Relationship Between Condition, Aggression, Signaling, Courtship, and Egg Laying in the Field Cricket, *Gryllus assimilis*

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Abstract

Sexual selection theory suggests males in good condition should be more successful than males in poor condition when competing with rivals for territories and mates. Understanding how condition influences the interplay between aggression, mate attraction, and courtship displays could help explain why variation is maintained in traits that confer fitness. Using laboratory-reared Jamaican field crickets, *Gryllus assimilis*, we found that fine-scale temporal components of mate attraction signals were positively correlated with body condition (residual body mass) and body size; signaling effort was positively correlated with both body condition and fine-scale temporal signaling components; aggression was positively correlated with signaling effort; number of eggs laid was positively correlated with female body size, male body condition and aggression. Together our correlative study suggests that variation in body condition and size may drive some of the variation in cricket mate attraction signaling and aggression. Given condition and body size are influenced by foraging ability, nutrient availability and the organism's ability to uptake and retain these essential nutrients could explain some of the persistent variation in fitness conferring traits.

Introduction

Many male secondary sexual traits evolve through sexual selection, via male–male competition and/or female choice (Darwin 1871; Andersson 1994; Safran & Hauber 2007). Traits used in competition and attraction should be honest indicators of condition because of the energetic costs involved in their development, maintenance, and expression (Maynard Smith & Harper 1988; Berglund et al. 1996; Rowe & Houle 1996). Individuals in good condition should have a larger resource pool than those in poor condition. They should, therefore, be better able to afford the elevated costs of exaggerating their secondary sexual traits (Rowe & Houle 1996). Given this condition dependence, males in good condition should be better at competing with rivals and attracting mates (Berglund et al. 1996; Kodric-Brown 1996; Maynard Smith & Harper 2003; Simon 2011).

Most sexual selection studies have focused individually on male–male competition or mate choice (reviewed by Andersson 1994; Hunt et al. 2009). However, in many animals, both competition and choice are important components of mating. Relatively, fewer studies have focused on the interplay between competition and choice (e.g., Berglund et al. 1996; Kodric-Brown 1996; Forsgren 1997; Candolin 1999; Vanhooydonck et al. 2005; Brumm & Ritschard 2011; Simon 2011). Understanding the interplay between traits used in intrasexual and intersexual competition is important because indicator models of sexual selection suggest they should exhibit positive relationships (Zahavi 1975, 1977, 1992; Kodric-Brown & Brown 1984; Pomiankowski 1988; Michod & Hasson 1990). While most of the research exploring the relationship between competition and mate choice reveals positive relationships (e.g., chaffinch birds, *Fringilla coelebs*:

Brumm & Ritschard 2011; stickleback fish, *Gasterosteus aculeatus*: Candolin 1999; and anoles lizards, *Anolis sagrei*, *Anolis grahami* and *Anolis lineatopus*: Simon 2011; Vanhooydonck et al. 2005), Forsgren (1997) revealed a negative relationship between competitive behaviors and mating success in her studies on sand gobies, *Pomatoschistus minutus*. This negative relationship seems to be driven by parental care: Dominant males were not superior caregivers; females preferred males who were good parents (Forsgren 1997).

Crickets are an ideal system to explore the relationships between aggression, signaling, condition, and reproduction because they allow easy quantification and manipulation of all their behaviors. Crickets compete in aggressive contests (fights) to gain access to mating territories, produce long-range acoustic signals to attract potential mates (phonotaxis; Alexander 1961), and then use short-range courtship songs and antennal stroking to entice females to mate (Shackleton et al. 2005). Females appear to select mates based on male body size, pheromones, and fighting and singing ability, (Crankshaw 1979; Gray 1997; Nelson & Nolen 1997; Savage et al. 2004; Leonard & Hedrick 2010). To date, most cricket studies have not explored the interplay between all of these factors, instead focusing either only on aggression (Parker 1974; Adamo & Hoy 1995; Hofmann & Schildberger 2001; Briffa 2007; Jang et al. 2008), only on acoustic mate attraction signaling (Wagner et al. 1995; Bertram 2000; Robinson & Hall 2002; Bertram & Warren 2005; Bertram & Bowen 2006), the interplay between acoustic mate attraction signaling and mating (Balakrishnan & Pollack 1996; Hack 1997; Gray & Eckhardt 2001; Holzer et al. 2003; Simmons 2004), or the interplay between aggression and mating (Bateman & Toms 1998; Kortet & Hedrick 2005; Shackleton et al. 2005; Brown et al. 2006). The only research to explore the interplay between mate signaling and cricket aggression was described by Wilson et al. (2010) which investigated correlations between activity, exploratory behavior, antipredatory behavior, aggression, and mate signaling. Surprisingly, there was no relationship between aggression and mate signaling.

