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## Original Article

# Acoustic experience influences male and female pre- and postcopulatory behaviors in a bushcricket

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Sexual signals can provide information about the range and number of conspecifics in the social environment, and individuals may use such information to adjust their behavior accordingly. However, the extent to which both males and females adjust their behavior remains relatively unexplored. We used the chorusing bushcricket *Ephippiger diurnus* to test whether variation in acoustic signal experience contributes to plasticity in 1) precopulatory and 2) postcopulatory reproductive behaviors. Males produce calls containing 1–8 syllables that are repeated rhythmically within local choruses. During mating, males transfer a large spermatophore to the female, which she then consumes and removes prior to laying eggs. We reared field-collected individuals from late-instar nymphs through adulthood in one of 5 acoustic environments that varied in call syllable number: silent, low, mid, high, and mixed. We found that males 1) increased call rates in the absence of competitors, 2) increased spermatophore size in the presence of calls with a relatively low syllable number, and 3) decreased spermatophore size when exposed to calls with a variable syllable number. Females 1) became more selective as variation in potential mates increased, 2) increased egg investment when exposed to silence or calls with an average syllable number, and 3) decreased investment when exposed to calls with a relatively high syllable number, indicative of attractive males being available. The adjustments that males and females make due to acoustic experience may impact the course of sexual selection by generating feedback loops between the sexes, and in turn may contribute to divergence between populations.

**Key words:** experience-mediated plasticity, mate choice, nuptial gifts, preference functions.

## INTRODUCTION

Phenotypic plasticity allows individuals to adjust to variation in environmental conditions. Social environments provide a particularly dynamic source of environmental variation because they can greatly vary as a function of the individuals creating them and due to the complex interactions between individuals (West-Eberhard 1983). Information from such interactions can in turn greatly influence the development of individual phenotypes. By garnering demographic information through these social interactions, such as the range and number quality and quantity of competitors in their environment, individuals can use this information to adjust their own behavior accordingly (Danchin et al. 2004). For example, conspecific interactions can induce changes in behaviorally plastic traits such as the expression of alternative male reproductive tactics (Bailey et al. 2010) and foraging behavior (Withers et al. 2008).

Sexual advertisement signals are a common feature of social environments that can provide information about other individuals in the vicinity, and how individuals respond to those signals is an important feature of fitness (West-Eberhard 1983; Andersson 1994; Kokko et al. 2006; West-Eberhard 2014). Consequently, individuals often adjust their behavior in response to such experience (Bretman et al. 2011; Rodríguez et al. 2013). In chorusing species, these signals provide a continuous source of feedback to both males and females about potential competitors and/or available mates. By eavesdropping on the signals of others, males can gather information about their competitors, whereas females can gather information about the range of available mates in the environment.

Despite accumulating evidence that males and females adjust their own signaling and mate preference behavior, respectively, in response to signal experience with conspecifics in their environment (Hebets and Sullivan-Beckers 2010; Bretman et al. 2011; Verzijden et al. 2012; Kahn et al. 2013; Rodríguez et al. 2013), our understanding of how individuals respond to social environments is incomplete. Recent work on both males and females indicates

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that potential adjustments made by one sex may strengthen or counter the adjustments of the other (Verzijden et al. 2008; Kahn et al. 2013; Svensson et al. 2014). For example, whereas sexually naive male damselflies discriminate against heterospecific females, females learn to discriminate against heterospecifics through exposure (Svensson et al. 2014). Because female damselflies are more plastic in mate preferences than males, they can generate greater population divergence in mate preferences and reinforce male discrimination of females (Svensson et al. 2014).

In addition to adjustments to signaling and/or mate preference behaviors, there is a suite of postcopulatory adjustments that individuals may make in response to social feedback. For example, a female may adjust how long she retains a spermatophore after mating with a given male, and such postcopulatory adjustments may be concealed from the other sex (Rebar et al. 2011). Thus, understanding the impact that reproductive plasticity may have on population dynamics requires a comprehensive approach involving both participants in the mating equation and extending from pre- to postcopulatory reproductive behaviors.

Here, we study a chorusing insect to learn how males and females may use acoustic signals in their environment to adjust their pre- and postcopulatory reproductive behaviors. We studied a population of the flightless bushcricket *Ephippiger diurnus* (Orthoptera: Tettigoniidae). This species consists of small, isolated populations found in the Pyrenees mountains and along the Mediterranean coast of France (Spooner and Ritchie 2006). Males engage in daily choruses in which each male calls rhythmically, often in alternation with his nearest neighbor. The calls are comprised of syllables, and males in a given population produce calls having a typical syllable number, ranging from 1 to 8 syllables across the complex (Duijm 1990; Ritchie 1991, 1992). Furthermore, between-male variation in syllable number increases as the population mean syllable number increases (Ritchie 1996). Pair formation is typically initiated by female phonotaxis to a calling male, and on contact, she may initiate mating by mounting the male. Females from polysyllabic populations prefer males that produce calls with more syllables than the population mean (Ritchie 1996; Party et al. 2014). On mating, males transfer an extremely large nuptial gift, a spermatophore weighing up to 40% of his body weight (Busnel and Dumortier 1954; Duijm et al. 1983) that contains a sperm-filled ampulla and a nutrient-rich spermatophylax (Gwynne 2001). Females readily consume the spermatophylax after mating (Busnel and Dumortier 1954; Wedell 1994) and begin to lay eggs only after removing the ampulla.

A small number of reproductive behaviors in *E. diurnus* can thus capture most of the reproductive investment that males and females make in pair formation and mating. From the male perspective, advertisement calls constitute most of their mating effort in attracting a mate and nuptial gifts the bulk of their parental effort. Although it is unclear in *E. diurnus* whether spermatophore and ejaculate investment constitutes parental investment (i.e., nutrition) or mating investment (i.e., ejaculate protection and/or prolongation of female refractory period) (Quinn and Sakaluk 1986), either one is an investment that requires males to divert energy from other potential allocations. As for females, phonotaxis to calling males constitutes the majority of their energy in locating a suitable mate, and egg deposition captures most of their reproductive investment in any 1 mating.

We experimentally manipulated male and female experience of acoustic environments from late juveniles through adulthood. This timeline falls within the natural window during which individuals may be exposed to conspecific calls (Rebar D, Barbosa F,

Greenfield MD, personal observation), and nymphs at this age hear the same range of frequencies as they do as adults, but at reduced sensitivity (Rössler 1992). We focus on the general phenomenon of acoustically based plasticity, rather than on the specific timing of when such adjustments in reproductive traits may occur, to test the hypothesis that males and females use acoustic experience to adjust their 1) precopulatory and 2) postcopulatory behaviors. We constructed acoustic environments that varied in male call syllable number, the most divergent adult male trait among populations of *E. diurnus* (Ritchie 1991) and then tested for the effects of those environments on male and female behaviors.

