

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

Lauren P. Fitzsimmons<sup>1</sup> & Susan M. Bertram

(Department of Biology, Carleton University, Ottawa, ON, Canada K1S 5B6)

(Accepted: 12 July 2011)

---

## Summary

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

*Keywords:* acoustic signal, age, bioacoustics, life history, mating effort, senescence, sexual selection.

## 1. Introduction

Much research in behavioural ecology is dedicated to investigating what information males communicate to females through their sexual displays. Fisher (1958) proposed that female preference for male displays might increase her chance of choosing a high-quality male with whom to mate.

---

<sup>1</sup>) Corresponding author's e-mail address: lauren.p.fitzsimmons@gmail.com

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

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

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

every male is included in our analyses. We provide the most thorough exploration of lifetime calling patterns in crickets to date.

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

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

We examined the relationship between calling effort and longevity in *G. veletis* to assess whether males that invest heavily in calling effort pay a longevity cost or whether high-effort callers live longer than low-effort callers. We also investigated whether longevity was related to the onset of calling, and whether longevity was related to male body size or condition. We explore the relationship between calling effort and longevity with a higher degree of precision in the measure of calling effort than previous studies

(Hunt et al., 2004; Judge et al., 2008) since we recorded males continuously throughout their lives.

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

## 2. Material and methods

### 2.1. *Experimental animals*

We captured adult male and female *G. veletis* in Ottawa, ON, Canada (45°19'N, 75°40'W) in May and June 2008. Males used in this experiment were the first generation of lab-reared offspring from the field-captured individuals. Late-instar nymph males were removed from the colony and checked daily for adult eclosion. Adult males were individually housed in circular, clear plastic 540 ml containers. Crickets were provided with water and food (Harlan Teklad Laboratory Rodent Diet No. 8604: 24% crude protein, 4% crude fat, 4.5% crude fibre) ad libitum and were held in a temperature-controlled room ( $30 \pm 2^\circ\text{C}$ ).

### 2.2. *Acoustic mate attraction displays*

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

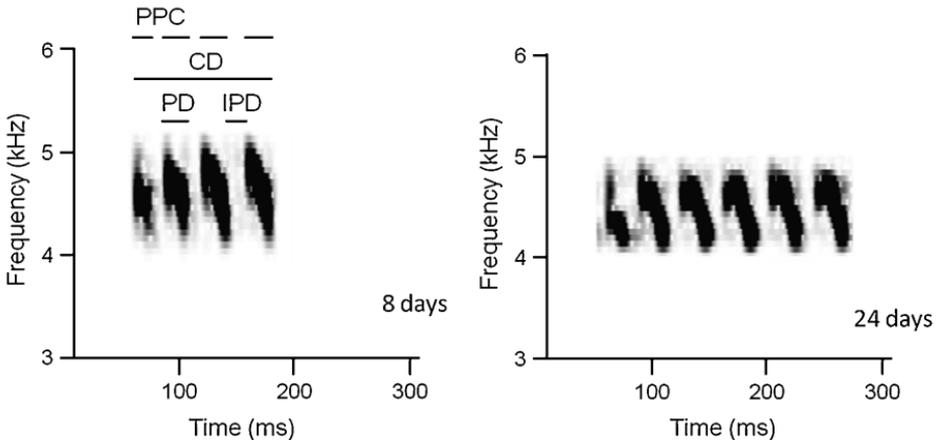
To quantify whether cricket calls change as males age, we continuously recorded long-distance acoustic mate attraction signals until day 35 of adulthood using real-time electronic analysis ( $N = 64$  males). To quantify the relationship between signalling and longevity, a subset of the males was left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

**Table 1.** CricketSong recording settings and thresholds for *G. veletis*.

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

Crickets produce sound by rubbing their forewings together. The fine-scale structure of cricket calls is determined by wing properties and the rate and pattern of wing movement. Each closing stroke produces a pulse of sound, and pulses are concatenated together to produce chirps. A series of chirps is concatenated together to produce a bout of song. We define a song as a series of chirps in which the male does not pause for longer than 30 s; once a male pauses for 30 s or longer, he is considered to have begun a new song bout (following Bertram & Warren, 2005; Bertram & Bowen, 2006). Because our recording system continuously monitored males' acoustic behaviour, our measure of daily time spent calling quantifies the total amount of time each male spent calling during a 24-h period.

