CHAPTER 2

Interactive effects of age and body size on calling song traits of male field crickets, *Gryllus bimaculatus* De Geer (Orthoptera: Gryllidae)

Abstract

Some authors suggested that female crickets prefer to mate with older males, based on their call traits. This study aims to determine whether calling song characteristics of the field cricket *Gryllus bimaculatus* change with age and whether the calling song can be used by females to discriminate between songs of young and old males. I make use of sound recordings of fifteen captive males, recorded throughout their entire lives, as well as records of all calling activity during their entire lives. Older males have slower chirp rates with shorter syllables and longer inter-syllable intervals, reflecting a general slowing down in most temporal call traits. There was a strong interaction between size and age of a male with old, large males calling at slower chirp rates than young, small males. Large males also called for significantly longer periods than small males. Highest calling activity occurred at 22 days after adult eclosion. Males with shorter life spans did not compensate for lost reproductive opportunities by calling faster or for longer periods than males with longer life spans. Although most males showed rigid circadian rhythms in calling throughout their life spans, there was a large degree of between-male variation in circadian rhythm. Females can, therefore, potentially select old males on the basis of call traits. In contrast, two call traits crucial for mate recognition (calling song frequency and syllable period) remained constant throughout the realistic outdoor cricket life span of 50 days, suggesting different modes of selection on this type of signaling trait.

Keywords: *Gryllus bimaculatus*, Calling song, Body size, Male age

Introduction

Male field crickets, *Gryllus bimaculatus*, produce calling songs to attract receptive females (Alexander, 1962). The calling song consists of discrete chirps, each of which comprises a number of syllables. Each syllable is produced by a complete wing-closing movement, where the teeth of the plectrum from one wing traverses the file of the other wing. Inter-syllable intervals are due to wing-opening movements (Bennet-Clark, 1989). Simmons (1988a) suggested that female crickets use male calling song to assess male quality, and showed that females preferred calling songs of large males. Following the ‘good genes’ model of sexual selection, males should be chosen on the basis of genetic quality, of which longevity is an important measure (Hansen & Price, 1995). Kokko (1998) showed through modelling that a strong correlation between genetic quality and survival should be expected. Long-lived males may have a smaller chance of possessing deleterious mutations (Manning, 1985). Females could use age-dependent male ornaments as an indicator of male fitness with older males having a higher fitness than younger males (Manning, 1985). In the willow warbler, *Phylloscopus trochilus*, and the European starling, *Sturnus vulgaris*, females use song repertoire size as an indicator of male age which, could in turn, convey information about the male’s condition, competitive experience, reproductive experience and genetic quality (Gil *et al*., 2001; Mountjoy & Lemon, 1995). On the other hand, the outcome of a simulation model by Beck & Powell (2000) suggested that the ’good-genes’ model of sexual selection could not explain female preference for older males in species where males only provide sperm. Accordingly, female preference for young to intermediate mates is more likely, because male genetic quality could decrease with age, based on the assumption that many mutations have deleterious effects. Also, males of lower genetic quality show a decrease in reproductive success as they age (Alatalo *et al*., 1986; Beck & Powell, 2000; Price & Hansen, 1998).

The effects of male age in crickets have been studied by a number of workers. Some authors have suggested mechanisms explaining the higher mating success of older males, but in some cases it is unclear why females would prefer older males as there may be negative consequences, for example, older *G. bimaculatus* males produce fewer progeny (Simmons, 1988b). Although older *G. bimaculatus* males have a significantly lower daily mating rate than younger males (Simmons, 1988b), they have a higher mating success than younger males

(Simmons & Zuk, 1992). In *G. veletis* and *G. pennsylvanicus* males found paired with females in the wild were older and less infected with gregarine parasites (Zuk, 1988) than were solitary calling males (Zuk, 1987). While gregarine infection had no influence on the mating success of older *G. bimaculatus* males, younger infected males did not mate and young uninfected males only had a small chance of mating success (Simmons & Zuk, 1992). In *G. integer*, young males call less actively than older ones (Bertram, 2000) since the calling song attracts parasitoid flies, and the risk of being parasitized outweighs the cost of mating at a young age. However, another explanation for this could be that younger males call less to avoid aggressive interactions with older males (Dixon & Cade, 1986).

There are two possible mechanisms explaining why female *G. bimaculatus* mate with older males: (a) they use a cue in the calling song of the male to determine his age or (b) females only show a post-copulation preference by accepting larger sperm quantities from preferred males (Simmons & Zuk, 1992). The present study investigates the first of these mechanisms by determining age effects on the calling behaviour of male *G. bimaculatus*.

The aims of this study are:

1. To determine whether calling song traits in the field cricket, *G. bimaculatus*, change with age; a requirement for females using calling songs to detect male age.
2. To determine how much of the population-level variation in calling song traits can be explained by male age and the interaction between age and male size.
3. To determine the amount of time males invest in producing calling songs, in order to determine whether males with shorter life spans call more intensively each day in order to compensate for reduced mating opportunities.

Materials and methods

Cricket rearing

Fifteen penultimate *G. bimaculatus* males were randomly chosen from a laboratory-reared colony in a climate room (27°C ± 2°C) with 12h:12h Light:Dark (L:D) regime. Each male was placed in a 2-litre container in the climate room with egg carton for shelter, food (Pronutro® and fish flakes) and water *ad libitum*. During this period they were exposed to the calling songs of all the males in the colony. At nine days post adult eclosion, each male was transferred to a sound-damped recording chamber.

Calling song recordings

Sound-damped recording chambers (31 cm x 35 cm x 26 cm; acoustic isolation 50.13 ± 1.73 dB (mean ± SE) between neighbouring chambers) were maintained at 25°C ± 1°C using an electric heating element. Each chamber was insulated with glass fibre mat (thickness 5 cm) and equipped with a Behringer XM200S dynamic microphone (50 Hz – 10 kHz ± 3 dB; Behringer Spezielle Studiotechnik GmbH, Germany). Each male was placed in a gauze container (11 cm x 11 cm x 12 cm) with a cardboard floor and a small piece of egg carton for shelter. Fresh food (Pronutro®) and water were provided every second day. A 12h:12h L:D light regime was implemented using an 8000 mCd light-emitting diode in the roof of each gauze container. When a male called, the sound was recorded automatically on the hard disk of a computer, utilizing a Maya 4 sound card (Audiotrak, Korea). Each male was monitored for its life span, i.e. life span is measured from 10 days post adult eclosion until death. Three sound recordings of 30 seconds duration each were made each night during which a particular male called, with between-recording intervals of at least 5 minutes. The equipment also kept a continuous record of all minutes during which a male called for longer than 40 seconds and the number of minutes spent calling per hour was monitored continuously throughout each male's life span.

Calling song characteristics and morphometric measurements

Canary V1.2.4 (Cornell Laboratory of Ornithology, Ithaca, New York) was used on an Apple Macintosh computer for spectrographic analysis of the calls. The power spectrum (Figure 2.1a) and oscillogram (Figure 2.1b) of each recording were used as a basis for measuring the call traits (Table 2.1) of three consecutive chirps every third night. After its death, each cricket was measured and weighed. A Video Blaster FS200 video software kit (Creative Laboratories, Singapore; resolution = 16 microns) was used for the morphological measurements (Table 2.1). Thorax area was calculated as thorax width x thorax length (mm^2) and was used as an indicator of body size. Mass was assumed to represent wet mass. After the body measurements were taken, the wings of each male were removed and the harp size of the right wing was measured by calculating the right angled triangular area formed by points H1, H2 and H3 as noted in Ferreira & Ferguson (2002). Repeatability ± standard error was calculated to estimate the reliability of the measurement procedure, following Becker (1984). The call traits and body measurements (Table 2.1; excluding tibia-R, tibia-L and mass) were measured twice for 30 individuals. Repeat measurements were blind with respect to previous measurements and separated by more than 24 hours.