How might individuals be expected to adjust to such acoustic experience? Although no models look at strategic calling investment, empirical work on other species suggests that males invest more into calling with increasing competition (Jia et al. 2001; reviewed in Gerhardt and Huber 2002; Bertram et al. 2013; Callander et al. 2013), and sperm competition theory predicts a general trade-off between pre- and postcopulatory investment (Parker and Pizzari 2010; Kelly and Jennions 2011; Parker et al. 2012). Furthermore, males should increase ejaculate size when a rival male is present and then decrease ejaculate size as the number of rivals increases (Parker and Pizzari 2010; Kelly and Jennions 2011). Alternatively, the Beau Geste hypothesis posits that males use the number of different song types they hear (i.e., calls of different syllable number) to assess the density of competitors (Krebs 1977), and perceived denser environments would offer less mating opportunities because of increased competition and therefore be of lower quality. We thus predicted that males would adjust their calling behavior 1) by increasing their calling rate as the quality and quantity of competition increased and, in addition, that males would call maximally when they heard no competitors. However, we predicted that males would not adjust the number of syllables per call across acoustic environments. Previous work on *E. diurnus* has shown high syllable number repeatability, which is in marked contrast to call rate (Party et al. 2014; Barbosa et al. 2015), suggesting that other call features may be more plastic than call syllable number. We also predicted that males would adjust their spermatophores 2) by decreasing investment as the quality and quantity of rival males increased.

Females, like males, have similar investment options. Empirical work has documented that females are more willing to mate and have weaker preferences when mate availability is low (e.g., Karlsson et al. 2010; Fowler-Finn and Rodríguez 2012a, 2012b), becoming more selective with increasing mate availability (e.g., Fowler-Finn and Rodríguez 2012a). In addition, life-history theory predicts that individuals will trade-off current and future reproductive investment according to their expected future reproductive success (Stearns 1992). We thus predicted that females would adjust their mate preferences 1) by being the least selective when mates were least available, and more selective as the quality and quantity of mates increased. We also predicted that females would adjust their egg investment 2) by not only increasing their current investment in eggs when mates were least available but also increasing it when the most attractive mates were available.

## METHODS

### Study species and population

The geographically isolated populations of *E. diurnus* differ most notably in the average number of syllables produced per male call, with populations averaging from 1 to 7 syllables (Duijm 1990). We studied a population of *E. diurnus* located in Font Romeu, France

(42.49°N, 2.00°E; elevation 1620 m). Males from this population produced an average of 3.5 syllables per call and a mode of 3 syllables per call in 2013, and individual males can range anywhere from 2 to 8 syllables per call (see Results for details).

We collected animals in July 2014 as young nymphs up to the penultimate instar ( $N = 105$  males and 105 females) and brought them to the laboratory. We housed animals individually throughout the experiment, placing each individual in a plastic container ( $107 \times 119 \text{ mm}^2$ ) with acoustically transparent plastic mesh on one-third of the sidewall. We kept all individuals in the same climate-controlled walk-in chamber ( $3.5 \times 2.5 \times 2.5 \text{ m}^3$ ) maintained at 25 °C and a photoperiod of L:D 16:8h. We fed them cabbage, pollen, and fish flakes ad libitum and misted individuals daily with water.

### Social environments

We constructed 5 identical  $100 \times 100 \times 80 \text{ cm}^3$  boxes from 10-mm-thick particle board, leaving one side open in which a light and a speaker were placed pointing into the box, and then lined each wall with 6 cm of 35 kg/m<sup>3</sup> acoustic isolation foam (Flexolan, Diedorf, Germany). The boxes were constructed to partition the climate-controlled chamber into 5 acoustically isolated environments while minimizing differences in rearing environments between them other than the acoustic experience itself. We were unable to replicate each box due space limitations (number of boxes), and thus tried to account for any unintended effects between acoustic environments through the following. First, we verified acoustic isolation between acoustic environment boxes with a sound level meter (CEL-430/2; Casella Cel, Kempston, UK). We found that the noise level in each box at the position of the individuals when the acoustic stimuli from the other boxes were playing was less than the ambient noise generated by the ventilation system alone (42–44 vs. 48–50 dB, respectively). We thus considered the acoustic environments to be sufficiently isolated from one another. Second, we illuminated each box with the same type of solar bulb (Solar Glo PT2193; Rolf C. Hagen Inc., Mansfield, MA) to standardize lighting conditions as much as possible. As these bulbs radiate heat in addition to light, we also verified that temperatures did not differ between boxes (all boxes were 26 °C and 25 °C during the L:D cycle, respectively).

We randomly placed 21 late juvenile males and 21 late juvenile females up to the penultimate instar into each of the 5 acoustic environments (see below), turning each individual container such that the mesh sidewall faced the loudspeaker. All individuals remained in their respective acoustic environment throughout the experiment, except for recording, testing, and mating (see below). We presented each acoustic experience for 6h/day beginning 2h after the lights turned on, and all treatments were presented simultaneously. By housing individuals in separate containers, we ensured that interactions between individuals were restricted to acoustic cues only. Furthermore, we rotated the positions of the individuals within their respective boxes daily to homogenize experience and rearing conditions as much as possible. Given the shared ventilation system of the climate-controlled chamber, similar lighting conditions and temperatures across environments, and daily rotation of individuals in each environment, we consider any differences in responses of individuals between acoustic environments as a result of the acoustic experience itself rather than any microclimatic differences that may have existed.

We broadcast stimuli in each acoustic environment with a single horn tweeter (FT17H; Fostex Corp., Tokyo, Japan) that was centered in the open side of each box and used a multichannel portable playback device (Tascam DR-680; TEAC Corp., Tokyo, Japan)

connected to a custom built multichannel, high-frequency amplifier to drive the speakers. We first calibrated the peak amplitude of the stimuli presented in each environment to that representative of a male 1 m away (90 dB; Greenfield et al. 2004; Party et al. 2014) with a sound level meter. We then introduced individuals to their respective acoustic environment: silent, low, mid, high, and mixed. Acoustic environments varied in the number of syllables in the synthetic male calls that were broadcast to individuals. In the silent environment, individuals were never exposed to any acoustic stimuli. The low environment consisted of only 2 syllable calls, the mid environment only 3 syllable calls and corresponded to the population mode, and the high environment only 4 syllable calls. The mixed environment consisted of 2, 3, and 4 syllable calls. We constructed each chorus stimulus by starting with a single, standard syllable having average acoustic features for the population. We then repeated this syllable to create each polysyllabic call. We adjusted the amplitude to create 4 different calls (relative dB: 0, -4, -8, -12 dB) to simulate distance between calls in order to create the perception of a chorus. We presented calls in a pseudo-randomized order: Calls were randomized within blocks of 4 (one of each amplitude) and a call was presented every 2 s. This alternation of calls falls within the natural range of alternating calls between males (Brunel 2012; Party et al. 2014). We then repeated the block of the 4 calls of different amplitudes in a new randomized order such that all calls were broadcast in equal amounts.

