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ORIGINAL PAPER

No relationship between long-distance acoustic mate attraction signals and male fertility or female preference in spring field crickets

Lauren P. Fitzsimmons · Susan M. Bertram

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Abstract To father offspring, a male must succeed at two processes of sexual selection: (1) mate with a female and (2) fertilize her eggs. We investigated the relationships between pre- and post-copulatory male traits and female mating responses in wild-captured and laboratory-reared spring field crickets, *Gryllus veletis*. The phenotype-linked fertility hypothesis suggests that females may receive a direct benefit, enhanced fertilization efficiency, by mating with males that signal attractively. We measured fine-scale components of male acoustic mate attraction signals as well as how much time males spent signalling, measured female preference for males in mating trials and then quantified sperm number and viability. We found no relationship between male signalling traits and male fertility or female preference, providing no evidence for the phenotype-linked fertility hypothesis. We also found no difference in sperm metrics between wild-captured and laboratory-reared males. While female crickets may receive benefits by choosing males based on acoustic signal characteristics, whether the benefits are a result of genetic quality, seminal fluid contents or some other male trait remains unknown.

Keywords Phenotype-linked fertility · Life-history theory · Acoustic signal · Mate attraction · Sexual selection · Sperm competition · Y model

Introduction

Females often exhibit distinct preferences for male secondary sexual traits (Andersson 1994; Candolin 2003). Because of the costs of choosiness (reviewed in Jennions and Petrie 1997), females must gain a fitness advantage for preferences to evolve (Andersson 1994). Females may receive direct material benefits (e.g. parental care and enhanced fecundity; Heywood 1989), indirect genetic benefits (e.g. superior offspring attractiveness or viability; Fisher 1958; Zahavi 1975) or both by mating with preferred males. Direct benefits of female preference result in mating biases toward males that provide more resources, more parental care or that are more fertile (Heywood 1989; Wagner 2011).

Hypotheses suggesting direct benefits (e.g. enhanced fecundity and paternal care) as the mechanism of female mating bias evolution are conceptually simple and are widely supported by empirical data (Møller and Jennions 2001; Kokko et al. 2003). A potential direct benefit of female mate choice based on pre-copulatory sexual traits is enhanced fertilization efficiency from males that signal attractively. The phenotype-linked fertility hypothesis proposes that males advertise their sperm quality as it relates to fertilization efficiency, and their ability to provide enough sperm to fertilize an entire complement of ova (Sheldon 1994). Relative to other types of direct benefits (e.g. the good parent model; Hoelzer 1989), the phenotype-linked fertility hypothesis has received little empirical attention. Under this hypothesis, mate attraction traits and sperm quality are predicted to covary positively because both types of traits should be condition dependent (Sheldon 1994).

Life-history theory predicts that the direction of the relationship between two costly traits depends on the relative variation among individuals in resource acquisition and resource allocation (Y model; van Noordwijk and de Jong 1986). If acquisition is more variable than allocation, we expect a positive relationship between traits because individuals that acquire many resources are able to invest heavily into both traits. Positive relationships among traits with an underlying trade-off have been found in some empirical studies

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L. P. Fitzsimmons (✉) · S. M. Bertram
Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S2Y4, Canada
e-mail: lauren.p.fitzsimmons@gmail.com

(e.g. Spitze et al. 1991). Alternatively, if allocation is more variable than acquisition, we expect a negative relationship between traits because investing heavily in one trait leaves fewer resources available to invest into the other trait (e.g. King et al. 2011; Wagner et al. 2012). In the context of pre- and post-copulatory male sexual traits, a positive relationship between traits may therefore indicate relatively high variation in resource acquisition or a phenotype-linked fertility process, with or without a strong underlying trade-off (e.g. Ruther et al. 2009; Beausoleil et al. 2012). Conversely, a negative relationship between pre- and post-copulatory sexual traits may indicate high variation in resource allocation or a cost of mate choice to female fecundity, and suggests a strong trade-off (e.g. Evans 2010; Rowe et al. 2010; Simmons et al. 2011). While there is theoretical and empirical support for both positive and negative relationships between life-history traits, negative relationships among traits with an underlying trade-off appear to be more common, particularly in resource-limited environments, likely because of the costs of reproduction (Reznick et al. 2000).