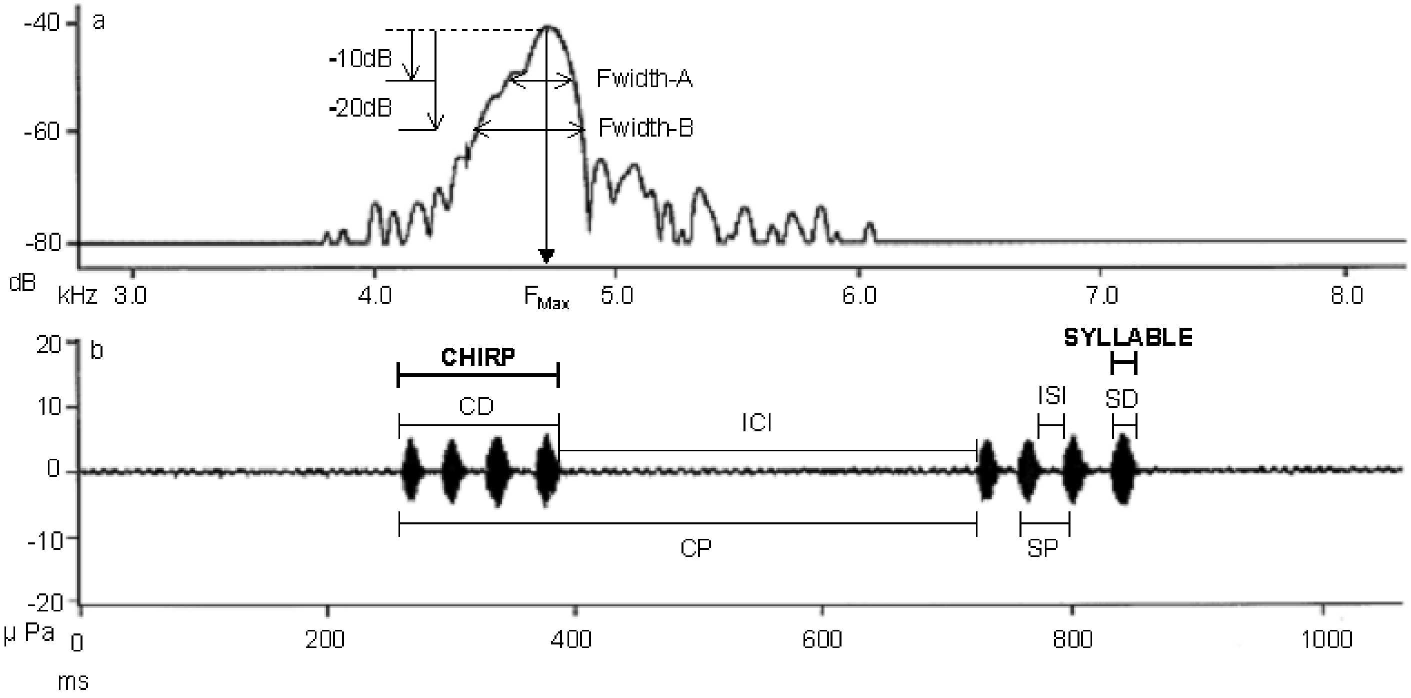


Figure 2.1. Graphical illustration of part of a male field cricket's, *G*. *bimaculatus*, calling song. A power spectrum (a) and an oscillogram (b) were used to measure the calling song characteristics (see Table 2.1).

Table 2.1. Description of the calling song characteristics, body measurements and wing measurement for the 15 *G. bimaculatus* males. The explained variance components for each of the call traits are shown, resulting from analysis of variance on a 6-day interval data set up to the adult age of 69 days. Error variance and unknown factors contributed to the remaining variation in the data set

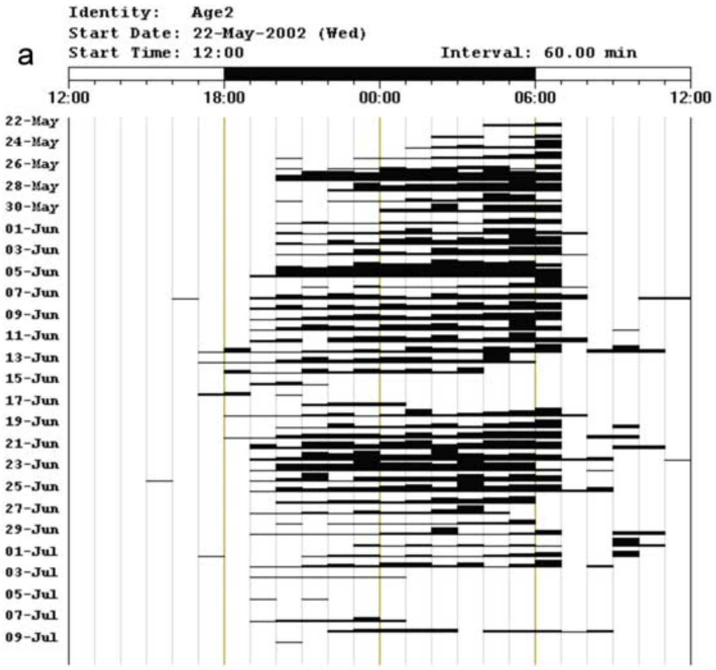
		Variance components	
Variable	Definition	Between individuals	Within individual (age effect)
Call traits			
F_{max} (kHz)	Emphasized frequency for each syllable (Fig. 2.1a)	0.16	0.12
Fwidth-A (kHz)	Frequency range at 10 dB below the amplitude of F_{max} (Fig. 2.1a)	0.52	0.20
Fwidth-B (kHz)	Frequency range at 20 dB below the amplitude of F_{max} (Fig. 2.1a)	0.51	0.16
ICI (s)	Interval between chirps (Fig. 2.1b)	0.11	0.20
CD (s)	Duration of one chirp (Fig. 2.1b)	0.37	0.19
CP (s)	Duration from the beginning of a chirp to the beginning of the next chirp (Fig. 2.1b)	0.17	0.26
SD (s)	Duration of one syllable (Fig. 2.1b)	0.57	0.13
ISI (s)	Interval between syllables (Fig. 2.1b)	0.27	0.39
SP (s)	Duration from the beginning of a syllable to the beginning of the next syllable (Fig. 2.1b)	0.52	0.06
S_C	Number of syllables per chirp	0.21	0.23
Body measurements			
Thorax width (mm)	Width of the thorax		
Thorax length (mm)	Length of the thorax		
Head width (mm)	Width of the head		
Head length (mm)	Length of the head		
Femur-R (mm)	Length of the right hind femur		
Femur-L (mm)	Length of the left hind femur		
Tibia-R (mm)	Length of the right hind tibia		
Tibia-L (mm)	Length of the left hind tibia		
Mass (g)	Wet mass of the cricket immediately after death		
Wing measurement			
Harp (mm^2)	Total area of the harp		

Statistical analyses

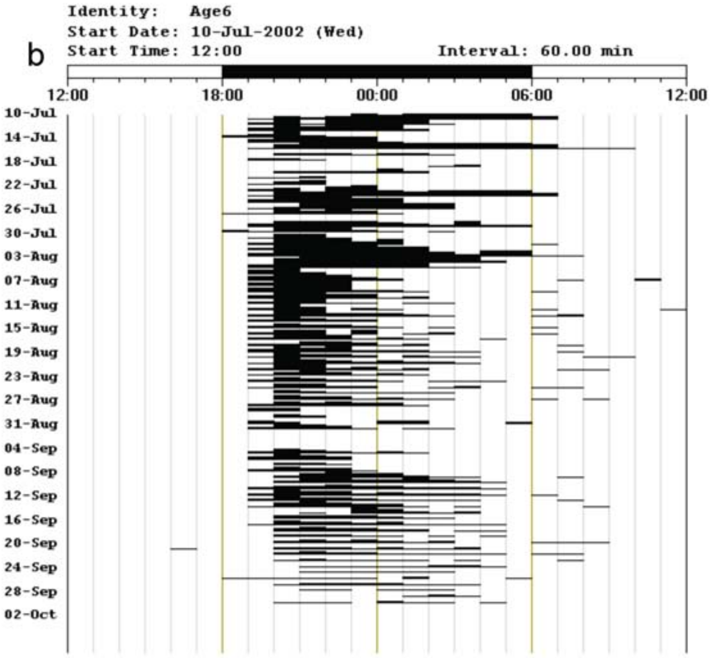
Circadian patterns of calling

Actiview^{TM} V1.2 (Mini Mitter Co., Oregon) was used to draw an actogram of each male's calling activity. All 15 actograms were inspected to determine a circadian calling pattern during the life span of each male. Males were classified accordingly into five circadian categories based on the following circadian patterns: (1) males calling mostly during the early morning hours, from midnight – 6am (Figure 2.2a), (2) males mostly calling during the early evening from 6pm – midnight (Figure 2.2b), (3) males whose calls were spread evenly throughout the night from 6pm – 6am (Figure 2.2c), (4) males that showed no pattern whatsoever throughout their life span and (5) males that changed their circadian patterns and shifted from early evening calling to early morning calling. Statistica V5.5 (StatSoft, Inc. (1999), Tulsa, USA) was used to perform a univariate analysis of variance (ANOVA) to compare the means of the call traits, thorax area and lifespan between the circadian calling pattern categories. This was done to determine firstly whether males with different circadian calling patterns also differ in their calling song traits, secondly whether small males have different circadian calling patterns compared with large males and lastly whether males with different circadian calling patterns have different life spans.