To quantify whether daily acoustic signalling parameters were affected by male age, we determined each male's mean values for time spent calling, calling effort, song duration, pulse rate, number of pulses per chirp, chirp duration, interchirp duration, pulse duration, interpulse duration, amplitude, and carrier frequency (Figure 1) for each full day of recording (measuring all sound pulses produced). Hourly calling parameter averages were weighted by the number of pulses produced in the hour. In this way, hours with many pulses were given heavier weight than hours when relatively few pulses were



**Figure 1.** Spectrograms of example *G. veletis* acoustic mate attraction calls measured in this study. One chirp is shown at each of two ages (8 and 24 days); these two chirps were produced by the same male. The chirp produced at age 28 days has more pulses, a longer chirp duration, slower pulse rate, and a lower carrier frequency than the chirp produced at 8 days, as was typical for older males in our study. Four of the fine-structural characteristics we measured are indicated on the upper chirp. PPC, number of pulses per chirp; CD, chirp duration; PD, pulse duration; IPD, interpulse duration.

produced. Pulse rate (No. pulses/s) was calculated at a later date using the equation: (No. pulses per chirp/chirp duration)  $\times$  1000.

### 2.3. *Body size*

Males were weighed on day 7 of adulthood using a Denver Instruments balance (Pinnacle Series model PI-314; precision  $\pm 0.1$  mg). We measured head width, pronotum length, and pronotum width for each cricket at the end of the experiment (either 35 days old or following natural death; see below for details). Measurements were taken using a Zeiss Axio Observer inverted dissecting microscope and highly magnified photographs (AxioVision v4.8, Carl Zeiss, Jena, Germany; magnification approx.  $8.5\times$ , resolution approx.  $1.60\ \mu\text{m}$ ).

### 2.4. *Statistical methods*

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

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

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

To assess whether calls could be statistically classified to age classes, we performed a discriminant function analysis (DFA). We took daily mean values for all calling parameters over the expected natural lifespan (28 days), and classified them by week (4 levels). Due to multicollinearity of calling parameters, we performed a PCA that generated two new variables with eigenvalues  $> 1$  for use in the DFA (Tabachnick & Fidell, 2007). PC1 explained 79.4% of the variation in calling parameters, and was heavily loaded by all parameters (all loadings  $> |0.67|$ ; highest loadings: calling effort = 0.99, amplitude = 0.99, pulse rate = 0.98). PC2 explained 12.3% of the variation, and was loaded by pulse duration (0.65), carrier frequency ( $-0.54$ ), and chirp duration (0.51). We used a fraction of our data to generate a training function, and we then used a cross-validated approach to classify the remainder of our data using this function.

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

### 3. Results

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

#### 3.1. Question 1: do calls change as males age?

Our GLMM analyses revealed that male age explained significant amounts of variation for all calling parameters (range: 7–92%; Table 2). Age explained the most variation for time spent calling (92%), song duration (42%), chirp duration (41%), and interchirp duration (47%). Identity explained variation in all calling parameters but time spent calling (range: 11–60%), and was highest for carrier frequency (60%) and chirp duration (49%). Size was not a significant factor in any of the models, and condition was only significant in explaining 7% of the variation in song duration. The interactions between condition and age, and size and age, were significant in some models but never explained more than 0.2% of the variation for any calling parameter (Table 2).

Given that age explains a significant amount of variation for all calling parameters, we plotted the relationships between acoustic parameters and age to help understand how calls changed as males aged. We found that both the quantity of calling and the acoustic structure of calls changed as males aged (Table 2; Figure 1). Song duration and pulse rate decreased, while the number of pulses per chirp, chirp duration, and interchirp duration increased (Figure 2). Pulse duration increased with age, and interpulse duration peaked late in life. Both call amplitude and carrier frequency decreased with age (Figure 2). In general, older males called with reduced effort compared to young males. Older males had less time spent calling, shorter song durations, quieter songs, longer interchirp durations, and longer interpulse durations. However, some of the changes reflect higher effort in older males: older males produced more pulses per chirp, and consequently longer chirp durations, as well as longer pulse durations. The small peaks in some parameters at old ages, such as time spent calling (Figure 2a), likely reflect noise due to small sample sizes at those times (e.g., only two males were sampled beyond day 80), and likely do not represent meaningful changes in call parameters over time.