Given the importance of these hypotheses in explaining variation in sexually selected traits, we investigated the relationship between acoustic mate attraction signals and male fertility and the relationship between male fertility and female mating responses in crickets. Male field crickets signal acoustically to attract females and repel rival males (Alexander 1961). Females use the acoustic properties of male mate attraction signals to distinguish between potential mates, and tend to prefer males who invest the most effort into acoustic signalling (e.g. time spent signalling; Cade and Cade 1992; Rodríguez-Muñoz et al. 2010). Crickets provide a useful system to study the relationship between pre- and post-copulatory traits because females mate multiply, females receive only ejaculates from their mates and we know how ejaculate quality influences fertilization success in several species (e.g. Sakaluk and Eggert 1996; Schaus and Sakaluk 2001). Previous cricket studies examining the relationship between pre-copulatory mate attraction traits and sperm traits have found mixed results in terms of the pattern of association between traits (Table 1).

We used wild-captured and first-generation laboratory-reared spring field crickets (*Gryllus veletis*) to investigate relationships between pre- and post-copulatory male traits and female mating responses. Specifically, we quantified singing activity and the fine-scale components of males' long-distance mate attraction songs, paired each male with a female and quantified female latency to mate, and then collected the transferred spermatophore to estimate male fertility. We used these data to examine the relationship between male song traits and male fertility, and the relationship between male fertility and female mating responses. A requirement of the phenotype-linked fertility hypothesis is females exhibit preference for the pre-copulatory traits of interest (Sheldon 1994). The traits that comprise the multicomponent acoustic mate attraction signal in crickets are likely to be targets of selection by females and potential candidates for honest signals of sperm quality. Because female preference functions for *G. veletis* have yet to be quantified, we assumed *G. veletis* females prefer particular song traits known to be preferred by females in related species. Female field crickets exhibit distinct preferences for males that signal most often (e.g. *Gryllus campestris*; Rodríguez-Muñoz et al. 2010) and for fine-scale components of male songs, such as high chirp rates and long chirp durations (e.g. *Gryllus lineaticeps*; reviewed in Wagner 2011), long bout durations (e.g. *Gryllus integer*; Hedrick 1986) and loud signals (e.g. *Gryllus texensis*; Cade 1981). Our study is the first to examine how long-distance acoustic mate attraction traits covary with sperm traits and female preference in a natural cricket population, as well as the first to examine whether wild-captured males exhibit different sperm traits than laboratory-reared males.

Methods

Experimental animals

We captured adult male and female *G. veletis* in Ottawa, ON, Canada (45°19′N, 75°40′W) in May and June 2010. We also used the first generation of laboratory-reared offspring from the wild-captured individuals. Late-instar nymphs were removed from the colony, isolated by sex and checked daily for adult eclosion. Adults were individually housed in circular, clear plastic 540-mL containers. Crickets were provided with ad libitum water and food (Harlan Teklad Laboratory Rodent Diet No. 8604: 24 % crude protein, 4 % crude fat, 4.5 % crude fibre) and were held in a temperature-controlled room (30±2 °C).