a


Identity: Age2
Start Date: 22-May-2002 (Wed)
Start Time: 12:00
Interval: 60.00 min
12:00
18:00
00:00
06:00
12:00
22-May
24-May
26-May
28-May
30-May
01-Jun
03-Jun
05-Jun
07-Jun
09-Jun
11-Jun
13-Jun
15-Jun
17-Jun
19-Jun
21-Jun
23-Jun
25-Jun
27-Jun
29-Jun
01-Jul
03-Jul
05-Jul
07-Jul
09-Jul

b


Identity: Age6
Start Date: 10-Jul-2002 (Wed)
Start Time: 12:00
Interval: 60.00 min
12:00
18:00
00:00
06:00
12:00
10-Jul
14-Jul
18-Jul
22-Jul
26-Jul
30-Jul
03-Aug
07-Aug
11-Aug
15-Aug
19-Aug
23-Aug
27-Aug
31-Aug
04-Sep
08-Sep
12-Sep
16-Sep
20-Sep
24-Sep
28-Sep
02-Oct

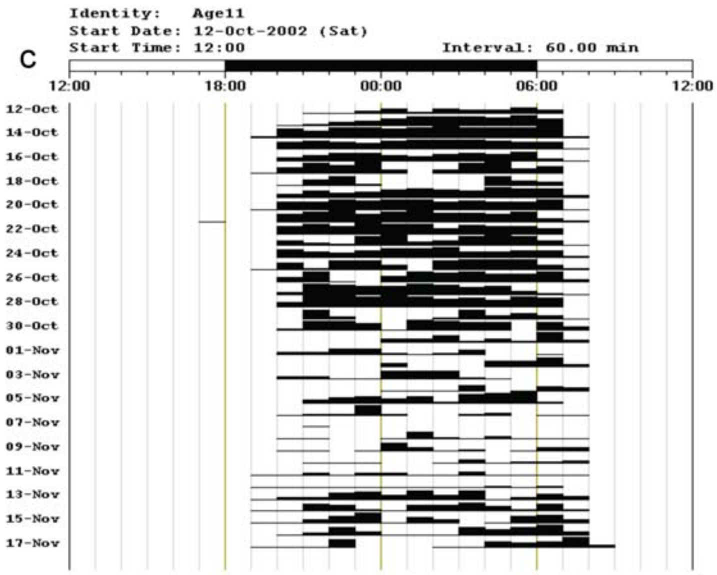


Figure 2.2. Three actograms showing between-male and within-male variation in the circadian calling activity of *G. bimaculatus*: (a) early-morning calling (category 1), (b) early-evening calling (category 2) and (c) calling throughout the night (category 3).

Age effects on calling activity

Because of a large degree of between-male variation in calling activity per day, raw data were transformed to relative calling activity (RCA). RCA reflected the time that each male spent calling per day as a fraction of the highest calling activity logged for that individual, i.e. (number of seconds called per day) / (largest number of seconds called per day during its lifetime). Mean RCA was calculated at 2-day intervals for each of the 15 males.

Age and body size effects on calling song characteristics

The call traits of each male were analysed at 3-day intervals, using Statistica. Male calls were studied over two spans of ageing after adult eclosion. A forward stepwise multiple regression analysis was performed on call traits for the periods 10 – 49 days and 10 – 140 days post adult eclosion to determine whether temporal changes in the call traits (Table 2.1) are correlated with male age and/or male body size. Following the results of the multiple regression analysis on the 10 – 140 days data set, a non-linear multiple regression was performed on the call traits that were significantly influenced by both male age and body size, using NLREG V5.4 (Sherrod, 2002). In addition, Pearson product moment correlations (Statistica) were performed for each male, to determine any significant correlation between male age (for the period 10 – 49 days) and call traits. A Bonferroni adjustment was applied to detect significant correlations (Rice, 1989).

The call traits of each male were grouped into 6-day age classes spanning the period nine to 69 days (because some males did not call ten days after adult eclosion but did call nine days after adult eclosion, the call recordings for the ninth day was used for these analyses). SAS V8.02 (Proc Varcomp; SAS Institute Inc., Cary, NC, USA) was used to determine the within-individual and between-individual variance components for the call traits of the males using restricted maximum-likelihood estimation (REML).

Relationship between life span, body size and calling activity

Pearson product moment correlation analysis was performed, using Statistica, to identify any significant correlation of the morphometric measurements (Table 2.1) with life span. This gave an indication whether small males had a longer or shorter life span than large males. Statistica was also used to calculate Pearson product moment correlation between the mean number of seconds called per day and life span. This gave an indication whether males with a short life span called for longer or shorter durations than males with a long life span. To determine the effect of male size and life span on calling activity, a forward stepwise multiple regression analysis was performed, using Statistica, with mean number of seconds called per day as the dependent variable and body size and life span as independent variables.

Effect of male size and male age on calling duration and calling intensity

To relate *calling intensity* (the mean number of minutes called during a particular hour) and *calling duration* (the mean number of hours called per day, which included only those hours with a minimum calling duration of 100 seconds) with male size and male age, forward stepwise multiple regression analyses were performed (Statistica) using body size and male age as the independent variables and calling duration and calling intensity as the dependent variable, respectively.

Relationship between calling duration, calling intensity and chirp rate

Statistica was used to perform forward stepwise multiple regression analysis to relate the effects of calling duration and calling intensity on the *chirp rate* (number of chirps per second) of a particular male.

Results

Repeatability of measurements of call traits and body measurements

There were significant differences between the 30 individuals for all the call traits ($21846.6 > F_{29, 57} > 64.54$, $P < 0.001$) and body measurements ($115.75 > F_{29, 57} > 7.31$, $P < 0.001$). The call traits and body measurements were highly repeatable (call traits: $1.0 \pm 0.0 > r > 0.985 \pm 0.006$; body measurements: $0.984 \pm 0.006 > r > 0.759 \pm 0.079$).

Circadian patterns of calling

There was a large degree of between-male variation in calling activity per day (Figure 2.2). Males started calling within two hours after the Light:Dark transition at 6pm and terminated calling within one hour prior until three hours after the Dark:Light transition at 6am. The majority of the males called either early in the evening (category 2, $n = 4$; Figure 2.2b) or throughout the whole night (category 3, $n = 4$; Figure 2.2c). The remainder of the males called either early in the morning (category 1, $n = 2$; Figure 2.2a) or showed irregular patterns of calling behaviour (category 4, $n = 3$; category 5, $n = 2$).

Due to the small sample sizes of categories four and five, they were combined for the ANOVA. Univariate ANOVA revealed no significant differences between the four circadian groups for the call traits, thorax area and life span. The circadian patterns of calling behaviour of small males did not differ significantly from large males (thorax area: $F_{3, 14} = 0.07$, $P > 0.05$, $n = 15$). In addition, older males did not have different circadian calling patterns compared with younger males (lifespan: $F_{3, 14} = 0.71$, $P > 0.05$, $n = 15$). There were also no significant differences between the circadian groups for the temporal ($3.09 > F_{3, 14} > 0.32$, $P > 0.07$, $n = 15$) or spectral call traits ($0.43 > F_{3, 14} > 0.1$, $P > 0.76$, $n = 15$).

Age effects on calling activity

There was a large degree of within-male variation for the time spent calling per day (Figure 2.2). Males showed two peaks for calling activity throughout their life spans (Figure 2.3). The first peak was at 22 days (RCA = 0.551 ± 0.063 (mean ± SE); $n = 15$) and the second peak was at 72 days (RCA = 0.463 ± 0.111 (mean ± SE); $n = 4$). When the mean number of seconds called per day was plotted against age (i.e. using untransformed data, not RCA), the same trend in calling activity was observed.