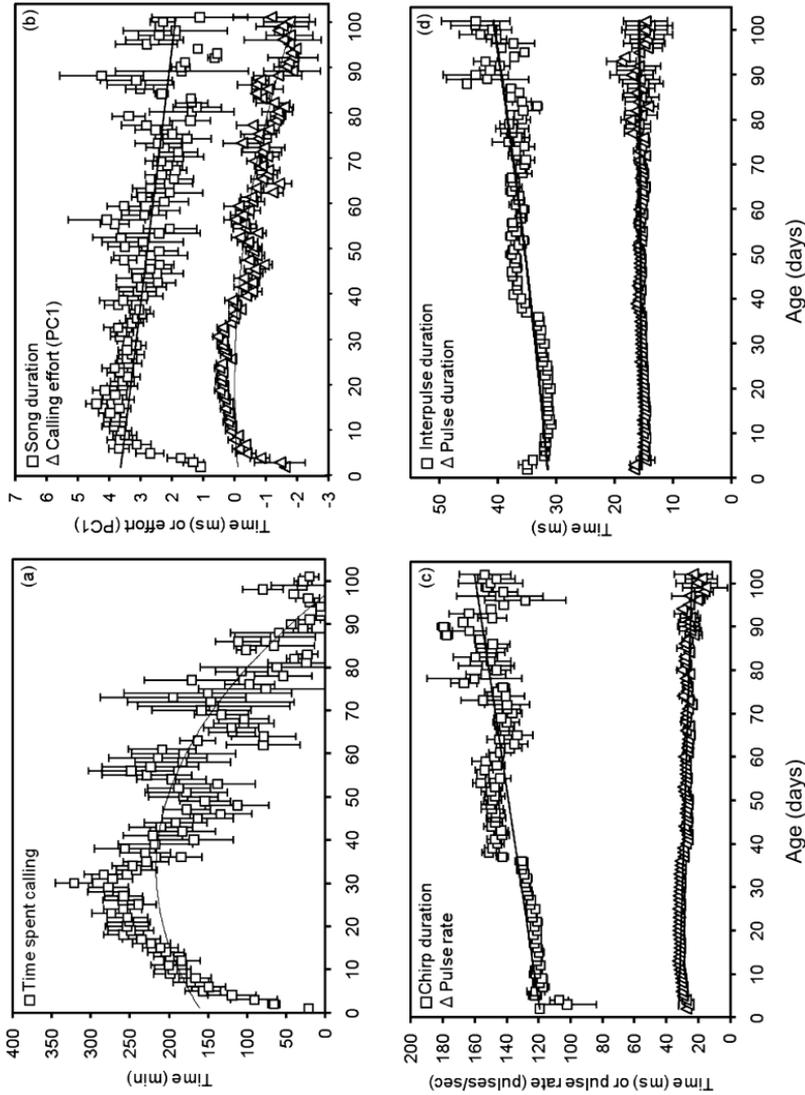
**Table 2.** The influence of male age, identity, condition, and body size on *G. veletis* acoustic mate attraction signal parameters using generalized linear mixed models ( $N = 62$  males).