Here, we quantified male acoustic long-distance mate attraction signaling, aggression, courtship, and number of eggs laid using Jamaican field crickets, *Gryllus assimilis*. Our study addresses the following questions: Does body size or condition (residual body mass) influence mate attraction, aggression, courtship, or offspring production? Is mate attraction signaling correlated with aggression or courtship? Are the winners of aggressive interactions more likely to signal more, court more, mate faster, or produce more offspring than losers? Does winning or losing a fight alter subsequent mate attraction signaling? We make the following predictions: (1) Body size and condition will positive correlate with male mate attraction displays, aggressive displays, courtship displays, and offspring production; (2) mate attraction signaling will be positive correlated with aggressive displays and courtship displays; and (3) winners of aggressive interactions will have higher signaling efforts, will court more, and will produce more offspring than losers of aggressive interactions. Answering these questions allows us to: determine how sexually selected traits are influenced by condition; quantify the interplay between traits used in competition, mate choice, and courtship; and gain further understanding about how these aggression and mate attraction traits may interact to influence offspring production.

Methods

Field Collection and Laboratory Rearing

We captured adult *G. assimilis* in Austin, TX, USA in Sep. 2008. Crickets were housed in communal plastic bins and allowed to mate freely; females laid eggs in moist sand. Eggs were transported back to Carleton University within 10 d of field-capture and offspring were reared in large colonies in a environmental-controlled room in Carleton University's greenhouse (temperature = 25 ± 2°C; lighting was set to 14:10 h light/dark cycle). We used third-generation laboratory-reared crickets for this experiment.

Colonies were checked daily and newly molted adults were removed and housed individually in clear plastic containers (diameter: 11 cm, height: 7 cm), with screened lids to allow acoustic signal recording. Crickets were provided with *ad libitum* food (powdered Harlan Teklad Rodent diet #8604, Harlan Laboratories, Indianapolis, IN, USA) and water. On day 7 post-imaginal molt, each male was weighed (details described in the following sections). Their pronotum was then painted with distinct nail polish to enable identification during aggressive contests.

Acoustic Signaling Behavior

Males had their acoustic long-distance mate attraction signals recorded from 7 to 21 nights post-imaginal molt using our Electronic Acoustic Recording system II (EARs II), which monitors up to 96 males simulta-

neously. Starting on day 7 post-imaginal molt, each cricket was acoustically isolated from the others by placing individual cricket containers into Styrofoam enclosures with 5.1 cm thick walls lined with 2.5 cm thick acoustic foam. Inside each cricket's Styrofoam enclosure, a single LED light provided the 14:10 h light/dark cycle. A microphone attached to the light ensured that each cricket's acoustic mate attraction signals was continuously recorded. Microphones were monitored in real time using a computer program (CricketSong; developed for our laboratory by Cambridge Electronic Design Ltd., Unit 4, Science Park, Cambridge, UK). CricketSong analyzed all acoustic mate attraction signals, calculating the following traits: Time spent signaling over each 24-h period (min), pulse duration (ms), interpulse duration (ms), pulses per chirp, chirp duration (ms), interchirp duration (ms), amplitude (dB), and carrier frequency (Hz) (Fig. 1). For further details on the functioning of the EARs II refer to Whattam & Bertram (2011) (described as NEARs in Whattam & Bertram 2011). Each male's acoustic long-distance mate attraction signals was quantified 24 h a day over the 2-wk period. Recordings were stopped for 15 min a day to replenish the males' food and water supply.

Aggressive Contests

On day 14 of adulthood (after seven nights of recording), all males were temporarily removed from the EARs II to either quantify their aggressive interactions or act as non-fighting controls. Aggressive contests were conducted in a small, sand-covered Plexiglas arena (15 cm L × 15 cm W × 21 cm H) with two compartments separated by a removable divider.
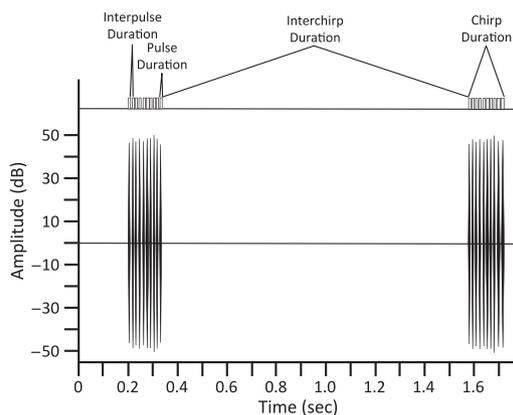

Fig. 1: *Gryllus assimilis* long distance acoustic mate attraction chirps showing a subset of the signaling parameters quantified.

All contests were filmed from above using a color video camera (Canon HG10, Canon, Mississauga, Ontario, Canada) mounted on a tripod. We covered the outside of the arena with white paper, so crickets were not disturbed and conducted trials between 10:00 and 12:00 h. We paired opponents by minimizing mass differences to within a 25% difference (following Jang et al. 2008; Hack 1997). We paired 124 males into 62 aggressive contests with each male being used in one contest. Opponents were placed on opposite sides of the divider and given 2 min to acclimatize to the arena. The divider was then carefully removed (without disturbing either male) allowing males to contact each other.