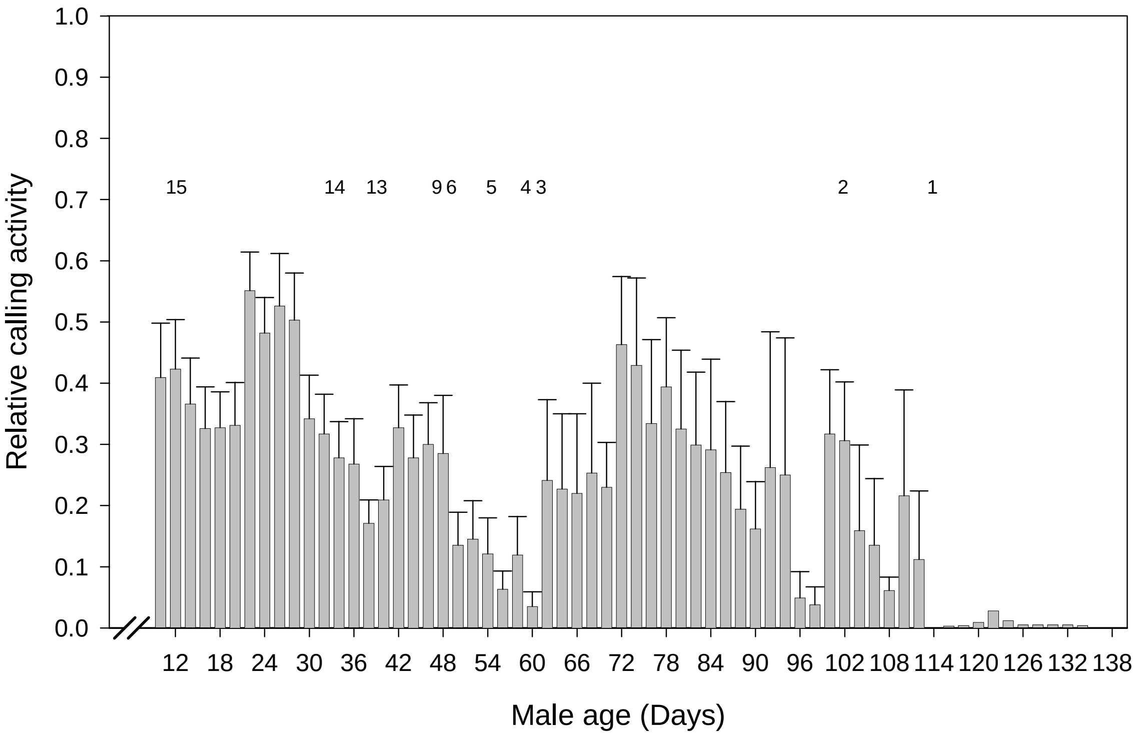


Figure 2.3. The relative calling activity (RCA) reflecting the time duration that *G. bimaculatus* males spent calling per day. Horizontal bars represent mean values calculated for all the males at 2-day intervals, while Y-error bars indicate standard error. Values above the Y-error bars represent the sample size of males that reached that particular age.

Age and body size effects on calling song characteristics

(i) *Ten – 49 days post adult eclosion*

Forward stepwise multiple regression analyses found only a significant correlation of body size (thorax area) with two chirp traits (CD and CP) and bandwidth. After controlling for body size, all the temporal call traits except syllable period (Figure 2.4) and the spectral call traits correlated significantly with male age. The sharp increase in the values of the standard errors of the call traits after 50 days (Figure 2.5) suggested that the temporal changes taking place during the first 50 days after adult eclosion are more informative than temporal changes at an older age.

Chirp call traits: While older males produced significantly longer inter-chirp intervals there was no significant correlation of thorax area with inter-chirp interval ($F_{2, 136} = 5.70$, $P < 0.01$, $n = 139$; male age partial correlation: r = 0.23, $P < 0.01$; thorax area partial correlation: r = 0.14, $P > 0.05$). Both older and larger males produced longer chirp durations compared with younger and smaller males ($F_{2, 136} = 10.41$, $P < 0.001$, $n = 139$; male age partial correlation: r = 0.28, $P < 0.001$; thorax area partial correlation: r = 0.23, $P < 0.01$). This increase in chirp duration resulted in the significantly longer chirp periods produced by these males ($F_{2, 136} = 13.10$, $P < 0.001$, $n = 139$; male age partial correlation: r = 0.33, $P < 0.001$; thorax area partial correlation: r = 0.22, $P < 0.01$). Older males produced significantly more syllables per chirp than younger males, while there was no significant correlation of number of syllables per chirp with thorax area ($F_{2, 136} = 9.89$, $P < 0.001$, $n = 139$; male age partial correlation: r = 0.32, $P < 0.001$; thorax area partial correlation: r = 0.16, $P > 0.05$).

Syllable call traits: There was no significant correlation of syllable period with either male age or thorax area ($F_{1, 137} = 3.71$, $P > 0.05$, $n = 139$; male age partial correlation: r = 0.16, $P > 0.05$; thorax area partial correlation: r = 0.22, $P > 0.05$). While syllable duration decreased significantly with male aging, the correlation of syllable duration with thorax area was not significant ($F_{1, 137} = 12.86$, $P < 0.001$, $n = 139$; male age partial correlation: r = -0.29, $P < 0.001$; thorax area partial correlation: r = -0.05, $P > 0.05$). Inter-syllable interval increased significantly with male ageing, while thorax area was not significantly correlated with this temporal call trait ($F_{1, 137} = 12.86$, $P < 0.001$, $n = 139$; male age partial correlation: r = 0.43, $P < 0.001$; thorax area partial correlation: r = 0.05, $P > 0.05$)

Spectral call traits: Calling song frequency did not correlate significantly with either male age or thorax area ($F_{2, 136} = 0.01$, $P > 0.05$, $n = 139$; male age partial correlation: $r = -0.01$, $P > 0.05$; thorax area partial correlation: $r = 0.004$, $P > 0.05$). Larger males produced significantly wider bandwidths than smaller males, while male age did not correlate significantly with bandwidth (Fwidth-A: $F_{1, 137} = 8.40$, $P < 0.01$, $n = 139$; male age partial correlation: $r = -0.02$, $P > 0.05$; male size partial correlation: $r = 0.24$, $P < 0.01$; Fwidth-B: $F_{1, 137} = 16.64$, $P < 0.001$, $n = 139$; male age partial correlation: $r = -0.06$, $P > 0.05$; male size partial correlation: $r = 0.33$, $P < 0.001$).

Pearson product moment correlations performed separately for each male revealed similar results to the multiple regression analyses performed on the combined data set. These results are reported in three sections below:

a) *Chirp call traits:* Twelve of the males showed positive trends towards either producing more syllables per chirp, longer chirp durations and longer chirp periods with age while 13 produced longer inter-chirp intervals with age. However, results for only seven of the 15 individuals were significant at $P < 0.05$. Three males produced significantly more syllables per chirp ($r > 0.59$, $P < 0.05$) as well as significantly longer chirps ($r > 0.59$, $P < 0.05$). On the contrary, two males produced significantly shorter chirps ($r = -0.65$, $P < 0.05$) as they aged. One male produced significantly longer inter-chirp intervals ($r = 0.75$, $P < 0.01$). Chirp period increased significantly with male age in four males ($r > 0.61$, $P < 0.05$). After a Bonferroni adjustment the negative correlations were not significant while one male produced significantly more syllables per chirp ($r = 0.87$, $P < 0.01$), two males produced longer chirp durations ($r > 0.66$, $P < 0.01$), one male produced longer inter-chirp intervals ($r = 0.75$, $P < 0.01$) and two males produced longer chirp periods ($r > 0.73$, $P < 0.01$) with age.

b) *Syllable call traits:* Similar trends were observed among individuals for each of the syllable traits with male ageing. Fourteen males produced shorter syllable durations as well as longer inter-syllable intervals with male age while ten males produced longer syllable periods as they aged. Nine individuals had significant correlations between male age and syllable traits. Seven males had significantly shorter syllable durations ($r < -0.56$, $P < 0.05$) of which five also had significantly longer inter-syllable intervals ($r > 0.70$, $P < 0.05$) as they aged. Two more males had significant correlations between male age and syllable period ($r > 0.68$, $P < 0.05$), of which one had longer inter-syllable intervals as he aged ($r = 0.63$, $P < 0.05$). After a Bonferroni adjustment, there was a significant correlation between male age and syllable period for two males ($r > 0.68$, $P < 0.05$). Two more males produced significantly shorter syllable durations (r < -0.75, $P < 0.01$). One of these as well as a third male produced significantly longer inter-syllable intervals (r > 0.81, $P < 0.01$, $n = 2$) as they aged.

c) *Spectral call traits:* Individual correlations between the spectral call traits and male age revealed no clear trends. Some males produced higher calling song frequencies and broader bandwidths as they aged, while others produced lower calling song frequencies and narrower bandwidths with age. These trends were still observed after a Bonferroni adjustment.