Acoustic parameter	Effect	Estimated variance	df	$t$ /Wald Z	$p$	% Variance explained
Time spent calling	Age	28785.04		10.79	<0.001	92.44
	Identity	2105.16		1.03	0.30	
	Condition	-5.45	96.75	-0.38	0.71	
	Size	-0.74	55.85	-0.16	0.88	
	Condition $\times$ Age	-0.083	157.71	-0.29	0.77	
	Size $\times$ Age	-0.003	153.00	-0.31	0.76	
Calling effort	Age	0.96		15.36	<0.001	36.51
	Identity	0.64		4.10	<0.001	24.38
	Condition	-0.12	74.61	-0.91	0.36	
	Size	-0.007	52.66	-0.016	0.99	
	Condition $\times$ Age	0.005	196.68	2.36	0.019	0.18
	Size $\times$ Age	-0.002	169.40	-3.85	<0.001	0.008
Song duration	Age	2.75		21.52	<0.001	41.68
	Identity	1.93		4.50	<0.001	29.27
	Condition	-0.44	68.66	-2.14	0.036	6.63
	Size	0.062	52.85	0.84	0.40	
	Condition $\times$ Age	0.0070	308.60	2.50	0.013	0.10
	Size $\times$ Age	-0.0004	275.40	-5.46	<0.001	0.006
Pulse rate	Age	9.79		15.73	<0.001	18.52
	Identity	18.59		4.74	<0.001	35.15
	Condition	-0.47	61.74	-0.79	0.43	
	Size	-0.10	52.73	-0.46	0.65	
	Condition $\times$ Age	0.012	186.87	1.93	0.055	
	Size $\times$ Age	-0.0012	163.19	-7.48	<0.001	0.002
Pulses per chirp	Age	0.36		14.90	<0.001	7.20
	Identity	0.67		4.71	<0.001	13.36
	Condition	-0.065	62.53	-0.56	0.58	
	Size	0.013	52.97	0.31	0.76	
	Condition $\times$ Age	-0.0036	173.15	-0.28	0.78	
	Size $\times$ Age	-0.0006	150.58	-1.78	0.077	
Chirp duration	Age	297.14		13.85	<0.001	40.88
	Identity	349.42		4.38	<0.001	49.06
	Condition	0.067	67.21	0.024	0.98	
	Size	0.98	52.34	0.98	0.33	
	Condition $\times$ Age	-0.075	169.98	-2.02	0.045	0.010
	Size $\times$ Age	0.0045	143.70	4.56	<0.001	0.0006

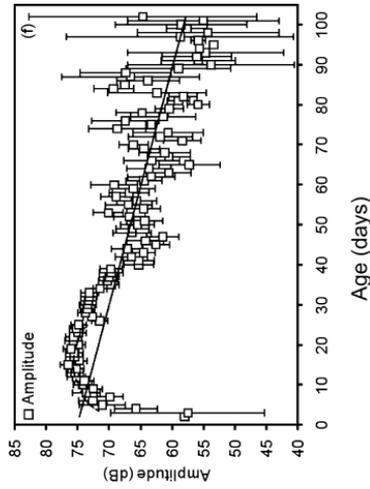
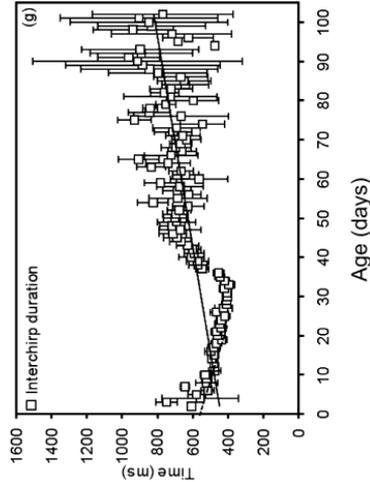
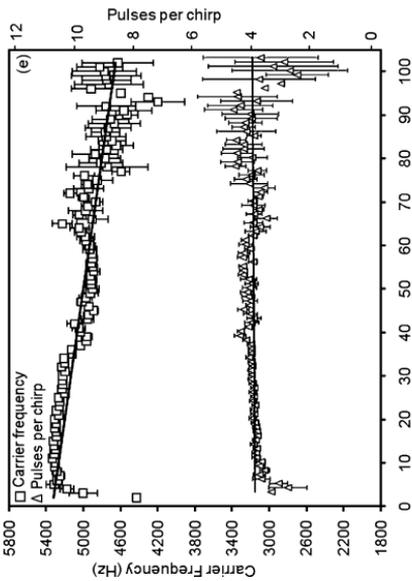
**Table 2.** (Continued.)