Acoustic recording

Wild-captured males were placed into individual containers in an electronic acoustic recording system on the morning after capture (EARSII developed for our laboratory by Cambridge Electronic Design Ltd., Cambridge, UK). Lab-reared males were placed in the EARSII recording system when they reached day 7 of adulthood. The EARSII 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 internally lined with 3.5-cm-thick acoustic foam) that contained the

Table 1 Previous studies, all performed on laboratory-reared individuals with unlimited access to high-quality food, have found mixed results in the directionality of association between mate attraction traits and sperm traits in crickets

Species	Finding	Reference	Direction of relationship	Analysis method
Acheta domesticus	No relationship between sperm number or viability and long-distance acoustic signal characteristics	Klaus et al. 2011	–	Phenotypic correlations
Teleogryllus oceanicus	Males with more attractive courtship songs have less viable sperm	Simmons et al. 2010	Negative	Quantitative genetics
Teleogryllus oceanicus	Dominant males have higher sperm viability rates	Thomas and Simmons 2009	Positive	Phenotypic correlations
Gryllodes sigillatus	Courtship call rate is unrelated to spermatophylax size	Ketola et al. 2007	–	Phenotypic correlations

microphone and the LED light. This design minimized the likelihood of individuals detecting their neighbours' signals. Further, although the signals 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.

We continuously recorded long-distance acoustic mate attraction signals for 7 days for each male before conducting mating trials. 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 Ltd., Cambridge, UK). Sounds were recorded at 31.25 kHz.

Crickets produce sound by rubbing their forewings together. The fine-scale structure of cricket signals 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 (Pfau and Koch 1994). We quantified average values of seven signal parameters for each male: chirp duration, interchirp duration, pulses per chirp, pulse duration, interpulse duration, carrier frequency and amplitude. We established species-specific thresholds to classify pulse, interpulse, chirp and interchirp periods (see Fitzsimmons and Bertram 2011 for details). 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 1–8,000 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.

Because the EARSII continuously monitored males' acoustic behaviour, our measure of daily time spent signalling quantifies the total amount of time each male spent signalling during a 24-h period. Hourly signalling 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 produced. Pulse rate (No. of pulses per second) was calculated at a later date using the equation: (No. of pulses per chirp/chirp duration)×1,000.

Body size measurements

Crickets were weighed on the day they mated (day 14 for lab-reared males) using a Denver Instruments balance (Pinnacle Series model PI-314; precision, ±0.1 mg). We also measured head width, pronotum length and pronotum width for each cricket. Measurements were taken using a Zeiss Axio Observer inverted dissecting microscope and highly magnified photographs (AxioVision v4.8, Carl Zeiss, Jena, Germany; magnification approximately ×8.5, resolution approximately 1.60 μm).

Mating trials

We placed each male in an empty 540-mL container with a virgin female of known mass. The cricket pair was observed for up to an hour to determine when mating occurred. We recorded the time it took for the male to begin producing courtship songs and the time it took for the female to mount the male and successfully mate, from their initial meeting to the transfer of a spermatophore. Male field crickets transfer sperm in a discrete external spermatophore that remains attached to the female after mating (Alexander 1961). We calculated mating latency as the time from when the male began courting to when the female mounted the male and a spermatophore was successfully transferred. No-choice tests measure the time it takes a female to mate when she is placed with a single male. This approach can be beneficial because the female's choice is based on the full complement of chemical, acoustic and physical mating cues that are provided by a male. Further, female choice is determined from actual mating, not quantified indirectly from time spent

Table 2 Principal component analysis results for signalling and body size metrics

PCA	PC	Eigenvalue	% variance explained	Factors	Loadings
Signalling	PC1	2.08	30	Chirp duration	0.66
				Pulses per chirp	0.66
				Interpulse duration	0.26
	PC2	1.73	25	Interchirp duration	0.60
				Interpulse duration	0.53
				Amplitude	−0.51
	PC3	1.42	20	Carrier frequency	0.71
				Pulse duration	−0.68
				Chirp duration	0.15
Body size	PC1	3.18	80	Head width	0.55
				Pronotum length	0.55
				Pronotum width	0.55
				Mass	0.28

Analysis of signalling included seven parameters (amplitude, carrier frequency, pulses per chirp, chirp duration, interchirp duration, pulse duration and interpulse duration); we list the three parameters with the heaviest loadings for each signalling principal component

near a male or first male approached. Shackleton et al. (2005) provide an extensive review of the benefits of using no-choice tests and the large number of taxa where no-choice tests are utilized.