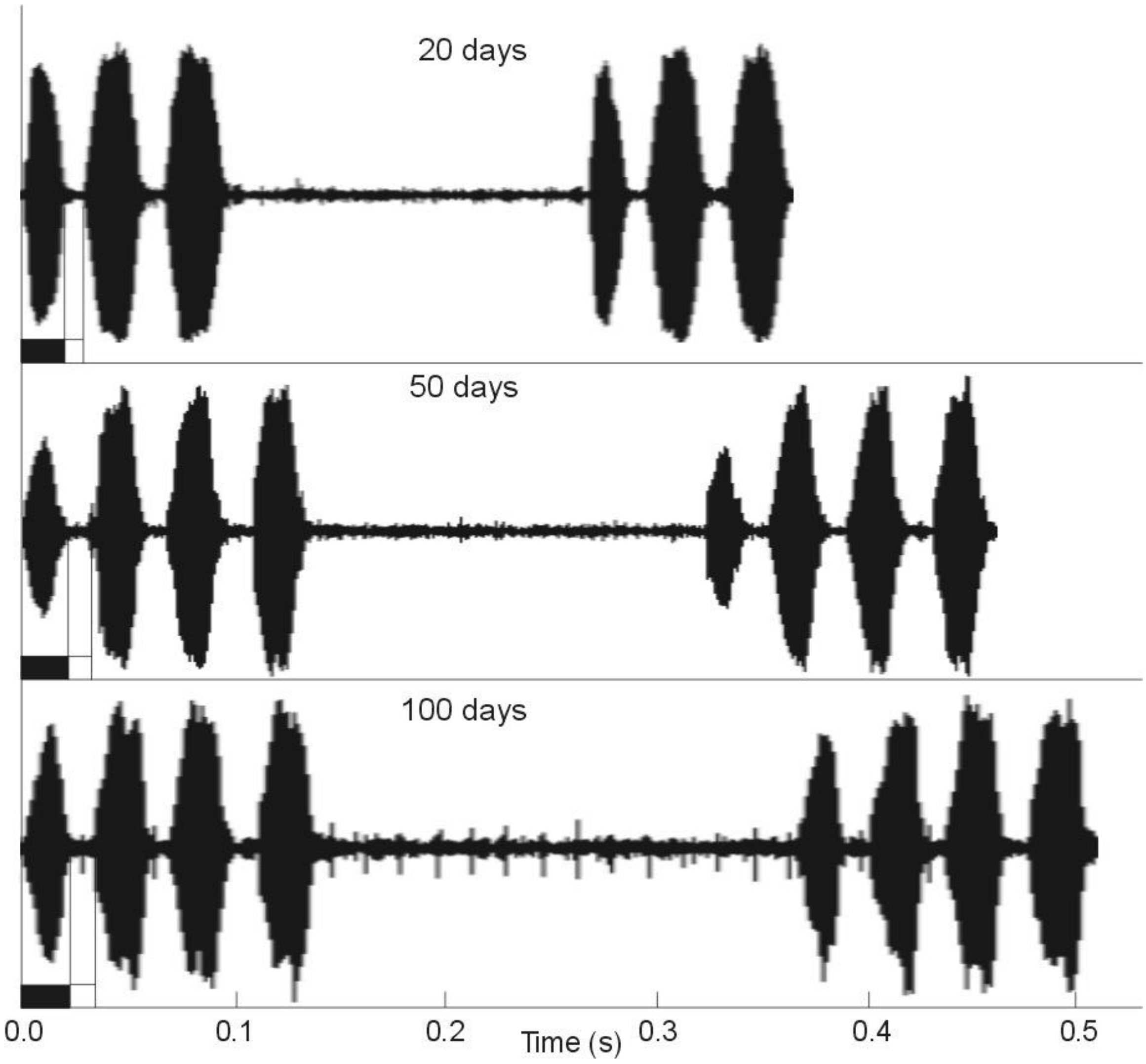


Figure 2.4. Graphical representation of the temporal changes in the calling song of adult male A1 at different ages (days after adult eclosion). Syllable duration (indicated by black bars) and inter-syllable interval (indicated by white bars) are shown for the first syllable at each time period.

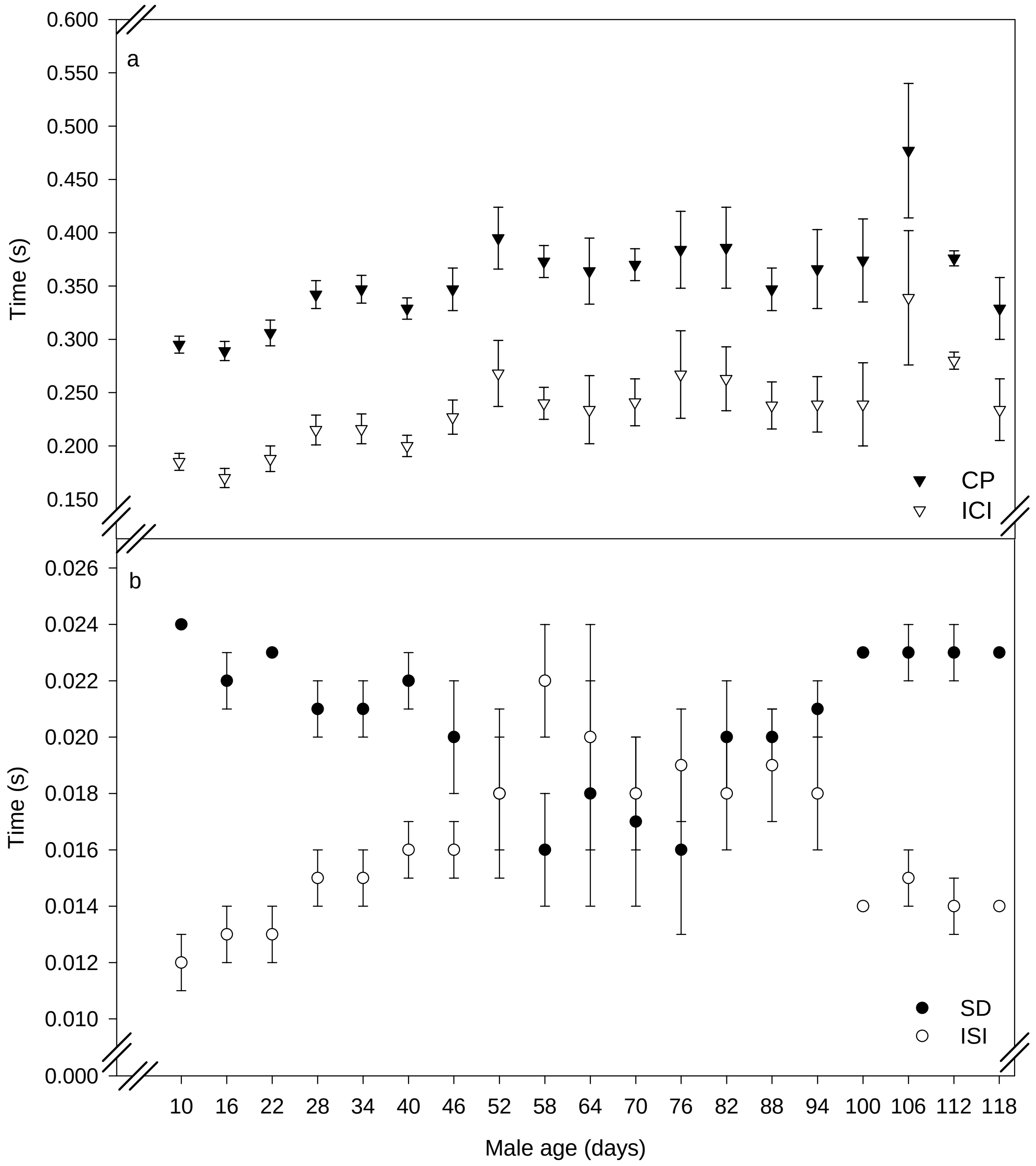


Figure 2.5. The relationship between male age and (a) two chirp and (b) two syllable traits of the calling song of male *G. bimaculatus*. Bullets indicate mean values, while the Y-error bars represent standard error.

(ii) *Ten – 140 days post adult eclosion*

The results of the statistical analyses over this range in life span should be interpreted with caution, since only three males had a life span of more than 62 days. Body size (thorax area) was significantly correlated with all the temporal and spectral call traits, except with syllable duration and calling song frequency. After controlling for body size, age had a significant correlation with two spectral call traits and all of the temporal call traits except number of syllables per chirp and chirp duration.

Chirp call traits: Although male age did not correlate significantly with chirp duration, there was a significantly correlation of thorax area with chirp duration ($F_{2, 201} = 8.14$, $P < 0.001$, $n = 204$; male age partial correlation: r = 0.07, $P > 0.05$; thorax area partial correlation: r = 0.26, $P < 0.001$). The same trend was also observed for syllables per chirp: there was no significant correlation of male age with syllables per chirp, while larger males produced significantly more syllables per chirp compared with smaller males ($F_{1, 202} = 8.80$, $P < 0.01$, $n = 204$; male age partial correlation: r = 0.06, $P > 0.05$; thorax area partial correlation: r = 0.20, $P < 0.01$). Older and larger males produced significantly longer inter-chirp intervals compared with younger and smaller males respectively ($F_{2, 201} = 28.10$, $P < 0.001$, $n = 204$; male age partial correlation: r = 0.42, $P < 0.001$; thorax area partial correlation: r = 0.22, $P < 0.01$). This gave rise to the significantly longer chirp periods produced by these males ($F_{2, 201} = 37.33$, $P < 0.001$, $n = 204$; male age partial correlation: r = 0.44, $P < 0.001$; thorax area partial correlation: r = 0.31, $P < 0.001$).

