al. 2007) and a key subject of cricket fighting in China (Hsu 1928-29), yet no study to date has reported sexual dimorphism in male weaponry for this species.

The current results suggest that maxillae span is under positive selection through male-male competition in *G. pennsylvanicus*. Head width (and thus likely maxillae span) is also under positive linear selection via female choice (Chapter 5). The apparent congruence between these two different mechanisms of sexual selection may be the reason that maxillae span is positively allometric in males when compared with other morphological traits (see Table A.3, Fig. A.5). In contrast, Bonduriansky and Rowe (2003) showed that male head elongation in a piophilid fly, *Prochyliza xanthostoma*, is under conflicting sexual selection. Males with relatively elongated heads were at a disadvantage in their first bout of combat against an opponent, whereas they were more attractive to females (Bonduriansky and Rowe 2003). Head length (nor indeed any sexual trait) is not positively allometric in this fly (Bonduriansky 2006), which may reflect a compromise between conflicting sexual selection pressures (Bonduriansky and Rowe 2003). The comparison of *G. pennsylvanicus* and *P. xanthostoma* highlights the need for studies of sexual traits within a group of related species, to draw conclusions about the different sexual selection pressures that shape morphology. Given the diversity of morphology (Alexander 1957) and life history (Alexander 1957, 1968; Masaki and Walker 1987; Walker and Masaki 1989; Roff et al. 2003), presence of alternative mating strategies (Cade 1981) and the existence of a phylogeny (Huang et al. 2000), North American gryllines represent an excellent taxon to test comparative hypotheses concerning the evolution of allometries.

Our results are consistent with reports of the practice of Chinese cricket fighting, which pointed to relatively larger heads (and thus probably greater maxillae span) as a trait that influences fight outcome (Chia 1260; Laufer 1927; Hsu 1928-29). Interestingly, there are reports that the Chinese cricket fighting community in Philadelphia, USA uses *G. pennsylvanicus* to practice their sport (Jin 1994). Chinese cricket fighting has provided testable hypotheses concerning male weaponry (this study) and the neurobiology of fight experience (Hofmann and Stevenson 2000), and may well yield further insights into animal behaviour research (Hofmann 1996).

Table A.3: Matrix of allometric slopes (above the diagonal) and Pearson correlation coefficients (below the diagonal) for the five morphological variables measured on 151 male and 75 female *G. pennsylvanicus* captured in the field. 99.5% confidence intervals are given in parentheses for the allometric slopes. All correlation coefficients are significant at $p < 0.001$).

Variable	Sex	LogMS	LogHW	LogPW	LogPL	LogMFL
Log Maxillae Span	Males		1.126 (1.057-1.199)	1.199 (1.106-1.301)	1.230 (1.106-1.371)	1.490 (1.327-1.681)
	Females		1.175 (0.992-1.398)	1.015 (0.839-1.228)	1.070 (0.847-1.357)	1.083 (0.872-1.350)
Log Head Width	Males	0.966		1.062 (0.975-1.158)	1.083 (0.989-1.186)	1.305 (1.177-1.452)
	Females	0.894		0.860 (0.707-1.041)	0.903 (0.740-1.098)	0.916 (0.765-1.095)
Log Pronotum Width	Males	0.945	0.939		1.019 (0.920-1.129)	1.228 (1.104-1.369)
	Females	0.874	0.869		1.057 (0.797-1.408)	1.071 (0.819-1.407)
Log Pronotum Length	Males	0.910	0.933	0.916		1.204 (1.084-1.340)
	Females	0.827	0.865	0.775		1.013 (0.825-1.244)
Log Mean Femur Length	Males	0.893	0.912	0.909	0.912	
	Females	0.844	0.885	0.789	0.860	