Sperm viability and number assays

We immediately removed the spermatophore from the female and placed it into 20 μL of Beadle saline (128.3 mM NaCl, 4.7 mM KCl, 23 mM $CaCl_2$). We then ruptured the spermatophore and gently forced it through a pipette five times to prevent sperm agglutination. To assess the number of sperm produced, we counted sperm from the spermatophore solution using an 'improved Neubauer chamber' haemocytometer under ×40 magnification (Zeiss AxioImager.M2m; Pitcher et al. 2007; Klaus et al. 2011). Sperm count was expressed as the total number of sperm per millilitre for each male. To assess sperm viability, we used a live/dead sperm viability assay (Invitrogen Molecular Probes; Garcia-Gonzalez and Simmons 2005; Thomas and Simmons 2007). Five microlitres of the spermatophore solution was mixed with 5 μL of 1:50 diluted 1-mm SYBR-14 stain and left in the dark for 10 min before 2 μL of 2.4-mm propidium iodide was added. The sample was then incubated in the dark for an additional 10 min before being observed under a fluorescence microscope with a blue excitation filter (λ=470 nm at 60 %). This assay stained live (i.e. viable) sperm green with SYBR-14, a membrane-permeant nucleic acid stain; dead sperm with damaged membranes were stained red with propidium iodide. On very rare occasions, sperm were stained both colours and, as the meaning of these moribund cells is unclear, they were not counted (Pitcher et al. 2007; Klaus et al. 2011). To minimize the effect of loss of viability that occurs after sperm leave the male's body, images of the sperm were taken immediately after staining, and we determined the live/dead ratio by viewing the images after the assay was completed (Holman 2009). We counted 100 sperm per male, and sperm viability was calculated as the proportion of live sperm. Because we

Table 3 Descriptive statistics for *Gryllus veletis* signalling parameters and sperm metrics

Parameter	Mean	SE	Minimum	Maximum
Pulse duration (ms)	15.98	0.16	12.49	18.59
Interpulse duration (ms)	34.56	0.28	29.97	42.34
Pulses per chirp	3.54	0.055	3	5
Chirp duration (ms)	117.91	1.97	89.59	159.3
Interchirp duration (ms)	574.49	18.50	257.5	1,009
Carrier frequency (Hz)	5,050.66	30.09	4,387	5,581
Amplitude (dB)	63.46	0.92	46.52	81.23
Daily time spent calling (min)	107.37	10.55	0	341.3
Sperm viability	0.75	0.025	0.08	1.00
Sperm per mL	9.61×10^5	6.98×10^4	0	3.70×10^6
Viable sperm per mL	7.59×10^5	6.41×10^5	0	2.55×10^6