The aggressive contest was recorded as having commenced at first body contact between males (following Jang et al. 2008). Our scale of aggressive behaviors ranged from zero to four: 0 withdraw; 1 antennal fencing; 2 kick; 3 mandible flare, chase, or mandible engagement (bite); and 4 grapple (following Adamo & Hoy 1995; Jang et al. 2008). Once trials were terminated, clear dominance was established between males (one individual retreated in two consecutive encounters), aggression had ceased, and all post-conflict displays had been completed. The contest duration was thus the entire observation period, from the start of the contest to the termination of the observation period following the conclusion of the contest. Once a contest was completed, males were transferred back into the EARs II for further acoustic monitoring (days 14–21). The walls of the aggressive arena were wiped down with 95% ethyl alcohol, and the sand raked to minimize the likelihood of pheromones affecting future fights.

Each male that fought was identified as having won or lost its aggressive contest: The individual that established dominance was identified as the winner; the retreating individual was identified as the loser. Nine pairs (9/62 = 15%) did not initiate aggressive interactions and were not included in further analyses. We computed the timing, duration, and frequency of all aggressive behaviors for each male in the remaining 53 contests (106 males) by playing the video in slow motion on a computer monitor. We then quantified each male's mean aggressive score, maximum aggressive score, and aggressive intensity. Mean aggressive score was an unweighted average of all the male's aggressive behaviors observed throughout the contest. Maximum aggressive score was the highest level of aggression the male reached during the contest. Aggression intensity was an average of all the male's aggressive behaviors observed throughout the contest weighted

by the amount of time each aggressive act was performed for. Aggression intensity was calculated by multiplying each behavior's aggressive score by the total number of seconds the behavior was performed for. These weighted aggression scores were then summed across all the behaviors each individual performed. That sum was subsequently divided by the total number of seconds the opponents were engaged in their aggressive contest to account for variable fight durations.

Non-fighting control males were placed in the same Plexiglas arena (15 cm L × 15 cm W × 21 cm H) as the males that fought, but they were placed in alone. They sat on one side to acclimatize for 2 min before the divider was carefully raised. Then, they roamed freely and explored the arena for 10 min. Control males were then placed back into the EARs II to analyze their mate attraction signals for a further seven consecutive days (Day 14–21).

Courtship and Mating

On day 21, all males were placed singly into a clear Plexiglas container (15 cm L × 15 cm W × 21 cm H) for 2 min to allow them to acclimatize and then paired randomly with a lone virgin female (female age = 10+ days post-imaginal molt). Mating trials were conducted between 09:00 and 12:00 h. Courtship and mating were filmed from above using a color video camera (Canon HG10). Behaviors were later examined in slow motion (50–75% slower than real time speed) to determine latency to first contact, latency to initiate courtship song, latency from 1st contact to 1st courtship song, number of courtship songs produced, latency from 1st courtship song to mount, total time spent mounted, and number of mounting attempts. The trial was continued until a spermatophore was transferred or courtship had ceased. Once mating had successfully occurred or courtship had ceased, males and females were transferred back to the male's container and housed together for 48 h (Day 21–23). Males were euthanized on day 23; females were housed for an additional 2 wk with food, water, and moist sand for oviposition. All eggs laid by each female over this 2-wk period were counted. Egg fertilization was not quantified.

Size and Condition

Males were weighed on day 7 post-imaginal molt (prior to initiating the experiment) using a Denver Instruments analytical balance (Pinnacle Series

model PI-314; precision ± 0.1 mg) (Denver Instruments, Bohemia, NY, USA). Males were euthanized (frozen) on day 23 post-imaginal molt (immediately following the 48-h mating period). We quantified male size following euthanization using a Zeiss Discovery V12 inverted dissecting microscope and highly magnified photographs (magnification: approx. 8.5×, resolution: approx. 1.60 μm, AxioVisionLE v4.8; Carl Zeiss Canada Ltd, Toronto, Ontario, Canada). Size measurements included head width, pronotum width and length, forewing length, femur length, scraper length, and harp area. Condition was quantified using the residuals from a standard least squares linear regression of body mass (wet mass) on the first principal component of body size (described in the following sections). Residual mass standardizes mean condition to 0 (Gray & Eckhardt 2001).