### Male call recording and analysis

Males were recorded in their container but in a different room from where they were reared. The recording room was maintained at the standard 25 °C (range from 24 to 25.5 °C), and it contained an active chorus of males from various populations. This chorus thus extended beyond the natural range of calls that males from this population would experience, and rather served to prompt the focal male to call. Males generally call in choruses and the likelihood that a male will call during a given interval while alone is relatively low. Our interest was on the influence of the acoustic environment, not adjustments made during calling to the active chorus. While that potential existed, all males experienced a similar chorus environment during recording. Furthermore, we separated each focal male by 5 m from the other singing males, the chorus, in the recording room in order to minimize his specific adjustments of call rhythm in response to his neighbor. We placed an electret microphone (LinearX M51, LinearX Systems, Tualatin, OR) directly above the mesh screen and recorded each male for a 2-min interval in which the male was actively calling. All males were recorded from 6 to 8 days postadult molt. We removed males from their respective environments in the morning before the acoustic stimuli began in order to equalize the time since the last acoustic experience and when we recorded each male across environments. Males were given a minimum of 1h to adjust to the recording room. Immediately after recording, we waxed the male's elytra together so that he could no longer produce calls and returned him to his assigned acoustic environment. We muted the males in order to limit the acoustic experience of individuals in each environment to the environment stimuli only as much as possible.

We analyzed the recorded calls using digital sound processing software (Adobe Audition 3.0, Adobe Systems Incorporated, San Jose, CA). We counted the total number of calls produced and the number of syllables per call, and used this information to calculate a male's call rate (calls/min) and average number of syllables per call. We also performed a repeatability analysis (Lessells and Boag

1987) on syllable number within each environment, using the first 20 calls/male.

### Statistical analysis

We performed one-way Anovas for syllable number and call rate to assess variation in those traits among social environments. In the case of overall significance, we performed a post hoc Tukey's Honest Significant Difference (HSD) test to determine which environments differed from one another. We provide a minimum and maximum effect size estimate for the variation in each response variable from all possible pairwise comparisons between acoustic environments. Effect size was first calculated as Cohen's  $d$ , using the difference of means for each environment pair. We then converted  $d$  to the standardized correlation  $r$  in order to express effect size in terms of small ( $r < 0.2$ ), medium ( $0.2 \leq r \leq 0.5$ ), or large ( $r \geq 0.5$ ) (Cohen 1988; Nakagawa and Cuthill 2007).

### Female mate preferences

We focused on female preferences for call syllable number because females from the various populations exhibit strong preferences for this call feature (Ritchie 1996; Party et al. 2014), whereas females do not express a preference for male call rate in all *E. diurnus* populations (Party et al. 2014). We isolated females from their respective acoustic environment at 13-day postadult molt for 24 h to increase the chance that they would be receptive during testing at 14 days, the typical age at which most females are receptive. Following this 24-h isolation period, we tested each female with 10 acoustic stimuli that ranged from 1 to 10 syllables and presented them in a randomized order. By randomizing the sequence for each female, we aimed to limit the potential for order effects that could result in biased female responses. This range of stimuli tests for female preferences beyond the known range of male calls across all populations of *E. diurnus*. We broadcast stimuli through a loudspeaker (Scanspeak; Avisoft Bioacoustics, Glienicke, Germany) and calibrated all stimuli to an amplitude of 85-dB sound pressure level (0 dB = 20  $\mu$ Pa) at the top of the sphere. Each stimulus presented 10 advertisement calls that only varied in syllable number. They were identical in call rate (20 calls/min), syllable length (122 ms), and length duration (30 s). We used a locomotion compensation sphere (TrackSphere LC-300; Syntech Equipment and Research, Kirchzarte, Germany) to test female preferences, which tracks female movement in response to a stimulus. We placed a female on top of the sphere, let her settle, and then presented a stimulus. An overhead camera detected her movement and continuously transmitted her position to motors mounted orthogonally to the sphere that compensated for her movement, such that a female remains on top of the sphere, and the movement information is recorded by computer software (TrackSphere software version 2.2; Syntech Equipment and Research). We used this information to calculate her net movement, in centimeters, toward each stimulus over the 30 s of testing.

We used the responses of a female to the 10 stimuli to construct her mate preference function. We constructed each preference function with nonparametric regression by generating cubic splines, curves that makes no assumption about the shape other than that it is smooth in nature. We calculated cubic splines with the `mgcv` package and a custom-written script in R 3.1.3 (R Development Core Team 2014), allowing the package to choose the smoothing parameter for each individual spline.

### Statistical analysis

We tested for variation in female mate preference functions by adopting a function-valued approach in which the entire preference function is used as the trait of interest (Meyer and Kirkpatrick 2005; Fowler-Finn and Rodríguez 2012a, 2012b; Stinchcombe and Kirkpatrick 2012; Rebar and Rodríguez 2013, 2014). We used a linear mixed model with female response to male stimuli as the dependent variable. We treated the acoustic environment as a fixed effect and female individuals nested within environment as a random effect, along with a linear and quadratic stimulus term, an environment  $\times$  linear stimulus term, and an environment  $\times$  quadratic stimulus term. Each female contributed 1 preference function to the model. The environment term describes differences in the overall responsiveness of females across acoustic environments. The 2 interaction terms test for variation in the shape of the preference functions. The environment  $\times$  stimulus term describes female response as a function of increasing or decreasing syllables per call (e.g., directional or open preference). The environment  $\times$  quadratic stimulus term describes female response as a curvilinear function of syllables per call (e.g., stabilizing or closed preference). Therefore, both interaction terms were of particular interest to us, as significant interaction terms allowed us to explore how the preference functions varied between environments.