Acoustic parameter	Effect	Estimated variance	df	<i>t</i> /Wald <i>Z</i>	<i>p</i>	% Variance explained
Interchirp duration	Age	27 059.00		14.09	<0.001	46.89
	Identity	25 011.27		4.29	<0.001	43.34
	Condition	15.80	71.44	0.66	0.51	
	Size	-1.13	53.26	-0.13	0.90	
	Condition × Age	-0.40	168.98	-1.15	0.25	
	Size × Age	0.012	146.62	1.34	0.18	
Pulse duration	Age	1.62		17.83	<0.001	9.04
	Identity	2.03		4.68	<0.001	11.31
	Condition	-0.16	64.90	-0.80	0.43	
	Size	0.040	53.42	0.52	0.60	
	Condition × Age	0.0032	221.49	1.32	0.19	
	Size × Age	-0.0001	195.35	-1.72	0.086	
Interpulse duration	Age	8.15		15.19	<0.001	18.12
	Identity	5.55		4.09	<0.001	12.34
	Condition	0.46	74.06	1.24	0.22	
	Size	0.023	52.40	0.18	0.86	
	Condition × Age	-0.015	191.70	-2.55	0.012	0.033
	Size × Age	0.0015	167.50	9.90	<0.001	0.0034
Amplitude	Age	63.59		18.59	<0.001	31.32
	Identity	64.77		4.58	<0.001	31.90
	Condition	-0.76	65.40	-0.68	0.50	
	Size	0.022	52.47	0.052	0.96	
	Condition × Age	0.046	236.35	3.13	0.002	0.022
	Size × Age	-0.0026	209.06	-6.79	<0.001	0.0013
Carrier frequency	Age	29 380.22		24.49	<0.001	32.89
	Identity	54 649.89		4.86	<0.001	60.56
	Condition	-21.27	59.04	-0.67	0.51	
	Size	-11.43	52.41	-0.95	0.34	
	Condition × Age	0.60	295.94	2.02	0.044	<0.001
	Size × Age	-0.062	264.28	-8.25	<0.001	<0.001

The analyses of how calling parameters changed as males aged during their natural lifetime revealed almost identical results to the analyses using our full data set (Table 3). The only notable difference was that identity explained a significant amount of variation in time spent calling (26%) when analyzing the natural lifetime but not the extended laboratory lifetime. As in the analyses with the full data set, age explained the most variation in time spent calling and song duration, whereas individual differences explained



**Figure 2.** The change in calling parameters as males aged: (a) time spent calling, (b) song duration and calling effort, (c) chirp duration and pulse rate, (d) interpulse duration and pulse duration, (e) carrier frequency and pulses per chirp, (f) amplitude and (g) interchirp duration.



**Figure 2.** (Continued.) A comparison of the different relationships for the expected lifespan of a cricket in the wild (28 days, short dashed line) and for the extended lifespan of crickets in the laboratory (up to 102 days, solid line) is shown for amplitude and interchirp duration. Values are daily means and error bars represent standard error. Untransformed data shown.

**Table 3.** The influence of male age, identity, condition, and body size on *G. veltis* acoustic mate attraction signal parameters using generalized linear mixed models ( $N = 62$  males; over expected natural lifespan of 28 days).

Acoustic parameter	Effect	Estimated variance	df	$t$ /Wald Z	$p$	% Variance explained
Time spent calling	Age	16 865.77		9.08	<0.001	72.66
	Identity	6031.57		2.42	0.016	25.99
	Condition	15.20	77.94	0.98	0.33	
	Size	-4.73	51.31	-0.89	0.38	
	Condition $\times$ Age	-1.06	99.72	-2.00	0.048	0.0046
	Size $\times$ Age	0.21	98.08	12.11	<0.001	0.0009
Calling effort	Age	0.68		11.59	<0.001	22.32
	Identity	0.66		4.07	<0.001	21.72
	Condition	0.021	83.29	0.16	0.88	
	Size	-0.036	52.10	-0.79	0.43	
	Condition $\times$ Age	0.0083	113.59	0.22	0.83	
	Size $\times$ Age	0.0010	110.73	8.18	<0.001	0.033
Song duration	Age	2.56		14.05	<0.001	38.40
	Identity	2.01		4.13	<0.001	30.16
	Condition	-0.084	87.07	-0.36	0.72	
	Size	-0.004	53.30	-0.051	0.96	
	Condition $\times$ Age	-0.004	147.68	-0.53	0.60	
	Size $\times$ Age	0.0009	144.90	4.05	<0.001	0.013
Pulse rate	Age	7.47		15.00	<0.001	14.88
	Identity	20.26		4.79	<0.001	40.38
	Condition	0.032	63.99	0.050	0.96	
	Size	-0.12	52.28	-0.53	0.60	
	Condition $\times$ Age	-0.014	151.74	-1.24	0.22	
	Size $\times$ Age	0.002	149.17	5.61	<0.001	0.004
Pulses per chirp	Age	0.25		12.52	<0.001	4.70
	Identity	0.61		4.74	<0.001	11.38
	Condition	0.049	68.50	0.44	0.66	
	Size	-0.007	53.64	-0.17	0.86	
	Condition $\times$ Age	-0.002	121.22	-1.09	0.28	
	Size $\times$ Age	0.0004	118.40	5.51	<0.001	0.007
Chirp duration	Age	337.58		4.46	<0.001	29.80
	Identity	337.58		4.46	<0.001	54.31
	Condition	0.97	74.06	0.35	0.73	
	Size	0.39	53.49	0.40	0.69	
	Condition $\times$ Age	-0.020	105.63	-0.30	0.76	
	Size $\times$ Age	0.0066	101.97	3.07	0.003	0.001