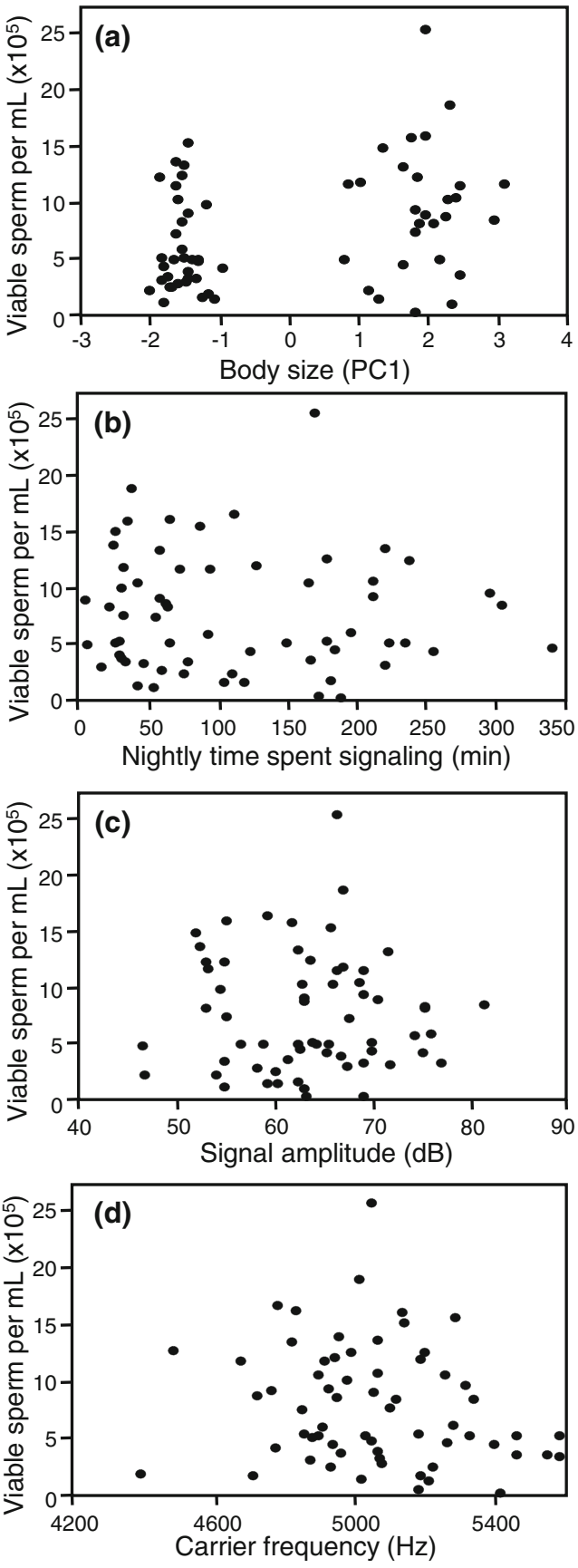

Fig. 1 The lack of relationship between the amount of viable sperm males produced in a mating trial and (**a**) his size (PC1), (**b**) how much time he spent producing long-distance mate attraction signals, (**c**) how loud he signalled and (**d**) at what carrier frequency he signalled

mechanically ruptured spermatophores, our sperm viability estimates may be lower than if sperm was allowed to evacuate freely without rupturing the spermatophores (Gress and Kelly 2011).

We conducted mating and sperm assays on 30 field-captured and 39 laboratory-reared males. The measures we used to estimate sperm quality were total number of sperm, sperm viability and number of living sperm (total number of sperm multiplied by sperm viability). We included number of living sperm as one of our measures because sperm viability and sperm number are suspected to be non-independent of one another, as they may share dependence on environmental and genetic factors (Gress and Kelly 2011) or may be linked simply because of experimental artefacts (Holman 2009). As such, combining the two measurements may offer a more accurate measure of ejaculate quality than either assay alone.

Statistical methods

Due to collinearity of the fine-scale signalling variables and body size metrics, we used principal components analysis (PCA) to reduce the number of factors in our models (Table 2). To address the question of whether sperm metrics are related to male signalling traits, we built general linear models (GLM) with fine-scale signalling metrics (PC1, PC2 and PC3), time spent signalling, male body size (PC1), whether the males were wild-captured or laboratory-reared, and the interactions between our four signalling metrics and whether males were wild-captured or laboratory-reared as the independent variables. We used mean values of the daily signalling PC scores for each male. To address the question of whether female mating responses are related to male sperm traits, we built GLM with mating latency (an indirect measure of female choice), female mass and whether the individuals were wild-captured or laboratory-reared. We performed separate GLM for each sperm metric (sperm number, sperm viability or the number of living sperm). We performed statistical analyses in JMP v10.0.0 (SAS, Cary, NC, USA).