Syllable call traits: Older males produced significantly shorter syllable durations while thorax area did not correlate significantly with syllable duration ($F_{2, 201} = 9.88$, $P < 0.001$, $n = 204$; male age partial correlation: r = -0.27, $P < 0.001$; thorax area partial correlation: r = -0.13, $P > 0.05$). Older and larger males produced significantly longer inter-syllable intervals than younger and smaller males respectively ($F_{2, 201} = 25.66$, $P < 0.001$, $n = 204$; male age partial correlation: r = 0.41, $P < 0.001$; thorax area partial correlation: r = 0.20, $P < 0.01$). This lead to the significantly longer syllable periods produced by them ($F_{2, 201} = 5.96$, $P < 0.01$, $n = 204$; male age partial correlation: r = 0.18, $P < 0.01$; thorax area partial correlation: r = 0.14, $P < 0.05$).

Spectral call traits: Older males produced significantly lower calling song frequencies than younger males, while thorax area did not correlate significantly with this spectral trait ($F_{2,\ 201} = 6.91$, $P < 0.01$, $n = 204$; male age partial correlation: r = -0.23, $P < 0.001$; thorax area partial correlation: r = 0.13, $P > 0.05$). While Fwidth-A only correlated significantly with thorax area and not male age, Fwidth-B on the other hand, correlated significantly with male age and thorax area respectively (Fwidth-A: $F_{2,\ 201} = 19.88$, $P < 0.001$, $n = 204$; male age partial correlation: r = -0.12, $P > 0.05$; thorax area partial correlation: r = 0.40, $P < 0.001$; Fwidth-B: $F_{2,\ 201} = 23.65$, $P < 0.001$, $n = 204$; male age partial correlation: r = -0.15, $P < 0.05$; thorax area partial correlation: r = 0.43, $P < 0.001$).

(iii) *Age and body size effects on calling song characteristics: Ten – 140 days post adult eclosion*

Nonlinear regression analysis predicted that an older, large male should call at a slower chirp and syllable rate than a younger, small male (CP: adjusted nonlinear $R^2 = 29.42\%$, Figure 2.6; SP: adjusted nonlinear $R^2 = 4.88\%$) and the rate at which chirp period and syllable period changes with male ageing is faster during the first 49 days of an adult male's life span, than after 49 days (Figure 2.6). These effects were mainly caused by the longer inter-chirp intervals and inter-syllable intervals that were produced by larger and older males (ICI: adjusted nonlinear $R^2 = 21.75\%$; ISI: adjusted nonlinear $R^2 = 25.55\%$). Bandwidth also changed at a faster rate in younger males, compared with older males, with older males producing narrower bandwidths than younger males. On the other hand, larger males produced wider bandwidths (Fwidth-A: adjusted nonlinear $R^2 = 14.30\%$; Fwidth-B: adjusted nonlinear $R^2 = 17.50\%$).

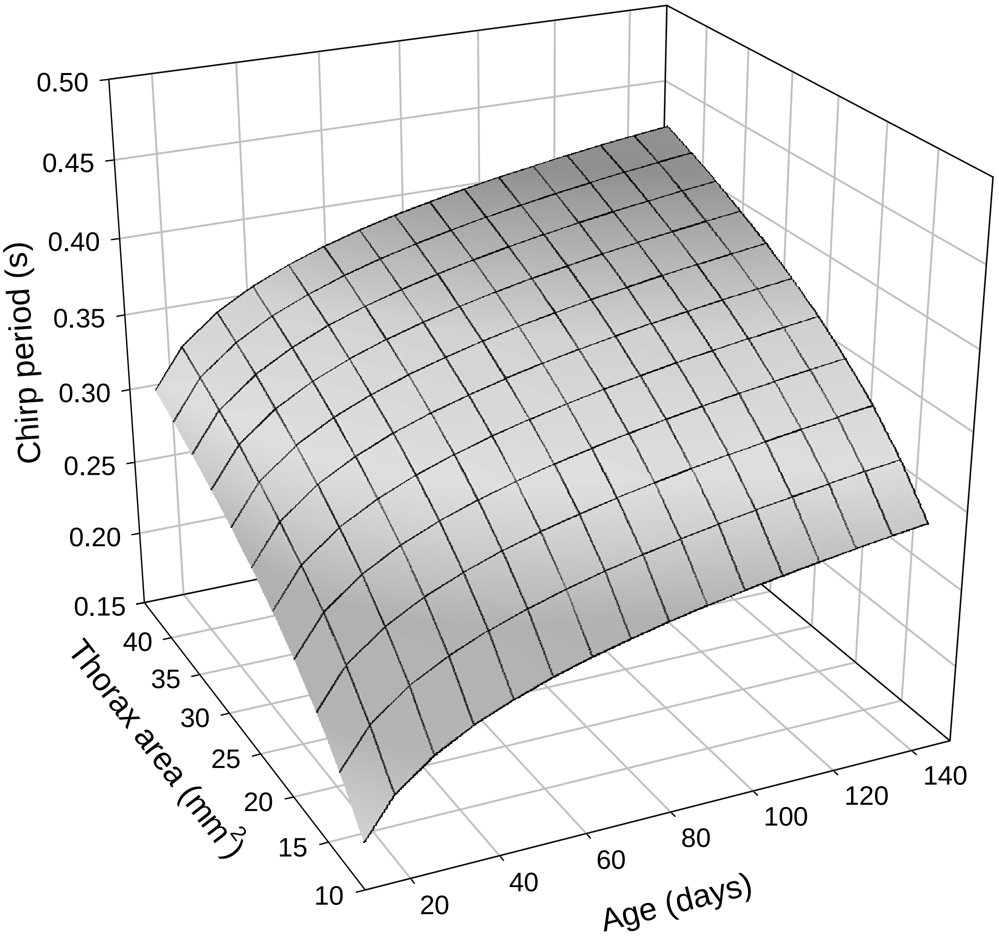


Figure 2.6. Predicted values for chirp period (adjusted nonlinear R^2 = 29.42%) of *G. bimaculatus* males as a function of body size (thorax area) and male age, based on nonlinear regression analysis (see results).

Between-individual variation in the calling song characteristics

Following REML analysis for nine – 69 days of adult age, the between-individual variance component contributed largely to the variation in bandwidth, chirp duration, syllable period and syllable duration (Table 2.1), while the within-individual variance component contributed largely to the variation in inter-syllable interval (Table 2.1). The variation in chirp period, inter-chirp interval, number of syllables per chirp and calling song frequency were mainly caused by the error variance component and unknown factors (Table 2.1).

Relationship between life span, body size and calling activity

There was no significant correlation between life span and any of the ten morphometric measurements (Table 2.1), i.e. life span was not size dependent ($-0.142 < r < 0.323$, $P > 0.05$, $n = 15$). There was also no detectable correlation between the mean number of seconds called per day and life span ($r = -0.08$, $P > 0.05$, $n = 15$; Figure 2.7a). After taking the effect of male size into account, forward stepwise multiple regression revealed that the partial correlation between the mean number of seconds called per day and life span was not significant ($r = -0.24$, > 0.05, $n = 15$). However, there was a significant partial correlation between the mean number of seconds called per day and thorax area ($r = 0.52$, $P < 0.05$, $n = 15$; Figure 2.7b), suggesting that while larger males spent more time calling per day than smaller males, life span had little influence on the daily calling activity of males.