We described variation in female preference functions in terms of 2 traits, peak preference and selectivity, that capture important features of preference function shape not detected by the linear mixed model. Peak preference represents the stimulus value that elicits the greatest response from a female while selectivity describes how females disfavor males as they deviate from her peak preference (Bailey 2008; Fowler-Finn and Rodríguez 2012b; Rebar and Rodríguez 2013; Rodríguez et al. 2013; Rebar and Rodríguez 2014). Selectivity was derived from 3 correlated traits: responsiveness, tolerance, and strength (Fowler-Finn and Rodríguez 2012b; Rebar and Rodríguez 2013; Rodríguez et al. 2013; Rebar and Rodríguez 2014). Responsiveness describes the overall height of the curve across all stimuli, tolerance describes the shape of the curve around the peak preference, and strength describes the steepness of the descent of the curve from the peak preference. We performed a Principal component analysis to create the composite trait termed selectivity; this first principal component had an eigenvalue of 1.93 that explained 64.2% of the variance. Responsiveness, tolerance, and strength loaded similarly and quite heavily on this axis (0.78, 0.82, and  $-0.80$ , respectively). We used these 2 traits, peak preference and selectivity, as response variables in one-way Anovas to assess variation in these traits arising from variation in acoustic experience.

### Male and female reproductive investment

Immediately after preference testing, we randomly paired the female with a male from the same acoustic environment. Previous work on *E. diurnus* has shown that young, virgin males do not adjust spermatophores based on their partner's attributes (Jarrige et al. 2013), and in our experiment, females could not assess male calls as males were muted with wax affixed to their elytra. However, because both individuals in each pair were exposed to the same acoustic environment, they could thus potentially influence the investment made by the other. Nonetheless, our approach approximates mating in the field, as the populations of *E. diurnus* are generally small and geographically isolated. In other words, an individual

would be exposed to and mate with another individual in the same acoustic environment.

We used an indirect approach to measure male spermatophore investment as to not interfere with the mating process, thus allowing us to measure a female's current reproductive investment by counting the eggs laid over the next 7 days. We weighed males and females just before pairing and immediately separated the pair after mating, weighing both the male and the female again. We estimated the weight of the spermatophore by calculating the difference in before and after weight for both the male and female, and then averaged the 2 values. We then placed fresh sand in the female's container for her to lay eggs in. The female and male were returned to their respective containers and placed back in their social environment. We collected eggs from females 7 days after mating, a typical minimum number of days before a female is sexually receptive again (Rebar D, Barbosa F, Greenfield MD, unpublished results).

### Statistical analysis

To test for potential adjustments in current reproductive investment, we performed one-way Anovas for spermatophore weight (percentage of body weight), egg number, and egg weight (percentage of body weight) to address variation in those traits among social environments. We first divided spermatophore weight by male pre-mating weight to make our measurement of reproductive investment relative to the size of each male. For female investment, we used egg number and total egg weight relative to the size of each female. In instances of overall significance, we performed a post hoc Tukey's HSD test to determine which environments differed from one another. As before, we provide a minimum and maximum effect size estimate for the range of variation in each response variable between acoustic environments by calculating Cohen's *d* as the difference of 2 environment means and then converting it to *r* (Cohen 1988; Nakagawa and Cuthill 2007). We carried out all statistical analyses with JMP 10 (SAS Institute Inc., Cary, NC).

## RESULTS

### Acoustic experience on male reproductive behaviors

One of 2 calling traits, call rate, was affected by the acoustic environments (Figure 1, Table 1). Consistent with our prediction, call rate was elevated the most by males that experienced no competitors (silent environment; Figure 1) and was significantly higher than males in the low, mid, and mixed environments (Figure 1). Males that experienced higher-quality competitors (high environment) called at a rate between the silent and other acoustic environments, although they were not significantly different from them (Figure 1). Male call syllable number did not change as a result of acoustic experience (Figure 1, Table 1), which matched our prediction, and males across all acoustic environments produced anywhere from 2 to 8 syllables per call. Repeatability estimates for syllable number were high, ranging from 0.59 to 0.83 within acoustic environments.

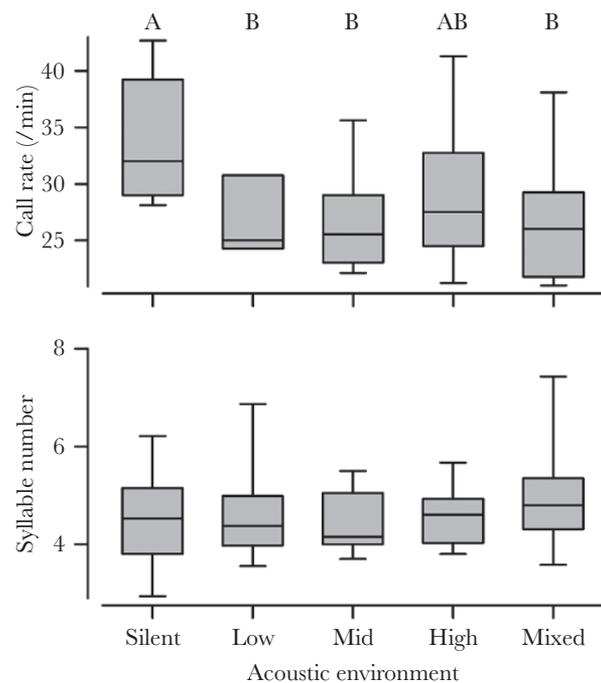
We investigated whether males really did adjust their call rates or whether it was a by-product of a trade-off with syllable number by looking at the relationship between the 2 traits. We found a negative correlation between them within each environment (*r* ranged from  $-0.15$  to  $-0.66$ ), but limited sample sizes restricted our interpretation. Therefore, we pooled across environments to look at the general relationship, finding that call rate and syllable number were negatively correlated ( $N = 48$ ,  $r = -0.47$ ,  $P < 0.001$ ). Because syllable number did not differ between environments, this

result does not contradict but rather strengthens our finding that differences in call rate between environments are due to acoustic experience. Individuals in the silent environment, despite showing a trade-off between call rate and syllable number ( $r = -0.46$ ), called at a faster rate than males in the other acoustic environments, and thus increased their total effort in mate attraction.

Acoustic experience also influenced male reproductive investment (Figure 2, Table 1). Male body mass did not significantly differ between treatments (one-way Anova;  $F_{4, 88} = 0.55$ ,  $P = 0.70$ ), but males produced significantly larger spermatophores, calculated as the percentage of body weight donated, when they experienced unattractive competitors (low environment; Figure 2) compared with when they experienced an increase in the quality and quantity of competitors (mixed environment; Figure 2). Males from the other 3 acoustic environments (silent, mid, high) produced intermediately sized spermatophores that did not significantly differ from those of males in the low or mixed environments (Figure 2).