Results

Our PCA for signalling produced three PC factors with eigenvalues above 1.0 that together explained 75 % of the variance in signal structure (Table 2). Examining the most heavily loaded signalling parameters for each component revealed

Table 4 Results of GLM reveal that *Gryllus veletis* sperm metrics are not related to signalling metrics, male body size or whether individuals were wild-captured or lab-reared

GLM	F_a	r^2_{adj}	P	Effects	Coefficient	t	P
Sperm number	1.11	0.02	0.37	Signalling PC1	2.3×10^5	1.57	0.12
				Signalling PC3	1.0×10^5	0.88	0.38
				Signalling PC2	-7.0×10^4	−0.60	0.55
				Male size	1.2×10^5	0.55	0.58
				Time spent signalling	−290	−0.22	0.83
				Field/lab	-8.4×10^4	−0.22	0.83
Sperm viability	0.80	−0.04	0.63	Signalling PC2	0.065	1.51	0.14
				Field/lab	0.15	1.06	0.30
				Time spent signalling	5.1×10^{-4}	1.02	0.31
				Signalling PC3	−0.036	−0.83	0.41
				Male size	−0.085	−0.83	0.41
				Signalling PC1	0.013	0.24	0.81
Number of viable sperm	0.98	−0.003	0.47	Signalling PC1	2.2×10^5	1.59	0.12
				Signalling PC3	8.3×10^4	0.75	0.45
				Time spent signalling	440	0.34	0.73
				Male size	6.0×10^4	0.28	0.78
				Signalling PC2	1.6×10^4	0.15	0.88
				Field/lab	2.2×10^4	0.06	0.95

[a] *df*: Sperm number: 10, 51; sperm viability and number of viable sperm: 10, 48

that males with high PC1 scores had long chirp durations and a high number of pulses per chirp, whereas males with high PC2 scores had signals with long interchirp and interpulse durations produced at low amplitudes. Males with high PC3 scores had signals with high carrier frequencies and short pulse durations (Table 2). Our body size PCA resulted in one PC factor that explained 80 % of the variation in male body size and was loaded positively and equally by head width, pronotum length and pronotum width (Table 2).

Descriptive statistics for fine-scale signal parameters and sperm metrics are reported in Table 3. Our GLM revealed that sperm number, sperm viability or the number of living sperm were not related to male fine-scale signalling traits, how much time males spent signalling, male body size or whether males were wild-captured or lab-reared (Fig. 1 and Table 4). None of the interactions between signalling traits and whether males were wild-captured or laboratory-reared were significant (all $P \geq 0.19$). Similarly, male sperm traits were not related to female mating latency, female mass or whether individuals were wild-captured or lab-reared (Fig. 2 and Table 5).

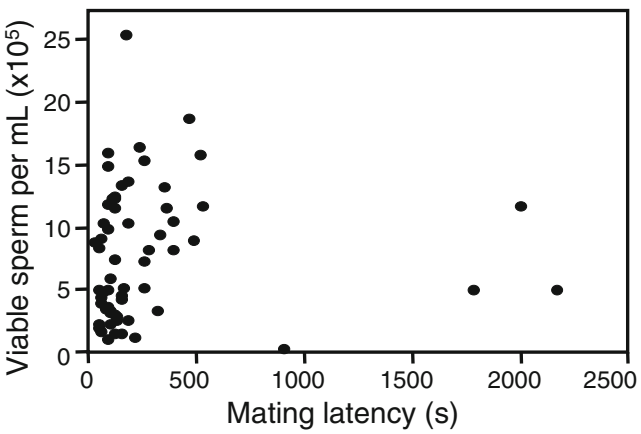

Fig. 2 The lack of relationship between the amount of viable sperm males produced in a mating trial and female mating response (latency to mate)

Discussion

We found no relationships between male acoustic mate attraction signals and male fertility, and no relationships between male fertility and female mating responses in spring field crickets. Our results do not provide supporting evidence for the phenotype-lined fertility hypothesis, and we are unable to speculate as to whether or not an underlying trade-off between mate attraction and male fertility exists. When should we expect relationships between life history traits? The resource environment is likely an important factor in determining what, if any, relationships exist because of the dependence on variances associated with resource acquisition and allocation (Y model; van Noordwijk and de Jong 1986). When acquisition is more variable than allocation, a positive relationship between traits should exist; when allocation is more variable than acquisition, a negative relationship between traits should exist. For example, King et al. (2011) found high genetic variation in both acquisition and allocation in *Gryllus firmus*, and found a trade-off between flight allocation and reproductive allocation only under resource-limited conditions. A