**Table 3.** (Continued.)

Acoustic parameter	Effect	Estimated variance	df	<i>t</i> /Wald <i>Z</i>	<i>p</i>	% Variance explained
Interchirp duration	Age	18 910.52		10.48	<0.001	42.36
	Identity	20 055.96		4.14	<0.001	44.93
	Condition	-3.16	86.62	-0.14	0.89	
	Size	1.18	54.90	0.15	0.88	
	Condition × Age	0.59	104.77	0.88	0.38	
	Size × Age	-0.18	101.60	-8.73	<0.001	0.0004
Pulse duration	Age	1.69		9.69	<0.001	8.77
	Identity	1.65		4.16	<0.001	8.54
	Condition	0.21	84.93	0.98	0.33	
	Size	0.0021	55.65	0.029	0.98	
	Condition × Age	-0.0093	84.97	-1.48	0.14	
	Size × Age	0.00007	82.38	0.36	0.72	
Interpulse duration	Age	5.15		10.42	<0.001	11.74
	Identity	4.89		3.94	<0.001	11.14
	Condition	0.038	85.24	0.10	0.92	
	Size	-0.0081	52.06	-0.006	0.99	
	Condition × Age	0.016	99.10	1.45	0.15	
	Size × Age	-0.0010	96.26	-2.84	0.005	0.002
Amplitude	Age	48.53		14.39	<0.001	25.95
	Identity	56.90		4.42	<0.001	30.42
	Condition	0.11	75.50	0.096	0.92	
	Size	-0.24	52.05	-0.59	0.56	
	Condition × Age	0.020	137.81	0.67	0.50	
	Size × Age	0.0052	135.48	5.57	<0.001	0.003
Carrier frequency	Age	27 084.10		12.57	<0.001	33.84
	Identity	47 078.76		4.60	<0.001	58.82
	Condition	-21.36	72.55	-0.66	0.51	
	Size	-11.26	53.46	-0.98	0.33	
	Condition × Age	0.18	118.28	0.25	0.81	
	Size × Age	0.0049	115.68	0.21	0.84	

more variation for pulse duration, chirp duration, amplitude, and carrier frequency (Table 3). When we plotted the relationships, many were similar in slope and direction, but a few changed entirely. For example, in the analyses limited to natural lifespan, daily calling effort peaked late instead of early, amplitude peaked mid-life instead of linearly decreasing, and interchirp duration decreased instead of increased (Figure 2f,g).

**Table 4.** Mean and standard error values for *G. veletis* long-distance acoustic calling parameters at three ages.

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

To account for potential change in the population structure over time, only males who were recorded at all three time points were included ( $N = 15$  males). All calling parameters changed significantly as males aged (see Table 2).

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

For descriptive purposes, mean values for calling parameters at age 10, 30, and 50 days are shown for 15 males that were recorded at all three ages (Table 4). Table 4 highlights how time spent calling, calling effort, song duration, and pulses per chirp peaked in mid-life and decreased with age, whereas silent intervals (interchirp duration and interpulse duration) increased as males aged. Amplitude and carrier frequency were also lowest when males were 50 days old.