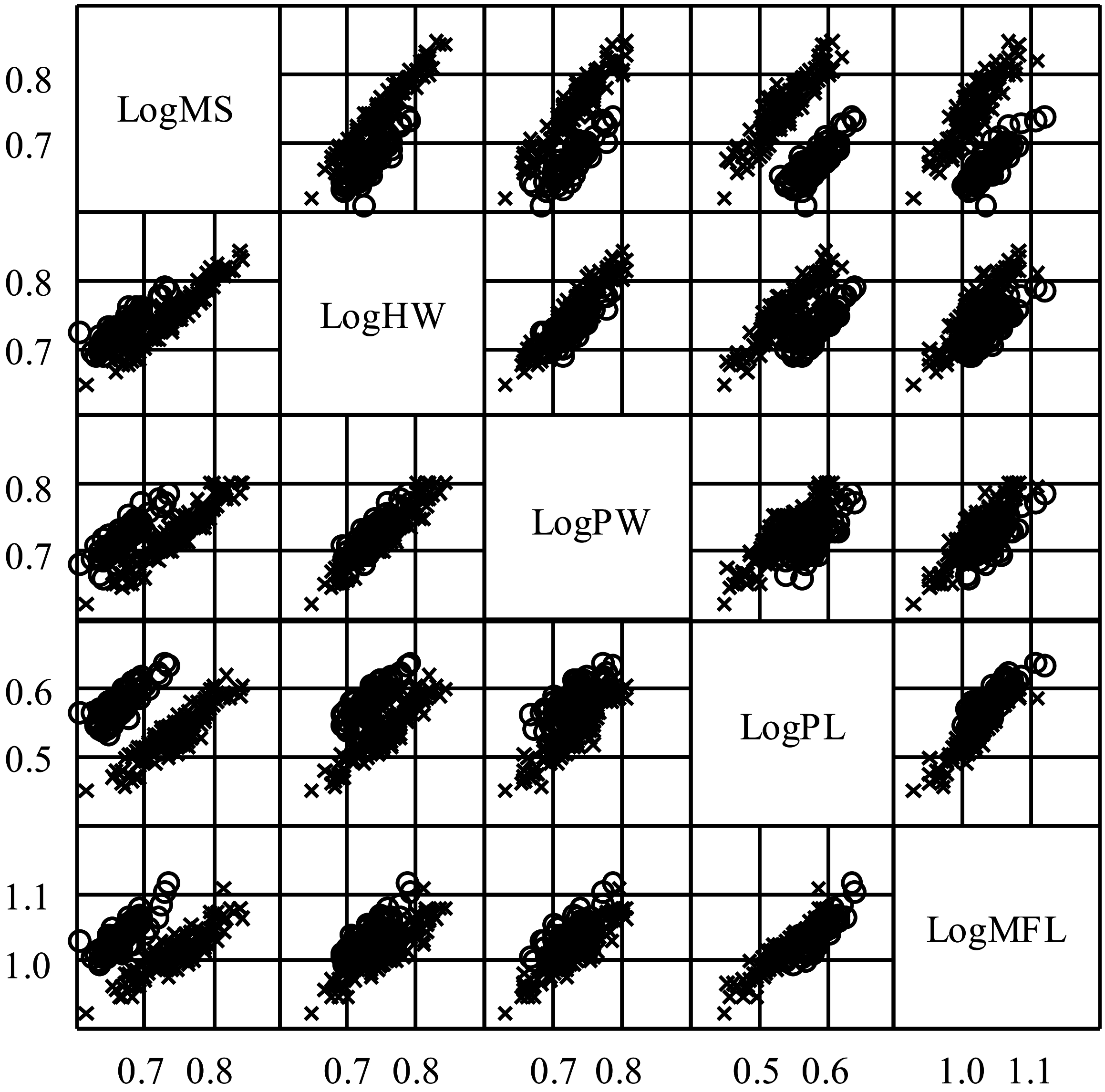

Figure A.5: Matrix of scatterplots for the log transformed morphological variables showing sexual dimorphism in a sample of 151 males (X) and 75 females (O). Abbreviations are as follows: LogMS = log maxillae span, LogHW = log head width, LogPW = log pronotum width, LogPL = log pronotum length, and LogMFL = log mean femur length.

A.6 Acknowledgements

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