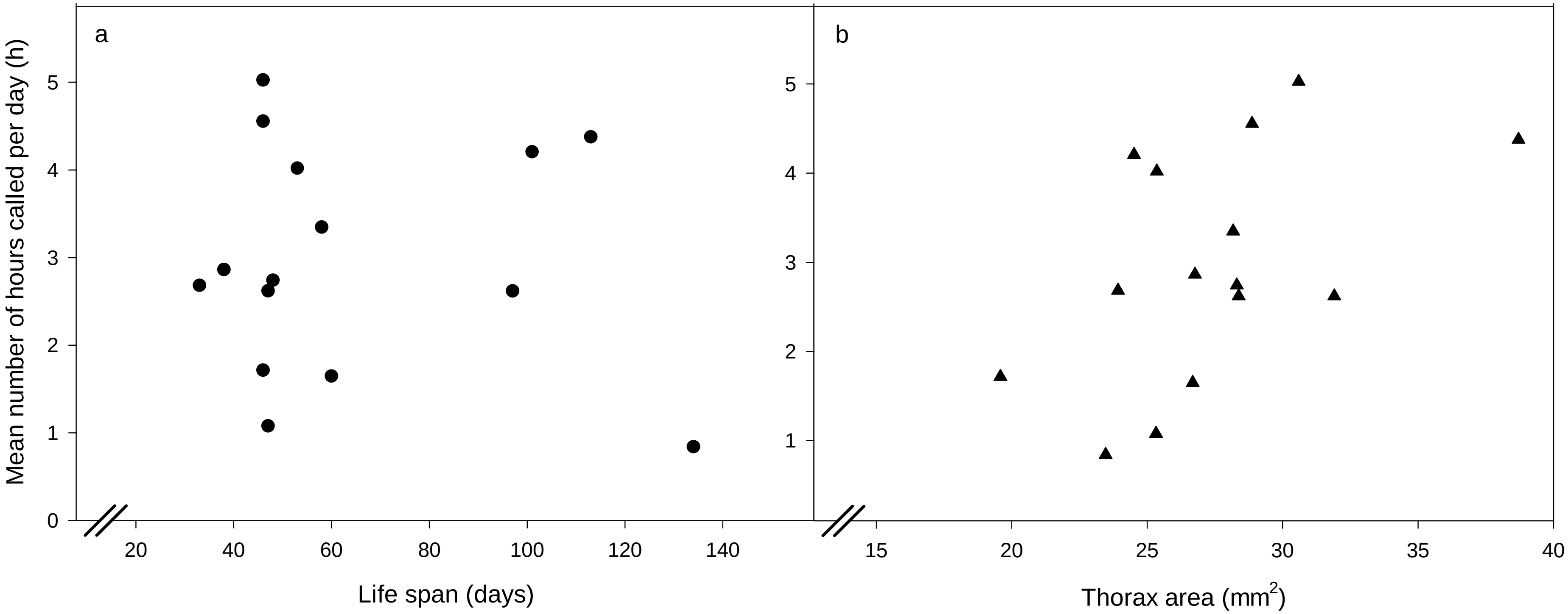


Figure 2.7. Mean number of hours (seconds were converted to hours for easier interpretation) that each *G. bimaculatus* male spent calling per day as a function of (a) life span and (b) male body size.

Effect of male size and male age on calling duration and calling intensity

Larger males spent significantly more hours per day calling (calling duration: partial correlation: r = 0.18, $P < 0.001$, $n = 655$) and more minutes per hour calling (calling intensity: partial correlation: r = 0.29, $P < 0.001$, $n = 654$), than smaller males. However, regardless of the effect of male size, calling intensity and calling duration decreased significantly with male age (calling intensity: partial correlation: r = -0.26, $P < 0.001$, $n = 654$; calling duration: partial correlation: r = -0.25, $P < 0.001$, $n = 655$).

Relationship between calling duration, calling intensity and chirp rate

Males that called at a faster chirp rate spent fewer minutes per hour calling than males with slower chirp rates (calling intensity: partial correlation: r = -0.19, $P < 0.01$, $n = 198$). However, this effect was not evident at a time scale longer than an hour (calling duration: partial correlation: r = -0.04, $P > 0.05$, $n = 197$).

Discussion

Effect of male age on the calling song characteristics

Two trends were observed in the temporal call traits within a realistic life span of 50 days for wild *G. bimaculatus* males (see below). Similar results were obtained irrespective of whether analyses were performed on a combined data set or separately on individual males. Firstly, at the chirp level, older males produced longer chirps and increased the intervals between chirps, resulting in longer chirp periods (Figure 2.5a). Secondly, at the syllable level, older males produced shorter syllables and longer intervals between syllables (Figure 2.5b). This suggests that a female could use syllable duration and chirp period to determine a male's age. The shorter syllables could be due to physical wear of the file, which resulted in a shorter contact period between the plectrum and the file. In the bushcricket, *Ephippiger ephippiger*, older males produced shorter syllables and had fewer pegs on the file, which form part of the stridulatory organs (Ritchie *et al*., 1995). Up to 49 days of age there was no significant effect of male age on the two calling song characteristics important for mate recognition in this species, namely syllable period and calling song frequency (Huber & Thorson, 1985; Schildberger *et al*., 1989). Calling song frequency either increased or decreased with male age (up to 49 days of age), suggesting that there are no directional changes that take place in calling song frequency with male ageing. Syllable period changed very little up to 49 days of age because, as syllable duration decreased, inter-syllable interval increased with male age (Figure 2.5b). Although two males had significantly longer syllable periods up to 49 days as they aged, a clear trend was only observed in one of them, while the other one had a variable syllable period. This supports the findings of Ferreira (2006, Chapter 3) that there is a large degree of within-individual stability in calling song frequency and syllable period (Table 2.1). The large within-individual variance component for inter-syllable interval largely reflects male ageing (Table 2.1). The inter-syllable interval is produced by the wing opening movement (Bennet-Clark, 1989) and ageing could cause older males to take longer in the stridulatory movements of their wings, compared with younger males. However, a physiological study is required to determine whether this is indeed the case. In contrast to my results, existing data describing the effect of age on cricket calls of several species do not show strong trends. Souroukis *et al*. (1992) found no significant correlation between syllable rate, percentage of missed syllables per trill and male age for *G. integer*. Supporting this, Martin *et al*. (2000) found no significant effect of male age on any of the call traits measured, for G. integer, except for a slight effect on duty cycle that could be explained by the experimental design or the fact that the result is not strongly significant. In *G. rubens*, syllable rate did not change significantly with age when measured at two days after adult eclosion and again after 25 days (Walker, 2000). The F_1 and F_2 male offspring of wild caught *G. integer* females showed no significant correlation between number of syllables per trill and male age (Gray & Cade, 1999). Ciceran *et al*. (1994) found no significant correlation of male age with syllable rate, number of syllables per chirp, inter-chirp duration or chirp duration in *G. pennsylvanicus*. No significant correlation of amplitude, number of syllables per chirp, frequency and inter-chirp interval with male age was found in the house cricket, *Acheta domesticus* (Gray, 1997). Brown *et al*. (1996) found no significant correlation of male age with frequency or syllable period in the black-horned tree cricket, *Oecanthus nigricornis*, but syllable duration decreased significantly with male ageing.

Relative calling activity throughout a male's life span

Two periods of intensive calling throughout the males' life spans were observed, firstly at around 22 days and secondly at around 72 days (Figure 2.3). The first peak at 22 days coincided with the highest median calling effort reported at 20 days after adult eclosion for *Teleogryllus commodus* reared on a high protein diet (Hunt *et al*., 2004). The second peak at 72 days is probably of no biological significance for three reasons. Firstly, the error in this estimate may be substantial due to the small sample size (*n* = 3) of animals at 72 days of age and older. Secondly, a single male contributed to most of the calling activity after 70 days of age and thirdly, it is highly unlikely that males become this old under natural conditions. Wild *G. bimaculatus* males caught by Simmons & Zuk (1992) had a mean (± SE) adult age of 12.6 ± 0.40 days (*n* = 97). Captive males in the present study had a mean (± SE) life span of 64.00 ± 7.98 days (*n* = 15), compared with Simmons's (1988b) study on lifetime mating success where captive *G. bimaculatus* males had a mean (± SE) life span of 37.46 ± 2.48 days (*n* = 10).

Relationships between male size, life span and calling activity

This study showed no significant correlation of male size with life span for *G. bimaculatus*, supporting Simmons's (1988b) results. No significant correlation was found between body mass and male life span for *G. pennsylvanicus* (Ciceran *et al*., 1994). In *T. commodus* male adult life span was affected by a combination of the protein quality of the rearing diet and reproductive effort, in the form of calling. Males reared on a high protein diet were heavier at adult eclosion but they invested more energy in calling during early adulthood and therefore died sooner (Hunt *et al*., 2004). This suggests that larger males do not necessarily live longer than smaller males. In addition, this study found no evidence that males with longer life spans have different circadian patterns of calling behaviour than males with shorter life spans. During my study, male size had a significant effect on calling activity, calling duration and calling intensity but not on the circadian pattern of calling behaviour. Larger males spent longer time periods per day calling than smaller males (Figure 2.7b), but this was not confined to early age as reported by Hunt *et al.* (2004). Large males could therefore increase their chances of mating through the longer durations of calling activity as shown by Hunt *et al.* (2004), where female *T. commodus* showed strong positive selection on calling effort in playback experiments in the wild. This study found no evidence that small males have different circadian patterns of calling behaviour compared with large males. This suggests that small and large males compete for the same females at a given time, since a small male does not avoid this competition by calling at a different time than a large male.

Interaction between body size and male age with respect to calling song characteristics

Several studies have shown that *G. bimaculatus* females prefer large males (Bateman *et al*., 2001; Simmons, 1986a, b) and Simmons (1988a) suggested that temporal traits could provide information relating to male size. Simmons & Zuk (1992) found that females preferred older males and suggested that females use male calling song as an indicator of male age. However, preference for older males could be an artifact of selection for large males or *vice versa*. Non-linear regression predicted that older, larger males produced slower chirp periods than younger, smaller males (Figure 2.6). It would therefore be necessary to control for male age or male size when female preference studies involving either one of these traits are conducted.