### 3.2. Question 2: what is the relationship between calling effort and longevity?

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

The onset of calling was not related to mean daily or lifetime calling effort (daily calling effort:  $r_{\text{adj}}^2 = -0.03$ ,  $F_{1,18} = 0.46$ ,  $p = 0.50$ ; lifetime calling effort:  $r_{\text{adj}}^2 = 0.02$ ,  $F_{1,18} = 1.30$ ,  $p = 0.27$ ). Longevity was not related to male body size or condition ( $r_{\text{adj}}^2 = -0.03$ ,  $F_{2,25} = 0.58$ ,  $p = 0.57$ ).

## 4. Discussion

### 4.1. Do calls change as males age?

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

A few hypotheses have been proposed to account for the changes in cricket signalling as males age. Older males are expected to invest more effort in reproduction as their residual reproductive value decreases as they age (Williams, 1966). However, our results do not support this idea, as we found that older males invested less into signalling than younger males. Cricket wings are subject to wear and tear as males age, which likely affects the fine structure of their calls (Jacot et al., 2007; Judge, 2011). Further, Verburt et al. (2011) suggest that since insect flight muscles degrade as males age, and the same muscles are used to produce calls, age-related degradation in flight muscles should lead to predictable changes in call structure. Our results support this hypothesis; older males called with longer interpulse and interchirp durations, increasing the silent portions of their songs, and investing less energy into their calls, as do male *G. bimaculatus* (Verburt et al.,

2011). This may be due to increased muscle recovery time and may be a strategy to conserve energy to perform longer song bouts than would otherwise be possible with degraded calling muscles. More work is needed to test whether wing wear or muscle degradation or both is responsible for the fine structural changes in cricket calls over their lifetime.

How do our results compare to previous studies of time spent calling in *G. veletis*? Cade & Wyatt (1984) reported that *G. veletis* males begin acoustic calling between 3 and 6 days past adult moult, with an average of 5.8 days; we found the same average onset of calling, but a larger range of onset of calling (2–13 days). In the earliest study of calling effort, Alexander & Meral (1967) found a few high-effort callers that called for ten hours per day, based on monitoring which males were calling every couple of hours in the field; our highest effort callers called for as much as fourteen hours per day based on continuous sampling. In the laboratory, Cade & Wyatt (1984) found that *G. veletis* males called for 7.5 h on average per night; in contrast, French & Cade (1989) monitored which males were calling every five minutes in the field, and found a mean time spent calling of 1.8 h per night. Our results fall between these two studies, with a mean of 3.6 h of calling per night. Our results are similar to those of Burpee & Sakaluk (1993), which reported that males called for 2.5 h on average in the laboratory.

Only one previous study investigated whether time spent calling changes as males age in *G. veletis*. Cade & Wyatt (1984) recorded males for 45 days and found that there was no change in the amount of time spent calling as males aged; in contrast, we found that time spent calling peaked at day 20 and decreased thereafter. There is no previous information on how any of the other calling parameters measured change as *G. veletis* age, although recent studies have investigated these relationships in other cricket species. We found that, in general, older males called with reduced effort compared to young males. Older males had lower time spent calling, shorter song durations, quieter songs, and longer periods of silence within their chirps (longer interchirp durations and interpulse durations). However, older males also produced more pulses per chirp, and consequently longer chirp durations, as well as longer pulse durations, which reflect higher effort. Our results are quite similar to those of Verburt et al. (2011), which reported that all *G. bimaculatus* call traits declined in energetic quality as males aged except pulses per chirp and chirp duration. Older male *G. bimaculatus* produced calling songs with decreased pulse durations, song durations, and amplitude,

and increased interchirp and intersong intervals. Verburt et al. (2011) note that the increase in number of pulses per chirp does not compensate for the decrease in pulse duration and increase in interchirp interval; the energetic content of the calls of *G. bimaculatus* decreases significantly with age.

#### 4.2. What is the relationship between calling effort and longevity?

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

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

#### Acknowledgements

We are grateful to Jay Fitzsimmons for cricket catching assistance and helpful comments on the manuscript. Funding was provided by a Natural Science and Engineering Research Council of Canada (NSERC) Canada Graduate Scholarship to L.P.F., a NSERC Discovery

Grant to S.M.B., the Canadian Foundation for Innovation to S.M.B., the Ontario Research Fund to S.M.B., and Carleton University.

