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Jamaican Field Cricket Mate Attraction Signals Provide Age Cues

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Abstract

Older males often have a mating advantage, either resulting from the fact that they live longer or resulting from the fact that they both live longer and signal this to females. Male field crickets signal acoustically to attract potential mates. Some field cricket mating signals provide cues about male age while others do not. We explored whether male Jamaican field crickets, *Gryllus assimilis*, mating signals change with age. Our results show that older males produce chirps with longer pulses, more pulses, at higher pulse and chirp rates, and their chirps are both longer and louder than those produced by younger males. Our findings suggest that Jamaican field cricket mating signals provide cues about male age, explaining between 10% and 54% of the variation in signaling traits. Females might be able to use these mating signal differences to distinguish between older and younger mates.

Introduction

In nature, older males often have elevated mating success (reviewed by Brooks & Kemp 2001). This older male advantage could result from (1) high-quality males living longer or (2) males signaling their age and females preferring older males because they confer some sort of benefit (Brooks & Kemp 2001; Judge 2011; Kokko & Lindstrom 1996; Trivers 1972). While both hypotheses predict a positive relationship between survival and quality, the second hypothesis also predicts that age-related behavioral changes honestly reflect male quality (Brooks & Kemp 2001; Judge 2011).

Male crickets often signal their age. Male crickets signal acoustically to attract a mate by raising their forewings and rubbing them together to produce pulses of sound. These pulses are then concatenated into chirps or trills. Females, in turn, use these mating signals to locate and distinguish among potential mates. Several studies have revealed that older male crickets signal differently than younger male crickets. Older males signal more often than younger males in *Teleogryllus commodus* (Maklakov et al. 2009), *G. texensis* (Bertram 2000), and *G. pennsylvanicus* (Judge et al. 2008). Older *G. campestris* males produce pulses with lower carrier frequencies, have fewer pulses per chirp, and have shorter chirp durations than younger males (Jacot et al. 2007). Further, older male *G. pennsylvanicus* signal with shorter pulse periods and durations, more pulses per chirp, and lower peak frequencies compared to younger males (Judge 2011). Given female crickets often preferentially mate with older males (reviewed by Judge 2011), the above findings suggest that the older male advantage could result from females using male signaling differences to preferentially select older males.

Why might male signals change with age? Two hypotheses have been put forth to date, one ultimate and one proximate. From an ultimate perspective, older males may increase the amount of energy they put into attracting a mate because their residual reproductive value declines with increasing age (Judge 2011; Williams 1966). From a proximate perspective, age-based signaling changes could result

from stridulatory apparatus wear (Judge 2011). However, wear-related changes are unlikely to completely explain age effects because males vary extensively in how much they signal (Bertram & Warren 2005; Bertram et al. 2009; Hunt et al. 2004; Judge et al. 2008). A male that signals 10 h a night should experience stridulatory wear at ten times the rate of a male that signals for an hour a night. Further, several cricket species do not signal their age (reviewed by Judge 2011; Verburgt et al. 2011), suggesting that if stridulatory wear is the underlying cause of the age-related signaling changes, wear must not occur in all cricket species.

Given some field cricket species' mating signals provide cues about male age while others do not, we tested whether male Jamaican field crickets, *G. assimilis*, alter their mating signals as they age. Only one study to date has explored factors influencing signaling in the Jamaican field cricket: it revealed that signals carry information about male size and condition (Whattam & Bertram 2011). Larger males signal with louder chirps that contain high pulse rates, lower interpulse durations, and lower carrier frequencies than smaller males. Further, better condition males (heavier males after body size controlled for) signal with louder chirps. To determine whether Jamaican field crickets also signal their age, we conducted a longitudinal study where we quantified every signal produced from seven to 21 d post-final molt in laboratory-reared crickets. We then used a linear mixed model to test the hypothesis that male signaling parameters change with age and/or are dependent on size and condition.