### Acoustic experience on female reproductive behaviors

Female mate preferences were affected by acoustic experience (Figure 3, Table 2), with females differing in their responsiveness across environments (environment term; Table 2). Across acoustic environments, preference functions were strongly directional, favoring higher call syllable numbers (environment  $\times$  stimulus term; Table 2), with peak preferences nearing the end of the tested range (Figure 3). Females did not adjust their peak preference for call syllable number (that which they find most attractive) due to acoustic experience, but females did adjust how selective they were (Figure 4, Table 3). As we predicted, females were significantly



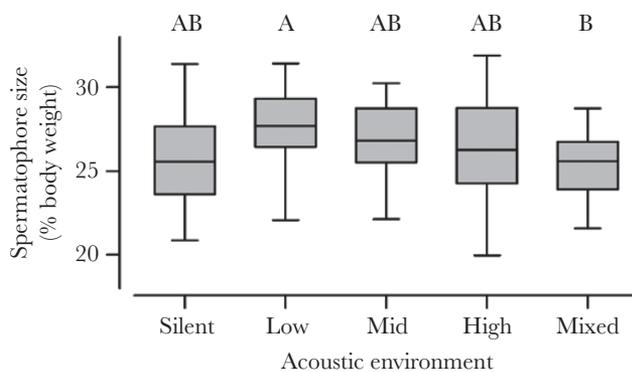
**Figure 1**

Box-and-whisker plots displaying the effect of acoustic environments on 2 traits describing male calling behavior. The box indicates the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and the line the median. The *y* axis represents the range of phenotypic variation in this study for each male trait. Acoustic environments not sharing a letter are significantly different (Tukey's HSD post hoc test).

**Table 1**  
**One-way Anovas testing for variation in *Ephippiger* male reproductive behaviors attributed to acoustic experience**

Trait	Source	df	MS	<i>F</i>	<i>P</i>	<i>r</i>
Call traits						
Syllable number	Environment	4	0.824	0.862	0.490	0.01–0.26
	Error	95	0.956			
Call rate	Environment	4	98.323	<b>3.637</b>	<b>0.0122</b>	<b>0.00–0.60</b>
	Error	43	27.032			
Reproductive investment						
Spermatophore size	Environment	4	54.671	<b>3.318</b>	<b>0.014</b>	<b>0.07–0.49</b>
	Error	86	16.478			

Two traits measuring male calling behavior, the number of syllables per call and call rate (calls per minute), and one trait measuring male reproductive investment, relative spermatophore size (percent of body weight given), are tested. The range of the effect size (*r*) from all pairwise comparisons is reported. Significant tests are in bold. df, degree of freedom; MS, mean square.



**Figure 2**

Acoustic experience on the relative size of male spermatophores. Spermatophore size was calculated as the percentage of body weight that a male gave to a female during mating. The box indicates the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and the line the median. The *y* axis represents the range of phenotypic variation for each male trait in the study. Acoustic environments not sharing a letter are significantly different (Tukey's HSD post hoc test).

more selective when many types of mates were available (mixed environment; Figure 4) than when no mates or midsyllabled mates were available (silent and mid environments; Figure 4).

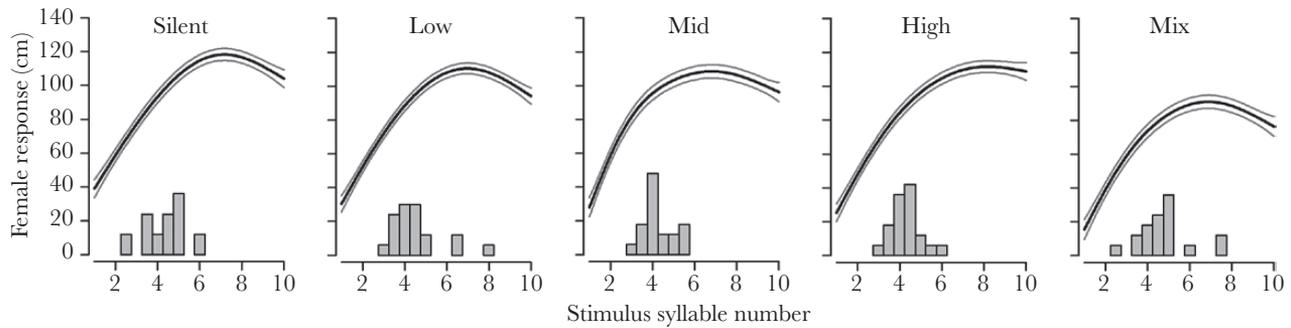
Female reproductive investment was also affected by acoustic experience (Figure 5, Table 3). Consistent with our prediction, females invested more in their current mating opportunity when reared not only in a silent environment but also in a midsyllable environment. Contrary to our prediction, these egg investments were significantly greater than when more attractive mates were available (high environment; Figure 5). The pattern of investment was consistent when measuring the total number of eggs laid or the percentage of body weight represented by those eggs (Figure 5), suggesting that females adjust egg number and not egg size according to acoustic experience. We confirmed that females were indeed adjusting egg number itself by looking at the relationships between egg number, egg size, and percentage of body weight. We found a very strong correlation between egg number and the percentage of body weight represented by eggs (Pearson product-moment correlation,  $N = 88$ ,  $r = 0.98$ ,  $P < 0.0001$ ; Supplementary Figure 1), but no relationship between egg number and egg size (Pearson product-moment correlation,  $N = 88$ ,  $r = 0.03$ ,  $P = 0.78$ ; Supplementary Figure 1) nor egg size and percentage of body weight (Pearson product-moment correlations,  $N = 88$ ,  $r = 0.10$ ,  $P = 0.34$ ; Supplementary Figure 1).

## DISCUSSION

We demonstrate that both males and females make adjustments to reproductive behaviors in response to a source of public information in their social environment. By manipulating their acoustic experience of conspecific male calls, we found that males adjust their pre- and postcopulatory behaviors (call rate and spermatophore size, respectively) in response to variation in perceived male competition. We found that females also adjust their pre- and postcopulatory behavior (preference selectivity and egg investment, respectively) in response to perceived variation in available mates.

### Acoustic experience on male reproductive behaviors

There is accumulating evidence that both real-time (i.e., in presence of) and longer-term experiences of signaling environments elicit male adjustments in signaling behavior (Bailey et al. 2010; Kasumovic et al. 2011; Bertram et al. 2013; Callander et al. 2013; Höbel 2015). A common finding is that males often increase signaling rates in response to longer-term experience of competitors (Bertram et al. 2013; Callander et al. 2013). Alternatively, one may expect males to maximally invest in attracting a mate in the absence of competition, and here, we observed such an effect: Males reared in the absence of competitors called at a higher rate. However, their call rate did not differ from males that experienced the most attractive competitors, suggesting that males may evaluate the quality of their competition to adjust their investment in calling. *Ephippiger diurnus* males typically make real-time adjustments to their call rate in order to alternate calls with the nearest neighbor (Greenfield and Snedden 2003), but our result suggests that there are longer-term effects of acoustic experience on "baseline" call rates. Because all males were recorded in the presence of a large chorus and without a near neighbor, the adjustments in call rate likely reflect a longer-term effect of acoustic experience (or lack thereof) on individuals. In low-density environments or when surrounded by high-quality competitors, male investment in acoustic expenditure (i.e., elevated call rate) may be key for attracting a mate. Recent work revealed that females from several, but not all, populations preferred males with faster call rates in 2-choice tests (Party et al. 2014). Whether this preference is a feature of our study population is unknown. Nonetheless, a male may increase his chance of attracting a mate simply by calling more overall. Such increased calling investment could be especially important in an environment where density is perceived to be very low or when the quality of the competition is perceived to be quite high.