Table 5 Results of GLM reveal that *Gryllus veletis* sperm metrics are not related to female choice (mating latency), female mass, or whether individuals were wild-captured or lab-reared

GLM	F_a	r^2_{adj}	P	Effects	Coefficient	t	P
Sperm number	2.02	0.04	0.12	Field/lab	1.8×10^{5}	2.34	0.02
				Mating latency	−79	−0.59	0.56
				Female mass	140	0.16	0.88
Sperm viability	0.58	−0.02	0.63	Field/lab	0.026	0.95	0.34
				Mating latency	-2.9×10^{-5}	−0.61	0.55
				Female mass	1.8×10^{-4}	0.56	0.58
Number of viable sperm	2.29	0.06	0.088	Field/lab	1.7×10^{5}	2.43	0.018
				Mating latency	−88	−0.73	0.47
				Female mass	270	0.34	0.74

[a] *df*: sperm number, 3, 61; sperm viability and number of viable sperm, 3, 58

positive relationship between pre- and post-copulatory traits, predicted by the phenotype-linked fertility hypothesis, may therefore only be expected under certain resource scenarios.

Previous studies examining relationships between pre- and post-copulatory traits in crickets have used laboratory-reared individuals raised on unlimited access to high-quality food, and the findings have been mixed (Table 1). Klaus et al. (2011) and Ketola et al. (2007) found no relationships between acoustic signals and sperm traits. However, using a quantitative genetic approach, Simmons et al. (2010) found a trade-off between close-range courtship call attractiveness and sperm viability. Our study is the first to examine the relationship between long-distance mate attraction signals and sperm traits using wild-captured crickets, which we expect to be more resource-limited than laboratory-reared individuals. However, following capture, we provided wild-captured crickets with high-quality food, possibly confounding natural relationships by enhancing the resource environment for all individuals. Future diet manipulation experiments should clarify relationships between traits, particularly if treatments mimic the variable and limiting resources likely found in nature.

To date, the only cricket study to reveal a positive relationship between pre- and post-copulatory traits in crickets is Thomas and Simmons (2009), which reports dominance and sperm viability covary in *Teleogryllus oceanicus* (Table 1). However, female crickets do not always prefer dominant males (e.g. Shackleton et al. 2005), male dominance is not a fixed trait in crickets (e.g. Killian and Allen 2008) and experiments in other taxa reveal that ejaculate quality is sensitive to the social environment rather than the result of intrinsic differences between males (e.g. Cornwallis and Birkhead 2007; Montrose et al. 2008). Thus, the positive relationship between male dominance and sperm viability reported by Thomas and Simmons (2009) may provide evidence for the phenotype-linked fertility hypothesis, but is complicated by the flexibility of male dominance and the potential for sperm traits to change according to changes in social status. Further, while females are often assumed to prefer dominant males, male dominance is not always attractive to females or correlated with direct or indirect benefits (reviewed in Qvarnström and Forsgren 1998), and a relationship between male dominance and female fecundity may be the result of females allocating more effort to reproduction after mating with a dominant male (Bretman et al. 2006). Future studies are needed to assess whether female crickets generally receive direct fertility benefits from dominant males.

We did not find a relationship between female mating responses and male fertility, which indicates that female *G. veletis* may not use male acoustic long-distance mate attraction signals as an indicator of fertilization potential. Further, our result that male fertility metrics were unrelated to mating latency suggests that females do not use short-distance signals (e.g. cuticular hydrocarbons and courtship songs) to assess male fertility, but this idea needs to be formally tested. In a recent study on variable field crickets (*G. lineaticeps*), Tolle and Wagner (2011) found that the relationship between male signal attractiveness and female fecundity benefit depends on the resource environment experienced by the male, making female assessment of direct benefits based on male acoustic signals difficult. This finding further highlights the importance of manipulating the resource environment to evaluate the direction of trait relationships.