Methods

We captured adult *G. assimilis* in Austin, Texas, USA. in September 2008. Crickets were transported back to Carleton University and housed in a large colony in a temperature-controlled greenhouse. We used third-generation laboratory-reared crickets for this experiment. We checked the colony daily to determine when individuals had molted from their final juvenile stage to adulthood. Upon final molt, each individual was housed alone in a clear plastic container (11 cm diameter × 7 cm height). Temperature was controlled at $\bar{X} \pm \text{SE} = 25 \pm 2$°C (range = 22–28°C with daily lows hovering between 22 and 24°C and daily highs hovering between 26 and 28°C). Lighting was set to 14:10 h light/dark cycle with lights on at 0600 h and lights off at 2000 h. Crickets were provided with *ad libitum* food (Harlan Teklad Rodent diet #8604) and water. Food and water were checked daily and replaced as necessary.

Mate Signaling

Males were placed into an Electronic Acoustic Recording system (EARs II) from 7 to 21 d (14 nights) post-final molt. The EARs II can monitor up to 96 males simultaneously. Each cricket was acoustically isolated from the others. Males were placed individually into Styrofoam enclosures with 5.1 cm thick walls lined with 2.5 cm thick acoustic foam. Inside each cricket's Styrofoam enclosure (but outside the cricket's plastic container), a single LED light provided each male with 12:12 h light/dark cycle. A microphone attached to the light allowed each cricket to have all of its acoustic mate attraction signals monitored throughout its time in the EARs II. Each microphone was continuously monitored in real time using a computer program (CricketSong, developed for our laboratory by Cambridge Electronic Design Ltd., Unit 4, Science Park, Milton Road, Cambridge, UK). CricketSong analyzed the mating signals calculating the daily averages of the following signaling traits: signaling time over each 24 h period (min), pulse duration (ms), interpulse duration (ms), pulse rate (pulses/s), pulses per chirp, chirp duration (ms), chirp rate (chirps/min), carrier frequency (Hz), and amplitude (dB). Refer to Whattam & Bertram (2011) for further details on the functioning of the EARs II (called NEARs in Whattam & Bertram 2011).

Males were removed from the EARs II for 10–15 min each day to clean their containers and replenish their food and water. On day 14, males were placed into an empty Plexiglas arena for a separate experiment on aggression and signaling (S. M. Bertram and V. Rook, unpubl. data). None of the males from this study ever came into contact with another individual – instead they were used as non-aggressive controls to help in quantifying how aggressive interactions influenced subsequent signaling. Following each male's 10 min in the empty arena, he was immediately placed back into his original (cleaned) container and the container placed back into the EARs II for continued recording.

Size, Weight, and Condition

Males were weighed to the nearest 0.1 mg on day 7 post-final molt (just prior to initiating the experiment) using a Denver Instruments balance (Pinnacle Series model PI-314; precision ± 0.1 mg). Males

were euthanized (frozen) on day 23 of adulthood, following 2 d of mating for a different experiment (S. M. Bertram and V. Rook, unpubl. data). We measured pronotum area to quantify size using a Zeiss Discovery V12 inverted dissecting microscope and highly magnified photographs (AXIO VISION v4.8, Carl Zeiss, Toronto, Ontario, Canada; magnification: ~8.5×, resolution: ~1.60 μm). Condition (residuals of mass) was quantified from a regression of weight at 7 d over pronotum area at death.

Statistical Analyses

We used a box cox transformation to normalize time spent signaling data. All other signaling components were normally distributed and did not require transformation. The effects of age, size, and condition on acoustic signaling were estimated via restricted maximum likelihood (REML) as implemented in the MIXED procedure of SPSS version 19.0.0 (IBM Corporation, Armonk, NY, USA) using the following mixed model:

$$Y_{ij} = \mu + I_i + A_{j(i)} + S_i + C_i, \quad (1)$$

where Y is the observed calling parameter of replicate (age) A nested within individual (I). Random effects included individual (I). Fixed effects included the intercept (μ), size (S), and condition (C). We corrected for multiple tests (10 tests) by adjusting the significance level to $\alpha < 0.018$ using the False Discovery Rate (FDR_{BY}) method (Benjamini & Yekutieli 2001). We used a FDR_{BY} adjustment rather than a Bonferroni adjustment because the Bonferroni adjustment is overly conservative (Benjamini et al. 2001; Nakagawa 2004; Narum 2006).