**Figure 3**

Preference functions of females that were reared in different acoustic environments. The phonotactic response (in centimeters) of each female to 10 synthetic playbacks that ranged from 1 to 10 syllables per call was used to construct the preference functions. The black and gray lines display the average preference function  $\pm$  standard error, respectively, of all females tested across the range of stimuli in each acoustic environment. A histogram displaying the mean syllables per call of each male reared in that environment is displayed under each preference function.

**Table 2**

**Linear mixed model testing for differences in the shape of *Ephippiger* female mate preference functions of female responses to playback stimuli according to the acoustic environment in which they developed**

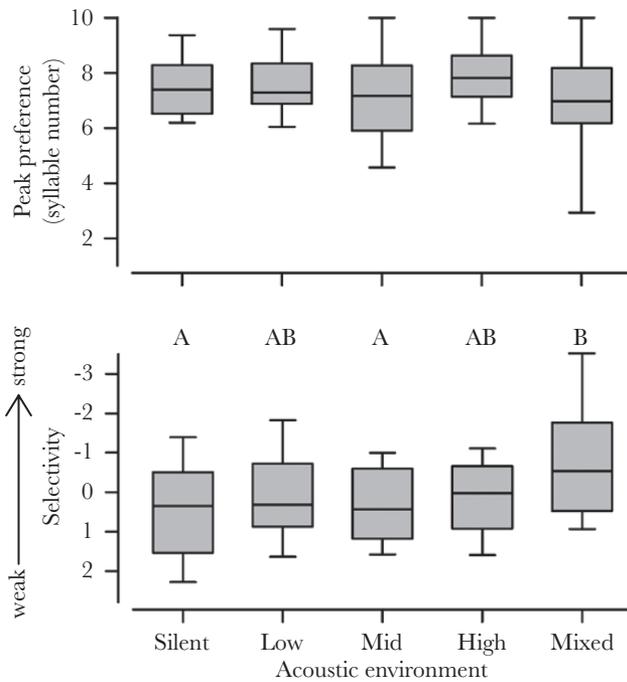
Source of variation	df	<i>F</i>	<i>P</i>
Whole model	98, 791	<b>55.09</b>	<b>&lt;0.0001</b>
Environment	4, 103.05	<b>3.02</b>	<b>0.0213</b>
Stimulus syllable	1, 791	<b>1114.77</b>	<b>&lt;0.0001</b>
Environment $\times$ stimulus syllable	4, 791	<b>4.75</b>	<b>0.0009</b>
Quadratic stimulus syllable	1, 791	<b>631.26</b>	<b>&lt;0.0001</b>
Environment $\times$ quadratic stimulus syllable	4, 791	0.80	0.5259
Individual (environment)	84, 791	<b>11.90</b>	<b>&lt;0.0001</b>

Acoustic environment is a fixed term, and individual is a random term nested within environment. The model includes a linear and quadratic stimulus syllable term, along with interactions of both with the acoustic environment. The environment term tests for differences in the responsiveness of females across social environments. The interaction terms tests for variation among the acoustic environments in the shape of the preference functions, with the stimulus interaction testing for linear (e.g., open) preference and the quadratic stimulus interaction testing for curvilinear (e.g., closed) preference. Significant values are in bold ( $P < 0.05$ ). df, degree of freedom.

Despite among-individual variation in call syllable number, we did not detect any adjustment by males in response to acoustic experience. Our high repeatability estimates, along with similarly high estimates from another polysyllabic population (Barbosa et al. 2015) suggest that males lack the ability to be socially plastic in their number of syllables per call. However, there was a surprising increase in mean call syllable number in this study (4.6 syllables/call average across environments) from that of males from the previous year (3.5 syllables/call). Females from polysyllabic populations prefer calls with higher syllable numbers than the population mean (Ritchie 1996; Party et al. 2014; this study), and this increase may be a result of ongoing directional selection on call syllable number. Alternatively, differences in developmental temperature may have resulted in the overall shift in syllable number (cf., Greenfield and Rodríguez 2004). Individuals developed at a constant, warmer temperature than the field-collected adults the previous year, and may thus have result in the increased syllables per call, a similar effect to that documented in the pulse rates of summer- and winter-developing katydids (Beckers and Schul 2008). However, whether developmental temperature plays a role in adult call phenotypes remains unexplored.

On the other end of investment, it is well accepted that males may strategically allocate their ejaculates based on perceived sperm competition risk (DelBarco-Trillo 2011; Kelly and Jennions 2011). By presenting calls of different amplitude to individuals in each acoustic environment, we were simulating distance between calls. Thus, the goal of our acoustic environments was to recreate a dense chorus of calling males. However, if males did not perceive amplitude differences as different males in their acoustic environment, then our environments would represent low density (silent environment), presence of competitors of varying quality (low, mid, high environments), and high density (mixed environment). Our results suggest this may have been the case: Although we did not detect a significant increase in spermatophore size to increased sperm competition risk (silent vs. low/mid/high environments), males did decrease spermatophore size with increased intensity of sperm competition (low vs. mixed environments) in a manner consistent with sperm competition theory (Parker and Pizzari 2010; Parker et al. 2012) and the perception of competitors according to the Beau Geste hypothesis (Krebs 1977). Furthermore, one might predict that males increase investment with increased competition quality. Although males in this study may have finely adjusted their spermatophores to perceived competition quality at the same level of sperm competition risk (low vs. mid vs. high), spermatophore size decreased with increasing competition quality. Albeit a nonsignificant result, a wider range of call environments may have revealed that males indeed pay attention to male competition quality in addition to competition risk and intensity. Only a few studies to date have demonstrated the social cues used by males to respond to rivals (Bailey et al. 2010; Bretman et al. 2011; Gray and Simmons 2013), and our results provide insight into the types of adjustments that males can make above and beyond just the presence or absence of rivals in their environment.