Statistical Analyses

All statistical analyses were performed using JMP v.9 (SAS, Cary, NC, USA). We used a box cox transformation to normalize time spent signaling data. All other traits were normally distributed and did not require transformation. We used principal component analyses to minimize correlations between traits. Specifically, we ran principal component analyses for traits associated with acoustic signaling, aggression, courtship, and size/condition measures. After saving the principal components for each of these behavior and size/condition measures, we incorporated them into standard least squares linear regression models to determine whether (1) variation in male body size and condition (residual body mass) explained signaling variation; (2) variation in male body size, condition, and signaling behavior explained aggressive variation; and (3) variation in male body size, condition, signaling behavior, aggression, and female body size explained courtship variation. We corrected for multiple tests (six regression models) by adjusting significance to $\alpha < 0.0204$ using the False Discovery Rate (FDR_{BY}) method (Benjamini & Yekutieli 2001). We used FDR_{BY} rather than Bonferroni adjustment because it is less conservative (Benjamini et al. 2001; Nakagawa 2004; Narum 2006). We used paired t-tests to determine whether aggressive contest winners and losers differed in body size, weight, signaling behavior, aggression, or subsequent courtship. We corrected for multiple tests (nine paired t-tests) by adjusting significance to $\alpha < 0.0177$ using FDR_{BY}. Lastly, we determined whether males changed their signaling (signaling effort and fine-scale signaling components)

following a fight by comparing week 1 of signaling to week 2 of winners, losers, and non-fighters (to control age effects) using analysis of variance. We corrected for multiple tests by adjusting significance to $\alpha < 0.0204$ using FDR_{BY}.

Results

Most males signaled acoustically to attract mates (160 of 187) and interacted with conspecific males aggressively (106 of 124). While most males courted females, only half of them successfully mated during the observation period (53%). Most females laid eggs (98%). Males varied extensively in their acoustic mate attraction signals, aggressive interactions, courtship displays, body size, weight, and condition (residual mass). For example, mean time spent signaling averaged more than 3 h a day, but ranged from no signaling to signaling for over 10 h a day. Time spent fighting averaged just under a minute but ranged from males that did not fight to males that fought for almost 5 min. Males also exhibited a twofold difference in size and a threefold difference in weight. Females were highly variable in their lifetime reproductive output, averaging over 300 eggs but ranging from laying zero eggs to laying over 600 eggs. Statistical moments, including means, standard errors, ranges, and coefficients of variation, are provided for all measured traits in the Table S1.

We used principal component analyses to reduce the large number of quantified traits associated with aggression, mate attraction signaling, courtship, and body size to more manageable numbers. The PC1 of fine-scale signaling traits accounted for 61% of the variation in mate attraction behavior (eigenvalue = 4.24). All fine-scale signaling components loaded onto PC1 in a manner indicative of optimizing sound production (pulse duration = 0.43, interpulse duration = 0.30, # pulses per chirp = 0.45, chirp duration = 0.40, interchirp duration = −0.26, amplitude = 0.40, and carrier frequency = −0.37). Males with higher PC1 scores signaled with longer pulses, shorter interpulses, had more pulses per chirp, longer chirps, shorter interchirps, signaled at higher amplitudes, and at lower carrier frequencies. We used fine-scale signaling PC1 and average time spent signaling to describe variation in acoustic mate attraction signals.

Aggression's first principal component (PC1) accounted for 76% of the variation (eigenvalue = 2.28) with all three aggressive scores loading onto PC1 in a similar manner ($\bar{x}$ = 0.61, maximum = 0.54, and intensity = 0.59). Males with

higher PC1 scores had higher mean, max, and intensity scores.

The PC1 for courtship, mounting, and spermatophore transfer accounted for 47% of the variation (eigenvalue = 2.37) and was indicative of courtship effort and mating success. Males with high PC1 scores spent more time producing courtship signals, were mounted more often by females, and spent more time mounted (Table 1). PC2 accounted for 18% of the courtship and mating variation (eigenvalue = 0.90) and was indicative of courtship latency. Males with high PC2 took longer to start courting females after contact. We used both PC1 and PC2 to describe courtship and mating. In a separate analysis, we also used latency to mount and time spent courting as dependent variable, following Shackleton et al. 2005. These measures provided the same results as those provided by our principal component analyses, so are not reported.

The first principal component of all size morphometrics accounted for 72% of the variation (eigenvalue = 5.79) and was indicative of overall body

Table 1: Principal component loading scores for courtship behaviors

Courtship parameter	PC1	PC2
Time to 1st courtship song	−0.30	**0.88**
Time to 1st mount	**0.42**	0.35
Time spent courtship singing	**0.47**	0.29
Total time mounted	**0.45**	−0.09
Number of mounting attempts	**0.56**	0.05

PC1 eigenvalue = 2.37 and accounted for 47% of the variation; PC2 eigenvalue = 0.90 and accounted for 18% of the variation. All values above |0.40| are bolded to reveal the key components loading on each principal component.