Results

All males signaled acoustically on at least one night. However, while most males signaled on all or almost all of the nights (45 signaled on all nights, two signaled on 13/14 nights, and two signaled on 12/14 nights), three of the males signaled on only five nights or less. These three males had significantly lower average daily signaling times (on the days they sang) than males who sang on most or all nights ($\bar{x} \pm$ SE = 1 ± 0.5 min/night vs. 224 ± 21 min/night, respectively; ANOVA $F = 7.127$, p = 0.0101, R^2_{adj} = 0.107, df = 1,50). The 'low-calling' males did not differ from other males in their size, weight, or condition (size: p = 0.055, weight: p = 0.176, condition: p = 0.872).

Table 1: Among male variation in signaling and size parameters measured

Signaling/size parameter	$\bar{x}$	SE	Min	Max	CV
Pulse duration (ms)	9.7	0.2	6.2	11.9	14.0
Interpulse duration (ms)	16.3	0.2	14.1	20.4	7.5
Pulse rate (pulses/s)	38.5	0.3	32.6	43.7	5.5
Pulses per chirp	7.9	0.1	5.0	9.3	12.6
Chirp duration (ms)	119.7	2.3	49.0	158.5	14.1
Chirp rate (chirps/min)	37.8	1.7	22.1	80.9	32.7
Interchirp duration (ms)	1610.5	63.9	692.6	2589.1	28.9
Carrier frequency (Hz)	3812.7	52.7	3362.6	5611.0	10.1
Amplitude (dB)	50.0	1.8	8.1	69.1	26.2
Signaling time (min)	189.8	19.2	0.1	551.6	73.6
Pronotum area (mm^2)	19.7	0.4	12.2	26.1	14.9
Weight (mg)	482.6	13.0	323.0	716.8	19.6

There was extensive variation within and across males in the properties of the acoustic mate attraction displays (Table 1). Our linear mixed models showed that 10–54% of the variation in mate signaling could be explained by male age (Table 2). Pulse duration, pulses per chirp, pulse rate, chirp duration, chirp rate, amplitude, and signaling time increased with male age, while interpulse duration, interchirp duration, and carrier frequency decreased with male age (Fig. 1).

Variation in mate signaling traits was also partially explained by differences in male body size (pronotum area). Interchirp duration decreased, chirp rate increased, and carrier frequency decreased with increasing size (Table 2). Random (individual-based) variation accounted for 4–60% of the total variation observed in the signaling traits (Table 2).

Discussion

Older males often experience higher mating success than younger males (reviewed in (Brooks & Kemp 2001; Judge 2011). Older male advantage may result from (1) highest quality males living longer or (2) males signaling their age and females preferentially selecting older males because they confer some fitness advantage (Brooks & Kemp 2001; Judge 2011). These hypotheses, while not mutually exclusive, both predict survival correlates with male quality. The second hypothesis also predicts that older males express different phenotypes than younger males. We tested whether male *G. assimilis* express different acoustic mating signals as they aged. Our findings suggest that male *G. assimilis* signals do, indeed, provide cues about male age. Older males signal with more energy, signaling more often through the day and night and producing chirps that have longer

Table 2: The influence of male age, size, condition on acoustic mate attraction signal parameters using a linear mixed models with individual as a random factor, age as a repeated measures, and body size and condition as covariates