Sperm competition theory also predicts a general trade-off between pre- and postcopulatory investment (Parker and Pizzari 2010; Parker et al. 2012). Because males were muted immediately after recording but did not mate until females matured about a week later, the temporal separation between trait measures precludes us from testing this prediction. However, work on a similar population of *E. diurnus* recently documented a trade-off between syllable number and spermatophore size, along with a further trade-off between those 2 traits and immunity (Barbosa et al. 2015). Further work on the links between pre- and postcopulatory investment in varying social environments is thus warranted.



**Figure 4** Acoustic experience on 2 traits describing the mate preference functions of *Ephippiger* females. The  $y$  axis for the traits represents the phenotypic range observed in this study for each trait. The box indicates the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and the line the median. Acoustic environments not sharing a letter are significantly different (Tukey's HSD post hoc test).

**Table 3**

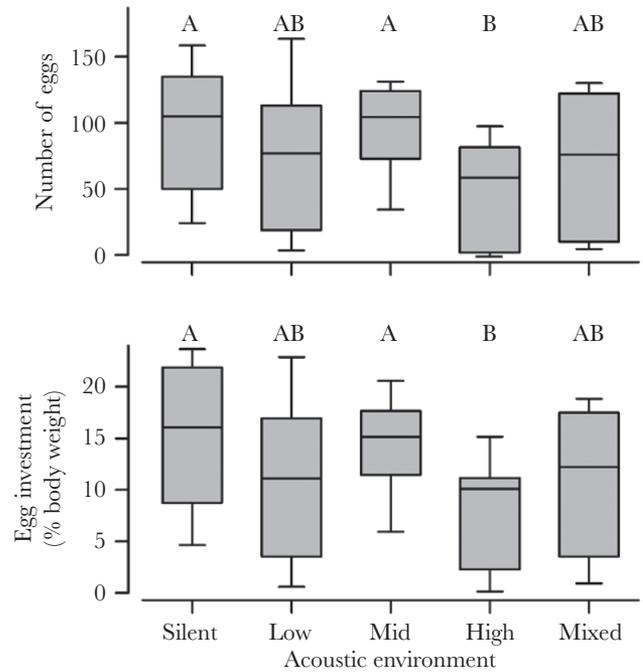
**One-way Anovas testing for variation in female reproductive behaviors attributed to differences in acoustic environments**

Trait	Source	df	MS	$F$	$P$	$r$
Mate preference	Environment	4	2.934	1.199	0.317	0.04–0.28
	Error	84	2.447			
Selectivity	Environment	4	5.471	<b>3.111</b>	<b>0.0194</b>	<b>0.01–0.38</b>
	Error	84	1.759			
Reproductive investment	Environment	4	7809.20	<b>3.733</b>	<b>0.008</b>	<b>0.06–0.48</b>
	Error	83	2092.02			
Egg investment	Environment	4	131.095	<b>3.103</b>	<b>0.0197</b>	<b>0.02–0.52</b>
	Error	83	42.243			

Two traits of female mate preference functions, peak preference and preference selectivity, and 2 related metrics measuring reproductive investment, the number of eggs laid over 7-day postmating and percentage of body weight invested in those eggs, are tested. Estimates for the minimum and maximum effect sizes ( $r$ ) from pairwise comparisons of acoustic environments are provided. Significant values are in bold. df, degree of freedom; MS, mean square.

### Acoustic experience on female reproductive behaviors

Females often adjust their mate preferences in response to experience with the mating signals of potential mates (Hebets and Sullivan-Beckers 2010), and numerous studies have demonstrated similar plasticity in preference selectivity as that seen here (Wagner et al. 2001; Bailey and Zuk 2008; Fowler-Finn and Rodríguez 2012a, 2012b). *Ephippiger* females were the most motivated and/or least selective in the absence of mates, but equally so in the presence of males of an average syllable number, and became more selective with increased variation in available mates. The lack of a shift in peak preference across acoustic environments by uniformly



**Figure 5** Acoustic experience on 2 measures of female reproductive investment. Eggs were counted over 7 days after mating, and egg investment was calculated as the percentage of female body weight allocated to those eggs. The box indicates the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and the line the median. Acoustic environments not sharing a letter are significantly different (Tukey's HSD post hoc test).

favoring high polysyllabic calls suggests that females exert consistent directional selection on male call syllable number.

Do females reinforce their high syllable preference with their postcopulatory behavior? Life-history theory predicts that individuals will trade-off current and future reproductive investment according to their expected future reproductive success (Stearns 1992). First, our result of increased female egg investment in the absence of acoustic cues is consistent with such theory, and regardless of the quality of the mate, females should shift their allocation to that mating opportunity as it may be her only opportunity. In the presence of acoustic cues, and contrary to our expectations, females with the most attractive perceived mates invested the least in their

current mating (high environment). Without reliable information about their current mate (i.e., male song), females may be shifting their investment toward future reproduction when those mates are perceived to be of higher quality, as represented by the acoustic environment. This behavior would thus reinforce their preference for calls with more syllables. Alternatively, females may have had a “disappointing” mating experience because of the lack of male song and phonotaxis toward the male of their choosing, and this contrasted with their “expectation” of a higher-quality mate. Egg investment may thus reflect an expectation–reality contrast above and beyond any allocation trade-off. Such dissonance may lead to an apparent disagreement between pre- and postcopulatory preference. Indeed, egg investment adjustments in the other acoustic environments indicate a more complex interaction of current and future potential. However, females may be adjusting their current investment to other cues present during mating. Recall that females received no acoustic cues from their mates (elytra were waxed), but females do antennate and evaluate males with their maxillary palps before mating. Recently, cuticular hydrocarbons (CHCs) have been shown to play a role in female mate preferences above and beyond song in a field cricket (Thomas and Simmons 2009; Simmons et al. 2013). In the absence of the major cue of male song available, females may use male CHCs to assess the quality of their current mate. If male CHCs positively correlate with male call syllable number such that they are more attractive to females, then males from the low treatment would contrast with the females’ experience in that treatment. Those males, because of their high average call syllable number, would be of higher quality than potential mates as represented by the acoustic playback. Females would thus be expected to invest more in their current mate in the low acoustic environment. Alternatively, females may be reproductively compensating for their mate’s unattractiveness (Harris and Uller 2009; Ratikainen and Kokko 2010). Our results strongly suggest that females may not be able to augment the quality of each offspring (larger eggs in this case) to counter a low-quality mate, and so investing in more offspring (number of eggs) in the low acoustic environment may reflect the only form of compensation that females can do to offset the perceived lower-quality males.

### Impact of male and female adjustments

Plasticity in male and female pre- and postcopulatory reproductive behaviors may alter the course of sexual selection, but which plastic adjustments persist depends on their fitness consequences. In other words, adaptive adjustments are likely to have consistent long-term impacts on sexual selection, but whether the documented plasticity here is adaptive or not remains unknown.