Table 2: Principal component analysis loading scores for the morphometrics traits

Morphometric trait	PC1	PC2	PC3
Pronotum width	**0.37**	−0.15	0.21
Pronotum length	**0.39**	−0.13	−0.11
Pronotum area	**0.38**	−0.26	−0.01
Head width	0.32	−0.28	**0.74**
Right leg length	**0.38**	−0.10	**−0.40**
Left leg length	**0.38**	−0.13	**−0.45**
Harp area	0.30	**0.64**	0.07
Scraper length	0.31	**0.61**	0.13

PC1 eigenvalue = 5.79 and accounted for 72% of the variation; PC2 eigenvalue = 0.94 and accounted for 12% of the variation; PC3 eigenvalue = 0.46 and accounted for 6% of the variation. All values above |0.40| are bolded to reveal the key components loading on each principal component.

size: males with high PC1 were larger (Table 2). PC2 accounted for 12% of the variation (eigenvalue = 0.94) and was indicative of signaling apparati: males with high PC2 had larger scrapers and harps relative to body size. PC3 accounted for 6% of the variation (eigenvalue = 0.46) and was associated with head size: males with higher PC3 had larger heads relative to body size. We utilized all three principal components along with condition (residuals of body mass) because they all potentially contribute to explaining morphological variation: mate attraction signaling (PC1 for body size, PC2 for signaling apparatus, and condition); aggressive interactions [PC1 for body size, PC3 for head size, and condition; following (Judge & Bonanno 2008)]; and courtship interactions (PC1, PC2, PC3, and condition).

Factors Influencing Variation in Behavior

A small but significant portion of the variation (6%) in fine-scale signaling structure (signaling PC1) was explained by variation in body size (size PC1), size of signaling apparati (size PC2), and condition (residual mass; Table 3; Fig. 2). Larger males, males with larger scrapers and harps, and males in relatively better condition (heavier relative to body size) produced acoustic mate attraction displays with longer pulses, more pulses per chirp, longer chirps, and lower carrier frequencies, and at louder amplitudes. A large and significant portion of the variation in total signaling time (33%) was explained by variation in body size and fine-scale signaling structure (Fig. 3; Table 3). Larger males and males that signaled with longer pulses, more pulses per chirp, longer chirps, lower carrier frequencies, and louder chirps signaled for more time each 24-h period. A small but significant portion of variation in aggressive behavior (10%) was explained by variation in fine-scale acoustic signaling traits. Males that signaled with longer pulses, more pulses per chirp, longer chirps, lower carrier frequencies, and louder chirps were more aggressive (Table 3; Fig. 3).

Our models to explain variation in courtship behavior were not statistically significant; however, our model that explained courtship latency exhibited a non-significant trend (p = 0.09; Table 3). Given only half of the males successfully mated during the observation period, we quantified whether there were differences between males that mated and males that did not. Successful males did not differ from unsuccessful males in any way (all p > 0.10 for fine-scale signaling PC1, time spent signaling, aggression PC1, size PC1-3, and condition). A significant amount of variation (18%) in number of eggs laid was explained by male condition, aggression, and female body mass (Table 3). Larger females, better condition males, and more aggressive males produced more offspring.

Differences Between Winners and Losers

Winners were more aggressive than losers, but winners did not differ from losers in their mate attraction signaling or courtship behaviors before the fights (Table 4). Aggression data are described in greater detail in a study that quantifies aggression in *G. assimilis* and compares it with other cricket species (Bertram et al. 2011).

We also determined whether winners or losers changed how they signaled for mates following the fight. Male fine-scale signaling did not change significantly, regardless of whether males won or lost their fight or acted as non-fighting controls (comparing signaling week 1 with week 2; losers: p = 0.29, F = 1.12, R^2_{adj} = 0.00; winners: p = 0.39, F = 0.74, R^2_{adj} = 0.00; non-fighters: p = 0.61, F = 0.27, R^2_{adj} = 0.01). Signaling time of fight losers increased significantly in the second week (week 1: 184.97 ± 17.37 vs. week 2: 241.32 ± 20.63; p = 0.04, F = 4.37, R^2_{adj} = 0.03). Winners and non-fighters showed the same trend of increasing signaling time, but these results were not quite significant (winners – week 1: 170.95 ± 19.13 vs. week 2: 220.68 ± 20.40; p = 0.08, F = 3.16, R^2_{adj} = 0.02; non-fighters – week 1: 170.02 ± 17.23 vs. week 2: 213.92 ± 19.16; p = 0.09, F = 2.90, R^2_{adj} = 0.01).

Discussion

Relationship Between Mate Attraction Signaling and Aggression

Indicator models of sexual selection suggest positive relationships should exist between aggressive behaviors and mate attraction signals (Zahavi 1975, 1977, 1992; Kodric-Brown & Brown 1984; Pomiankowski 1988; Michod & Hasson 1990). We found a positive relationship between aggression and mate attraction signaling: Males that signaled with longer pulses, more pulses per chirp, longer chirps, lower carrier frequencies, and louder chirps were more aggressive in their aggressive contests. Together our findings support the hypothesis that expression of traits used in mate attraction and aggression are reinforcing (Zahavi 1975, 1977, 1992; Kodric-

Table 3: Multivariate regression model results for factors influencing mate attraction signaling, aggression, courtship, and egg laying in the Jamaican field cricket, *Gryllus assimilis*. We corrected for multiple tests by adjusting the significance level to $\alpha < 0.0204$ using FDR_{BY} and bolded all models that were statistically significant