Parameter	Effect	Type	Estimated variance	df	*T* or Wald *Z*	p	% Variance explained	AIC	Total variance
Pulse duration	Size	Fixed	0.03	48.15	0.46	0.6476		2143.25	12.61
	Condition	Fixed	0.00	46.27	0.05	0.9571			
	Age (ID)	Repeated	1.33		12.83	<0.0001	10.54		
	ID	Random	1.73		4.23	<0.0001	13.75		
Interpulse duration	Size	Fixed	−0.01	45.62	−0.12	0.9044		2512.74	19.71
	Condition	Fixed	0.00	43.03	−0.18	0.8586			
	Age (ID)	Repeated	2.21		14.38	<0.0001	11.19		
	ID	Random	0.76		3.25	0.0011	3.86		
Pulse rate	Size	Fixed	−0.03	48.54	−0.30	0.7674		3456.27	52.43
	Condition	Fixed	0.00	45.85	0.19	0.8531			
	Age (ID)	Repeated	9.36		13.43	<0.0001	17.85		
	ID	Random	3.25		3.20	0.0014	6.20		
Pulses per chirp	Size	Fixed	0.09	46.48	1.84	0.0718		1741.78	8.31
	Condition	Fixed	0.00	44.53	−0.52	0.6054			
	Age (ID)	Repeated	0.81		11.32	<0.0001	9.71		
	ID	Random	0.85		3.95	0.0001	10.18		
Chirp duration	Size	Fixed	1.78	48.49	2.04	0.0470		5762.33	607.69
	Condition	Fixed	−0.03	46.35	−0.59	0.5552			
	Age (ID)	Repeated	263.24		12.65	<0.0001	43.32		
	ID	Random	257.59		4.07	<0.0001	42.39		
Interchirp duration	Size	Fixed	53.81	51.30	2.58	0.0129	0.02	10 278.31	322 544.63
	Condition	Fixed	−2.49	48.85	−1.89	0.0653			
	Age (ID)	Repeated	172 959.49		13.87	<0.0001	53.62		
	ID	Random	148 959.46		4.23	<0.0001	46.18		
Chirp rate	Size	Fixed	−2.15	51.55	−3.69	0.0005	0.59	5440.86	362.20
	Condition	Fixed	0.08	48.92	2.19	0.0330			
	Age (ID)	Repeated	175.29		11.41	<0.0001	48.40		
	ID	Random	102.27		3.66	0.0003	28.24		
Carrier frequency	Size	Fixed	−55.05	46.19	−3.63	0.0007	0.04	9501.67	142 650.22
	Condition	Fixed	0.84	44.47	0.87	0.3895			
	Age (ID)	Repeated	52 419.35		14.90	<0.0001	36.75		
	ID	Random	85 292.57		4.35	<0.0001	59.79		
Amplitude	Size	Fixed	1.02	47.52	1.79	0.0790		4925.53	221.93
	Condition	Fixed	−0.02	45.77	−0.61	0.5458			
	Age (ID)	Repeated	78.08		11.78	<0.0001	35.18		
	ID	Random	115.01		4.23	<0.0001	51.82		
Signaling Time	Size	Fixed	−6.40	47.42	−1.00	0.3239		8177.02	24 707.83
	Condition	Fixed	−0.10	45.74	−0.24	0.8106			
	Age (ID)	Repeated	9629.00		10.90	<0.0001	38.97		
	ID	Random	14 650.08		4.18	<0.0001	59.29		

Because of the ten statistical tests run, only models with $p < 0.018$ were considered significant.

pulses, shorter interpulses, more pulses per chirp, higher pulse rates, longer chirp durations, shorter interchirp durations, higher chirp rates, at lower carrier frequencies, and at louder amplitudes. Given that acoustic mate attraction signals change with age, our study provides partial support for the second hypothesis.

Several other researchers have shown that male acoustic mate attraction signals change with age, however, how male signals change with age appears to be species specific. Older male *G. pennsylvanicus* (Judge 2011), *G. campestris* (Jacot et al. 2007), *G. bimaculatus* (Verburgt et al. 2011), and *G. assimilis* (in this study) all signal at lower carrier frequencies than younger males. Older *G. campestris* males signal with fewer pulses per chirp, which results in shorter chirp durations (Jacot et al. 2007). Older *G. bimaculatus* signal with shorter pulse durations, higher interpulse durations, higher interchirp durations and have reduced signaling times than younger males