## References

- Alexander, R.D. & Meral, G.H. (1967). Seasonal and daily chirping cycles in the northern spring and fall field crickets, *Gryllus veletis* and *G. pennsylvanicus*. — *Ohio J. Sci.* 67: 200-209.
- Bertram, S.M. & Bowen, M. (2006). Field cricket species differences in the temporal patterns of long-distance mate attraction signals. — *Ethology* 112: 850-857.
- Bertram, S.M. & Warren, P.S. (2005). Trade-offs in signalling components differ with signalling effort. — *Anim. Behav.* 70: 477-484.
- Brooks, R. & Kemp, D.J. (2001). Can older males deliver the good genes? — *Trends Ecol. Evol.* 16: 308-313.
- Burpee, D.M. & Sakaluk, S.K. (1993). The effect of pair formation on diel calling patterns in two cricket species, *Gryllus veletis* and *Gryllodes signillatus* (Orthoptera: Gryllidae). — *J. Insect Behav.* 6: 431-440.
- Cade, W.H. & Wyatt, D.R. (1984). Factors affecting calling behaviour in field crickets, *Teleogryllus* and *Gryllus* (age, weight, density, and parasites). — *Behaviour* 88: 61-75.
- Crow, J.F. (2000). The origins, patterns, and implications of human spontaneous mutation. — *Nature Rev. Genet.* 1: 40-47.
- Fisher, R.A. (1958). *The genetical theory of natural selection.* — Dover Publications, New York, NY.
- Freckleton, R.P. (2002). On the misuse of residuals in ecology: regression of residuals vs. multiple regression. — *J. Anim. Ecol.* 71: 542-545.
- French, B.W. & Cade, W.H. (1989). Sexual selection at varying population densities in male field crickets, *Gryllus veletis* and *G. pennsylvanicus*. — *J. Insect Behav.* 2: 105-121.
- Gustafsson, L., Qvarnstrom, A. & Sheldon, B.C. (1995). Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. — *Nature* 375: 311-313.
- Hansen, T.F. & Price, D.K. (1995). Good genes and old age: do old mates provide superior genes? — *J. Evol. Biol.* 8: 759-778.
- Höglund, J. & Sheldon, B.C. (1998). The cost of reproduction and sexual selection. — *Oikos* 83: 478-483.
- Hoikkala, A., Saarikettu, M., Kotiaho, J.S. & Liimatainen, J.O. (2008). Age-related decrease in male reproductive success and song quality in *Drosophila montana*. — *Behav. Ecol.* 19: 94-99.
- 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.
- Jacot, A., Scheuber, H. & Brinkhof, M.W.G. (2007). The effect of age on a sexually selected acoustic display. — *Ethology* 113: 615-620.
- Jennions, M.D., Møller, A.P. & Petrie, M. (2001). Sexually selected traits and adult survival: a meta-analysis. — *Q. Rev. Biol.* 76: 3-36.
- Judge, K.A. (2011). Do male field crickets, *Gryllus pennsylvanicus*, signal their age? — *Anim. Behav.* 81: 185-194.

- Judge, K.A., Ting, J.J. & Gwynne, D.T. (2008). Condition dependence of male life span and calling effort in a field cricket. — *Evolution* 62: 868-878.
- Kotiaho, J.S. (2000). Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. — *Behav. Ecol. Sociobiol.* 48: 188-194.
- Selander, R.K. (1965). On mating systems and sexual selection. — *Am. Nat.* 99: 129-141.
- Tabachnick, B.G. & Fidell, L.S. (2007). *Using multivariate statistics.* — Pearson/Allyn & Bacon, Boston, MA.
- Verburgt, L., Ferreira, M. & Ferguson, J.W.H. (2011). Male field cricket song reflects age, allowing females to prefer young males. — *Anim. Behav.* 81: 19-29.
- Wagner, W.E. (1998). Measuring female mating preferences. — *Anim. Behav.* 55: 1029-1042.
- Williams, G.C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's Principle. — *Am. Nat.* 100: 687-690.
- Zahavi, A. (1975). Mate selection — a selection for a handicap. — *J. Theor. Biol.* 53: 205-214.
- Zuk, M. (1987). Age determination of adult field crickets: methodology and field applications. — *Can. J. Zool.* 65: 1564-1566.
-