Effect of life span and male age on calling activity

Hunt *et al*. (2004) found that the life span of males on a high protein diet (fish pellets, containing 45% protein) decreased with an increase in mean nightly calling effort. Conversely, my study investigated the effect of life span on calling activity and found no correlation between life span and the mean number of seconds that a male spent calling per day, suggesting that males of all life spans probably invest the same amount of time in calling, and that life span has little influence on the amount of time that a male spent calling (Figure 2.7a). Males in my study were fed Pronutro® containing 16% protein, reflecting a high protein diet. On the other hand, male age was a significant determinant of the time that a male spent calling per day: young males spent more hours per day and more minutes per hour calling than older males, potentially increasing their chances of mating success.

The role of energetic constraints on calling intensity and circadian pattern of calling

This study showed that chirp rate did influence calling intensity (number of minutes called per hour). Males with faster chirp rates spent fewer minutes per hour calling than males with slower chirp rates. The energetic cost of calling at a faster chirp rate could limit the amount of time that males spent calling per day, since the energetic cost of calling increases linearly with calling rate (syllables per second) in several cricket species (Prestwich, 1994). However, at a longer time scale, chirp rate did not influence calling duration (number of hours called per day) probably because the energetic constraints on calling are more important at the scale of short time intervals. Energetic and feeding constraints may impose an upper limit to the number of hours a cricket can call each night. Indeed, most of the males did not call for the whole duration of the night, but for substantially shorter periods. The mean start time ranged from 7pm to 8pm, while the mean stop time ranged from 5am to 8am (Figure 2.2). Although there were large between-male differences in the circadian patterns, most males showed consistency throughout their life spans regarding their individual circadian patterns. In addition, this study found no significant differences between the categories of circadian patterns of calling behaviour for any of the call traits, suggesting that time of day, in a controlled environment, does not affect calling song characteristics.

Conclusion

In conclusion, female field crickets could potentially distinguish older males from younger ones based on the shorter syllable durations and slower chirp rates produced by older males. These findings provide a mechanism for the hypothesis of Simmons & Zuk (1992) that female *G. bimaculatus* choose older males by using cues in the male calling song. In contrast, two of the calling song characteristics important for mate recognition in *G. bimaculatus* (Huber & Thorson, 1985; Schildberger *et al*., 1989) changed very little during the realistic free-living life span (50 days) of a male cricket. These are calling song frequency (nominally 4.8 kHz) and syllable period (nominally 38 milliseconds). The shortening of the syllable duration during the first 50 days is compensated for by a corresponding lengthening of inter-syllable interval, resulting in a constant syllable period. These differences in degree of constancy between call traits of male crickets probably reflect different selective forces that operate on separate parts of the cricket communication system. In-depth studies are required for a comprehensive understanding of age effects within the context of sexual selection in crickets.

Acknowledgements

I would like to thank Luke Verburgt for assisting with the call recordings. The National research foundation (NRF) and University of Pretoria is thanked for financial support.

References

Alatalo, R. V., Gustafsson, L. & Lundberg, A. 1986. Do females prefer older males in polygynous bird species? *The American Naturalist* **127**: 241-245.

Alexander, R. D. 1962. Evolutionary change in cricket acoustical communication. *Evolution* **16**: 443-467.

Bateman, P. W., Gilson, L. N. & Ferguson, J. W. H. 2001. Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Animal Behaviour* **61**: 631-637.

Beck, C. W. & Powell, L. A. 2000. Evolution of female mate choice based on male age: Are older males better mates? *Evolutionary Ecology Research* **2**: 107-118.

Becker, W. A. 1984. Repeatability. In: *Manual of quantitative genetics*, pp37-41. Fourth edition. Academic Enterprises, Pullman.

Bennet-Clark, H. C. 1989. Songs and the physics of sound production. In: *Cricket behavior and neurobiology*, pp228-261. Huber, F., Moore, T. E. & Loher, W. (Eds). Cornell University Press, New York.

Bertram, S. M. 2000. The influence of age and size on temporal mate signalling behaviour. *Animal Behaviour* **60**: 333-339.

Brown, W. D., Wideman, J., Andrade, M. C. B., Mason, A. C. & Gwynne, D. T. 1996. Female choice for an indicator of male size in the song of the black-horned tree cricket, *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evolution* **50**: 2400-2411.

Ciceran, M., Murray, A-M. & Rowell, G. 1994. Natural variation in the temporal patterning of calling song structure in the field cricket *Gryllus pennsylvanicus*: effects of temperature, age, mass, time of day, and nearest neighbour. *Canadian Journal of Zoology* **72**: 38-42.

Dixon, K. A. & Cade, W. H. 1986. Some factors influencing male-male aggression in the field cricket *Gryllus integer* (time of day, age, weight and sexual maturity). *Animal Behaviour* **34**: 340-346.

Ferreira, M. 2006. High levels of environmentally-induced variation in the calling song of the field cricket, *Gryllus bimaculatus* De Geer (Orthoptera: Gryllidae). Chapter 3, MSc thesis, University of Pretoria, Pretoria, South Africa.

Ferreira, M. & Ferguson, J. W. H. 2002. Geographic variation in the calling song of the field cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) and its relevance to mate recognition and mate choice. *Journal of Zoology London* **257**: 163-170.

Gil, D., Cobb, J. L. S. & Slater, P. J. B. 2001. Song characteristics are age dependent in the willow warbler, *Phylloscopus trochilus*. *Animal Behaviour* **62**: 689-694.

Gray, D. A. 1997. Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Animal Behaviour* **54**: 1553-1562.

Gray, D. A. & Cade, W. H. 1999. Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. *Evolution* **53**: 848-854.

Hansen, T. F. & Price, D. K. 1995. Good genes and old age: Do old mates provide superior genes? *Journal of Evolutionary Biology* **8**: 759-778.

Huber, F. & Thorson, J. 1985. Cricket auditory communication. *Scientific American* **253**: 60-68.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L. & Bussière, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**: 1024-1027.

Kokko, H. 1998. Good genes, old age and life-history trade-offs. *Evolutionary Ecology* **12**: 739-750.

Manning, J. T. 1985. Choosy females and correlates of male age. *Journal of Theoretical Biology* **116**: 349-354.

Martin, S. D., Gray, D. A. & Cade, W. H. 2000. Fine-scale temperature effects on cricket calling song. *Canadian Journal of Zoology* **78**: 706-712.

Mountjoy, D. J. & Lemon, R. E. 1995. Extended song learning in wild European starlings. *Animal Behaviour* **49**: 357-366.

Prestwich, K. N. 1994. The energetics of acoustic signaling in anurans and insects. *American Zoologist* **34**: 625-643.

Price, D. K. & Hansen, T. F. 1998. How does offspring quality change with age in male *Drosophila melanogaster*? *Behavior Genetics* **28**: 395-402.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223-225.

Ritchie, M. G., Couzin, I. D. & Snedden, W. A. 1995. What's in a song? Female bushcrickets discriminate against the song of older males. *Proceedings of the Royal Society of London Series* B **262**: 21-27.

Schildberger, K., Huber, F. & Wohlers, D. W. 1989. Central auditory pathway: neuronal correlates of phonotactic behaviour. In: *Cricket behaviour and neurobiology*, pp423-458. Huber, F., Moore, T. E. & Loher, W. (Eds). Cornell University Press, New York.

Sherrod, P. H. 2002. Nonlinear Regression Analysis Program, NLREG version 5.4. Philip H. Sherrod. Nashville, TN.

Simmons, L. W. 1986a. Female choice in the field cricket *Gryllus bimaculatus* (De Geer). *Animal Behaviour* **34**: 1463-1470.

Simmons, L. W. 1986b. Inter-male competition and mating success in the field cricket *Gryllus bimaculatus* (De Geer). *Animal Behaviour* **34**: 567-579.

Simmons, L. W. 1988a. The calling song of the field cricket, *Gryllus bimaculatus* (De Geer): constraints on transmission and its role in intermale competition and female choice. *Animal Behaviour* **36**: 380-394.

Simmons, L. W. 1988b. Male size, mating potential and lifetime reproductive success in the field cricket, *Gryllus bimaculatus* (De Geer). *Animal Behaviour* **36**: 372-379.

Simmons, L. W. & Zuk, M. 1992. Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. *Animal Behaviour* **44**: 1145-1152.

Souroukis, K., Cade, W. H. & Rowell, G. 1992. Factors that possibly influence variation in the calling song of field crickets: temperature, time, and male size, age, and wing morphology. *Canadian Journal of Zoology* **70**: 950-955.

Walker, T. J. 2000. Pulse rates in the songs of trilling field crickets (Orthoptera: Gryllidae: *Gryllus*). *Annals of the Entomological Society of America* **93**: 565-572.

Zuk, M. 1987. Variability in attractiveness of male field crickets (Orthoptera: Gryllidae) to females. *Animal Behaviour* **35**: 1240-1248.

Zuk, M. 1988. Parasite load, body size, and age of wild-caught male field crickets (Orthoptera: Gryllidae): effects on sexual selection. *Evolution* **42**: 969-976.