Behavior	Model parameters	df	F	p	R^2_{adj}
Fine-scale signaling structure	**Whole model**	**4,150**	**3.54**	**0.009**	**0.062**
	Condition overall size PC1	1	4.74	0.031	
	Condition signal apparati size PC2	1	4.58	0.034	
	Condition head size PC3	1	0.54	0.463	
	Condition residual mass	1	4.69	0.032	
Total signaling time	**Whole model**	**5,154**	**15.85**	**<0.001**	**0.325**
	Condition overall size PC1	1	8.53	0.004	
	Condition signal apparati size PC2	1	0.01	0.914	
	Condition head size PC3	1	0.05	0.828	
	Condition residual mass	1	0.21	0.644	
	Fine–scale signaling PC1	1	71.86	<0.001	
Aggression	**Whole model**	**6,90**	**2.68**	**0.019**	**0.095**
	Condition overall size PC1	1	1.50	0.224	
	Condition signal apparati size PC2	1	0.00	0.979	
	Condition head size PC3	1	0.00	0.955	
	Condition residual mass	1	0.04	0.837	
	Total signaling time (box cox)	1	0.31	0.577	
	Fine–scale signaling PC1	1	8.87	0.004	
Courtship effort	Whole model	8,50	1.34	0.246	0.045
	Condition overall size PC1	1	1.67	0.202	
	Condition signal apparati size PC2	1	0.05	0.832	
	Condition head size PC3	1	2.41	0.127	
	Condition residual mass	1	0.16	0.687	
	Female mass	1	1.16	0.288	
	Total signaling time (box cox)	1	6.64	0.013	
	Fine–scale signaling PC1	1	1.12	0.295	
	Aggression PC1	1	0.04	0.846	
Courtship lag	Whole model	8,50	1.86	0.088	0.106
	Condition overall size PC1	1	0.16	0.688	
	Condition signal apparati size PC2	1	0.00	0.959	
	Condition head size PC3	1	0.70	0.408	
	Condition residual mass	1	2.08	0.156	
	Female mass	1	0.53	0.471	
	Total signaling time (box cox)	1	9.01	0.004	
	Fine–scale signaling PC1	1	0.01	0.936	
	Aggression PC1	1	0.96	0.331	
Number eggs laid	**Whole model**	**8,84**	**3.45**	**0.002**	**0.176**
	Condition overall size PC1	1	0.05	0.824	
	Condition signal apparati size PC2	1	0.06	0.805	
	Condition head size PC3	1	1.46	0.230	
	Condition residual mass	1	4.02	0.048	
	Female mass	1	15.01	0.000	
	Total signaling time (box cox)	1	1.18	0.280	
	Fine–scale signaling PC1	1	0.26	0.613	
	Aggression PC1	1	5.13	0.026	

Brown & Brown 1984; Pomiankowski 1988; Michod & Hasson 1990).

Our findings agree with most other studies that have examined the interplay between mate attraction signaling and aggressive displays. For example, variation in aggressive interactions result in part from variation in song amplitude in male chaffinches (Brumm & Ritschard 2011), findings that corroborate a review article's suggestion of a tight link between bird song amplitude and aggression (Gil & Gahr 2002). Further, variation in frog aggressive interactions can often be partly explained by information conveyed in the spectral properties of male mate attraction signals (Arak 1983; Ramer
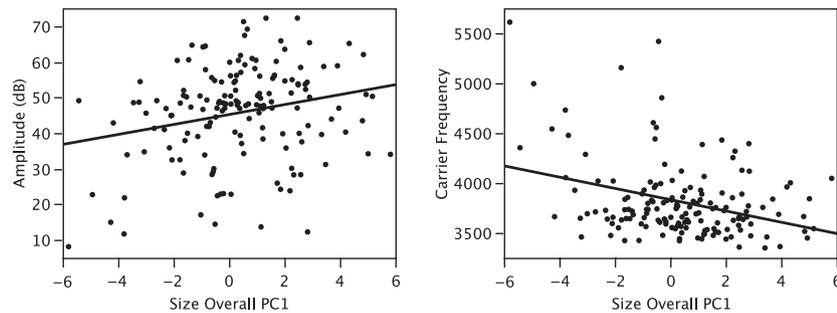
Fig. 2: Relationship between body size (size PC1) and amplitude (left) and carrier frequency (right). Larger males signaled louder and at lower carrier frequencies. Amplitude × overall size PC1 regression: p = 0.004, t = 2.97, df = 1,153, R^2_{adj} = 0.05; Carrier frequency × overall size PC1 regression: p < 0.001, t = −4.35, df = 1,153, R^2_{adj} = 0.10.