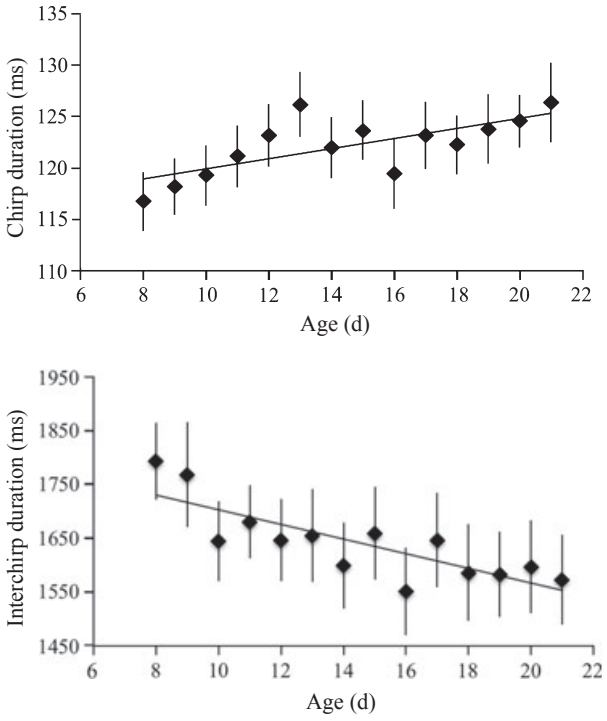

Fig. 1: How chirp duration and interchirp duration change with age in *Gryllus assimilis*.

(Verburgt et al. 2011). These findings suggest that older male *G. campestris* and *G. bimaculatus* put *less* overall energy into attracting a mate than younger males. In contrast, our findings reveal that older *G. assimilis* put *more* overall energy into attracting a mate than younger males, as they signal louder and more often and produce chirps that have longer pulses, more pulses, and are produced at a higher pulse rates and chirp rates than younger males. Older *G. pennsylvanicus* signal with more pulses per chirp and shorter pulse durations than younger males (Judge 2011), making it difficult to determine whether they put *more* or *less* overall energy into attracting a mate than younger males. Together these results may help researchers distinguish between species that are experiencing senescent-based declines in signaling with age vs. species that are enhancing their terminal reproductive investment by increasing their signaling effort with age.

Our linear mixed models also revealed that male body size (pronotum area) plays a role in explaining a small portion of the variation in mate signaling. We showed that variation in interchirp duration, chirp rate, and carrier frequency was subtly dependent on body size. These findings partially support earlier work. Whattam & Bertram (2011) found that males fed low quantities of food tended to be smaller with lower residuals of mass than males fed high quantities. Males fed high quantities of food signaled with longer and loud chirps at lower carrier frequencies (Whattam & Bertram 2011). While this study supported the Whattam & Bertram (2011) finding that body size influences carrier frequency, we did not find body size or condition influences for either chirp duration or amplitude. The differences between our findings and those of Whattam & Bertram (2011) may be attributable to the fact that Whattam & Bertram (2011) manipulated diet while crickets in our study had unlimited access to high quality food. Whattam & Bertram's (2011) findings are probably more indicative of condition in nature, where the quality and availability of food is expected vary temporally.

The question now exists as to whether females can use these differences in male acoustic signals to identify older or larger, or males in better condition. Little is known about female *G. assimilis* mating preferences. The time is thus ripe to quantify the signaling parameters that females use to select mates and the potential genetic benefits of those choices. If the trends continue across species that have been revealed by Judge (2011) and Verburgt et al. (2011), given older male *G. assimilis* seems to put more effort into attracting a mate, females may preferentially mate with older males.

Thought should also be given to whether females could, using acoustic mating signals alone, distinguish older males from younger ones, larger males from smaller ones, and/or males in better condition from males in poorer condition. While our work revealed that age explained some of the non-random variation in signaling components, substantial amounts of random individual-based variation remained unexplained by our regression models. Age-, size-, or condition-based differences in acoustic mate attraction displays have the potential, therefore, to be obscured by the extensive among male variation in signal components. Males may differ so much in their acoustic mate attraction displays that it may not be possible for females, using acoustic signals alone, to preferentially select older, larger, or better condition males.

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