A word of caution

Sperm traits have been shown to be phenotypically plastic in crickets. For example, sperm transfer depends on aspects of the social environment (e.g. the perceived risk and intensity of sperm competition; Schaus and Sakaluk 2001) and measures of female quality, including female size (Gwynne 1987), age (Simmons et al. 1993), attractiveness (Farmer and Barnard 2000) and mating status (Thomas and Simmons 2007). This plasticity complicates the examination of how traits are related to male fertility. We used males and females of unknown age and mating status in our trials with wild-captured individuals, so these factors may have influenced

sperm transfer during mating. We repeated the experiment with first-generation laboratory-reared individuals to control for age, adult social experience and mating status. We used young (~7 days old) virgin females and 14-day-old virgin males in our lab-reared trials, we held individuals separately during adulthood to control adult social experience and we accounted for variation in female size in our models. Our combined findings revealed that sperm traits were not related to whether males were wild-captured or laboratory-reared, or by male or female size. We only examined the first laboratory-reared generation, however, and differences due to rearing environment may only appear after several generations in captivity (e.g. Brent and Spurgeon 2011).

Can we assume traits are costly?

A major assumption of both the phenotype-linked fertility hypothesis and the Y model is that the traits of interest are costly. Here, we make the common assumptions that acoustic signalling, sperm production and female choice impose costs, but how costly these traits are relative to one another and other life-history traits remains unknown. Conspicuous acoustic signalling can impose costs related to the both the energetics of signalling and predation risk (Prestwich 1994). The metabolic cost of acoustic mate attraction signalling in field crickets has rarely been quantified, but there is evidence that signal components preferred by females are costly for males to produce (e.g. high chirp rate; Hoback and Wagner 1997). Sperm production is generally considered to be costly and condition dependent (reviewed in Wedell et al. 2002; Andersson and Simmons 2006). There is also evidence that males that are successful in pre-copulatory selection have higher costs of sperm production and become sperm depleted, resulting in reduced fertilization success (e.g. Preston et al. 2001). Female choice also imposes several costs, including time and energy spent assessing males, risk of injury from males and risk of predation (reviewed in Reynolds and Gross 1990). Female choice in non-resource-based mating systems has often been explained by potential indirect benefits to females (e.g. Andersson 1994), but the direct benefit of fertilization assurance alone may outweigh the costs of choice (Reynolds and Gross 1990; Kirkpatrick and Ryan 1991). In sum, there is good theoretical and empirical evidence for the costs of acoustic signalling, sperm production and female choice, but how these costs relate to the signalling of male fertility and female preference for male signals in crickets is a promising avenue for future experimental research.

Summary and future directions

Females must gain a fitness benefit for mating preferences to evolve, and fitness benefits are thought to occur either directly or indirectly via genetic benefits. The phenotype-linked fertility hypothesis suggests females can directly benefit from choosing certain males if males advertise their fertilization efficiency. We found pre- and post-copulatory traits did not covary, providing no support for the phenotype-linked fertility hypothesis. It is important to note, however, that life-history theory suggests the direction of the relationship between two costly traits should depend on the relative variation in resource acquisition and allocation. Future studies should therefore manipulate the resource environment to better examine the direction of the relationships between pre-copulatory mating signals, female mating responses and post-copulatory sperm traits. While female crickets may receive benefits by choosing males based on acoustic signal characteristics, whether the benefits are a result of genetic quality, seminal contents or some other male trait remains unknown. Future research should also examine trait relationships using a quantitative genetic approach (e.g. Evans 2010; Simmons et al. 2010; King et al. 2011; Wagner et al. 2012) in natural populations to better evaluate hypotheses related to life-history evolution (Reznick et al. 2000).

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