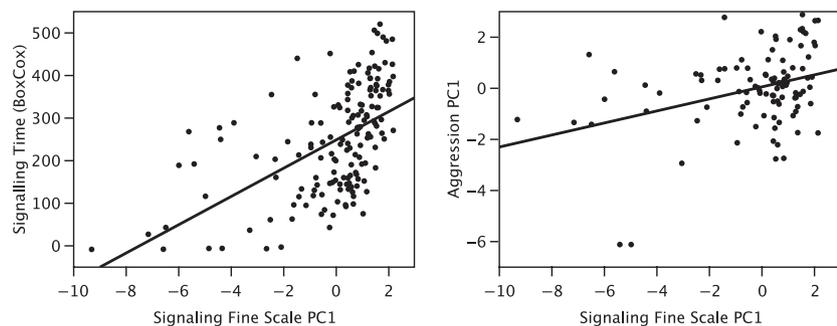**Fig. 3:** Relationship between fine-scale signaling parameters (signaling PC1) and total time spent signaling (left) and aggression (right). Males that produced longer pulses, longer chirps, louder chirps and signaled at lower carrier frequencies spent more total time signaling and were more aggressive in their fights. Signaling time × fine-scale signals PC1 regression: p < 0.001, t = 8.14, df = 1,158, R^2_{adj} = 0.29; Aggression × fine-scale signals PC1 regression: p < 0.001, t = 3.77, df = 1,00, R^2_{adj} = 0.12.

Table 4: Paired t-test results for behavioral or morphological differences between the winners and losers of the aggressive interactions. We corrected for multiple tests by adjusting the significance level to α < 0.0177 using FDR_{BY} and bolded all tests that were statistically significant

Behavior	Winner		Loser		Paired t-test results		
	$\bar{x}$	SE	$\bar{x}$	SE	t	p	df
Total signaling time	242.3	18.6	262.9	17.4	−0.82	0.415	47
Fine–scale signaling (PC1)	0.0	0.3	−0.4	0.3	0.84	0.408	47
Aggression (PC1)	**0.3**	**0.1**	**−0.3**	**0.3**	**2.91**	**0.005**	**52**
Courtship effort (PC1)	0.8	0.2	0.7	0.3	−1.61	0.124	18
Courtship lag (PC2)	0.1	0.2	0.1	0.2	0.75	0.464	18
Body size (PC1)	0.5	0.3	0.2	0.3	1.73	0.090	47
Signaling apparati size (PC2)	0.2	0.1	0.3	0.1	−0.70	0.487	47
Head size (PC3)	0.1	0.1	0.1	0.1	0.37	0.712	47
Condition (residual mass)	−4.6	5.8	−0.2	5.1	−0.89	0.377	47

et al. 1983; Robertson 1986; Given 1987; Wagner 1989).

Our study does not, however, agree with the only other cricket study to examine the relationship between mate attraction signaling and aggression. Wilson et al. (2010) quantified individual differences in cricket activity, exploration, mate attraction, aggression, and antipredator behavior. Wilson et al. (2010) found no relationship between aggression and mate attraction signaling. The apparent conflicting results between our two studies could have two possible causes. First, the study described by Wilson

et al. (2010) quantified mate attraction signaling using only signaling effort; they did not quantify fine-scale signaling structure, and our results revealed a relationship between aggression and fine-scale signaling components. Second, their study utilized European house crickets (*Acheta domesticus*) that were bred in captivity for numerous generations. These crickets may have experienced very different inbreeding and selection pressures compared with their wild counterparts (Gray 1997, 1999; Wilson et al. 2010). Given our finding of positive relationships between mate attraction signaling and aggression in the non-domesticated *G. assimilis*, we suggest that other wild field cricket populations may also show positive relationships between traits used in mate attraction and aggression. This idea requires further testing.

Do Sexually Selected Traits Reflect Condition?

Indicator models of sexual selection suggest that traits used in aggressive competitions and mate attraction should be honest indicators of a male's condition because of the energetic costs involved in their development, maintenance, and expression (Zahavi 1992; Berglund et al. 1996; Rowe & Houle 1996; Maynard Smith & Harper 2003). We found several sexually selected traits were condition dependent. Larger males, with relatively larger signaling apparati, and relatively higher residual mass produced mate attraction signals that were louder, had lower carrier frequencies, had longer pulse and chirp durations, and took shorter breaks between their pulses and chirps. Body size and fine-scale signaling features also influenced signaling effort as larger males signaled most often through the night.

Our findings that mate attraction signaling is dependent on body size and residual mass concur with an earlier manipulative study on *G. assimilis*. Whattam & Bertram (2011) fed males high (unlimited) or low amounts of food during development and into adulthood. Males that were fed unlimited food were larger and had higher residuals of mass than males that were fed low amounts of food. Males fed unlimited food also signaled with longer and louder chirps at lower carrier frequencies than males fed low amounts of food (Whattam & Bertram 2011). Together the results of the Whattam & Bertram (2011) study and our study suggest that acoustic mate attraction signals honestly reflect male condition.

Male condition should also be an indicator of how well a male performs in a fighting contest because males in better condition should allocate more energy to aggression and therefore last longer in a contest because they have a higher resource holding potential (Moretz 2003; Shackleton et al. 2005). We loosely weight-matched aggressive opponents to within 25% of their body mass (following Brown et al. 2006; Olsson 1992). Given minimal weight difference between opponents, it was not surprising that the relationship between condition and aggression was weak. Several studies have, however, revealed larger and/or heavier males have an advantage in aggressive interactions (e.g., Brown et al. 2006; Jang et al. 2008; Judge & Bonanno 2008). Larger *Gryllus pennsylvanicus*, for example, typically win aggressive contests against smaller males (Jang et al. 2008; Judge & Bonanno 2008). Further, male *A. domesticus* that are heavier or have higher residual mass are typically more aggressive than males that are lighter or have lower residual mass (Hack 1997; Brown et al. 2006).

Traits Influencing Mating Success and Reproductive Output

None of our models exploring factors influencing courtship and mating were statistically significant. This lack of statistical significance was surprising and suggests females might use different traits to assess suitors than those measured in our study, potential problems with our experimental design, and/or too small of a sample size. We address each of these possibilities in turn. First, male long-distance mate attraction signals, short-range courtship signals, and contact pheromones could be used by females to distinguish between potential mates (Alexander 1961; Kortet & Hedrick 2005). Our study quantified parameters associated with male long-distance mate attraction signals; it also quantified amount of time spent producing short-range courtship signals; it did not, however, quantify fine-scale properties of short-range courtship signals or male contact pheromones. Future work should establish how short-range courtship signals and contact pheromones influence courtship success in this species. Second, our experimental design for quantifying courtship and mating success could be improved. We placed potential mates together in a no-choice test and quantified latency to court, time spent courting, latency to mount, and latency to transfer a spermatophore (Shackleton et al. 2005). We did not, however, play long-distance mate attraction signals to the female prior to releasing her. Incorporating long-distance mate attraction signal information into the courtship protocol might influence female mating response.

Further, our no-choice test protocol requires further testing. While this no-choice protocol has been carefully tested in the black field cricket *Teleogryllus commodus* (Shackleton et al. 2005) and used to quantify female mating preference in the Pacific field cricket, *Teleogryllus oceanicus* (Rebar et al. 2011), and the European house cricket, *A. domesticus* (Klaus et al. 2011), it has not been rigorously tested in *G. assimilis*. Third, only half (53%) of our cricket pairs mated during the observation period. A larger number of copulations would have provided greater statistical power, possibly resulting in model significance.

Importantly, we found that males in better condition (higher residual mass) and males that were more aggressive had higher reproductive output (their mates laid more eggs). These findings support the hypothesis that reproductive output is influenced by reinforcing expressions of traits used in mate attraction and aggression (Zahavi 1975, 1977, 1992; Kodric-Brown & Brown 1984; Pomiankowski 1988; Michod & Hasson 1990). These findings also suggest that higher condition and competitively superior males may confer some form of fitness advantage to females (Mead & Arnold 2004; Andersson & Simmons 2006; Kokko et al. 2006; Hunt et al. 2009). Higher condition males may produce more viable sperm, and/or females may allow high condition male sperm to fertilize more eggs via cryptic female choice. Whether these indirect benefits result from females choosing mates or sperm that display certain attributes or are more competitive in aggressive interactions requires further testing (Berglund et al. 1996; Wiley & Poston 1996).

Conclusions and Future Directions

Traits associated with mate attraction, aggression, courtship and mating should be correlated if males' sexually selected traits are indicative of good genes (Zahavi 1975, 1977, 1992; Kodric-Brown & Brown 1984; Pomiankowski 1988; Michod & Hasson 1990). Our findings mostly support this hypothesis as variance in fine-scale mate attraction behaviors was explained by body size and residual mass; variance in aggression was explained by variance in signaling effort; and variance in reproductive output (number of eggs laid) was explained by variance in female mass, male residual mass, and male aggression. Together our findings suggest that reproductive success in *G. assimilis* is influenced by the interaction of multiple traits.

Given body size and residual mass directly influenced mate attraction displays and reproductive success, the ability to grow large and store energy reserves may be critically important to both male and female fitness. Future research should, therefore, investigate how diet influences variation in fitness. Several recent studies suggest that essential nutrients influence cricket growth, body size, egg laying, mate attraction, and survival. For example, Hunt et al. (2004) revealed that males signal more often but die younger when fed diets high in protein. Bertram et al. (2009) and Visanuvimol & Bertram (2011) revealed that males signal more often and females lay more eggs when fed diets high in phosphorus. Further, Maklakov et al. (2008) revealed that males signal more often when fed diets high in carbohydrates. The time is thus ripe to use a geometric framework to explore how multiple nutrients influence cricket behavior and subsequent fitness (Simpson et al. 2004).

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Statistical moments for all mate attraction signals, aggressive traits, courtship traits, mating traits, egg laying numbers, body size traits, and weight traits.

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