In addition, the timing of when such reproductive adjustments occur will also contribute to ongoing sexual selection. For example, males increased spermatophores when surrounded by unattractive males, but females varied the most in how they allocated egg investment in that same environment. Individuals may have modified their development as juveniles in response to acoustic cues, and this response may only be beneficial if the social environment remains relatively unchanged through adulthood. Alternatively, individuals may have modified their responses as adults, or even in addition to developmental responses, such that the overall response may be more beneficial than harmful or that such adjustments allow individuals to increase their potential should the social environment fluctuate temporally. Thus, the timing of such responses may be an important feature of how well the plastic responses benefit individual reproductive success.

Nonetheless, the nature of sexual selection may be impacted by potential feedback loops generated by the acoustically based adjustments that males and females make, and our results suggest that such feedback loops could be quite complex. *Ephippiger* populations are geographically isolated with minimal gene flow between populations (Spooner and Ritchie 2006), and social plasticity within and between populations may differ, generating different feedback loops and thus having important consequences for divergence in male and female reproductive behaviors. Furthermore, trade-offs between pre- and postcopulatory investment with other life-history traits, as recently documented in another polysyllabic population of *E. diurnus* (Barbosa et al. 2015), may contribute to the types of plasticity within and between populations. Whether such trade-offs are a common feature across populations that in turn may constrain trait plasticity or impact lifetime reproductive success remains unresolved. Nonetheless, further exploration of acoustically mediated plasticity in reproductive traits is thus required to further our understanding of the potential for complex feedback dynamics between male and female reproductive behaviors, and their importance in shaping and even promoting divergence among populations.

### SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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### REFERENCES

- Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.
- Bailey NW. 2008. Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. *Behav Ecol.* 19:960–966.
- Bailey NW, Gray B, Zuk M. 2010. Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Curr Biol.* 20:845–849.
- Bailey NW, Zuk M. 2008. Acoustic experience shapes female mate choice in field crickets. *Proc Biol Sci.* 275:2645–2650.
- Barbosa F, Rebar D, Greenfield MD. 2015. Reproduction and immunity trade-offs constrain mating signals and nuptial gift size in a bushcricket. *Behav Ecol.* doi: 10.1093/beheco/arv131.
- Beckers OM, Schul J. 2008. Developmental plasticity of mating calls enables acoustic communication in diverse environments. *Proc Biol Sci.* 275:1243–1248.
- Bertram SM, Harrison SJ, Thomson IR, Fitzsimmons LP. 2013. Adaptive plasticity in wild field cricket’s acoustic signaling. *PLoS One.* 8:e69247.
- Bretman A, Gage MJ, Chapman T. 2011. Quick-change artists: male plastic behavioural responses to rivals. *Trends Ecol Evol.* 26:467–473.
- Brunel O. 2012. De la communication acoustique au sein du groupe: contraintes et mécanismes [dissertation]. [Tours (France)]: Université François-Rabelais.

- Busnel RG, Dumortier B. 1954. Observations sur le comportement acousticosexuel de la ♀ d'*Ephippiger bitterensis*. *Compt Rend Soc Biol*. 148:1589–1592.
- Callander S, Kahn AT, Hunt J, Backwell PRY, Jennions MD. 2013. The effect of competitors on calling effort and life span in male field crickets. *Behav Ecol*. 24:1251–1259.
- Cohen J. 1988. *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates.
- Danchin E, Giraldeau LA, Valone TJ, Wagner RH. 2004. Public information: from nosy neighbors to cultural evolution. *Science*. 305:487–491.
- Delbarco-Trillo J. 2011. Adjustment of sperm allocation under high risk of sperm competition across taxa: a meta-analysis. *J Evol Biol*. 24:1706–1714.
- Duijm N. 1990. On some song characteristics in *Ephippiger* (Orthoptera: Tettigoniidae) and their geographic variation. *Neth J Zool*. 40:428–453.
- Duijm N, Oudman L, Veldstra B. 1983. Copulation in *Ephippiger* (Orthoptera, Tettigoniidae). *Tijdschr Voor Entomol*. 126:91–96.
- Fowler-Finn KD, Rodríguez RL. 2012a. The evolution of experience-mediated plasticity in mate preferences. *J Evol Biol*. 25:1855–1863.
- Fowler-Finn KD, Rodríguez RL. 2012b. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution*. 66:459–468.
- Gerhardt HC, Huber F. 2002. *Acoustic communication in insects and anurans*. Chicago (IL): University of Chicago Press.
- Gray B, Simmons LW. 2013. Acoustic cues alter perceived sperm competition risk in the field cricket *Teleogryllus oceanicus*. *Behav Ecol*. 24:982–986.
- Greenfield MD, Rodríguez RL. 2004. Genotype-environment interaction and the reliability of mating signals. *Anim Behav*. 68:1461–1468.
- Greenfield MD, Siegfried E, Snedden WA. 2004. Variation and repeatability of female choice in a chorusing katydid, *Ephippiger ephippiger*: an experimental exploration of the precedence effect. *Ethology*. 110:287–299.
- Greenfield MD, Snedden WA. 2003. Selective attention and the spatio-temporal structure of orthopteran choruses. *Behaviour*. 140:1–26.
- Gwynne DT. 2001. *Katydid and bush-crickets: reproductive behavior and evolution of the Tettigoniidae*. Ithaca (NY): Cornell University Press.
- Harris WE, Uller T. 2009. Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. *Philos Trans R Soc B*. 364:1039–1048.
- Hebets E, Sullivan-Beckers L. 2010. Mate choice and learning. In: Breed MD, Moore J, editors. *Encyclopedia of animal behavior*. Amsterdam (The Netherlands): Elsevier. p. 389–393.
- Höbel G. 2015. Socially mediated plasticity of chorusing behavior in the gladiator frog *Hypsiboas rosenbergi*. *Acta Ethol*. 18:145–152.
- Jarrige A, Greenfield MD, Goubault M. 2013. Male song as a predictor of the nuptial gift in bushcrickets: on the confounding influence of male choice. *Anim Behav*. 85:1427–1434.
- Jia F-Y, Greenfield MD, Collins RD. 2001. Ultrasonic signal competition between male wax moths. *J Insect Behav*. 14:19–33.
- Kahn AT, Dolstra T, Jennions MD, Backwell PRY. 2013. Strategic male courtship effort varies in concert with adaptive shifts in female mating preferences. *Behav Ecol*. 24:906–913.
- Karlsson K, Eroukhanoff F, Svensson EI. 2010. Phenotypic plasticity in response to the social environment: effects of density and sex ratio on mating behaviour following ecotype divergence. *PLoS One*. 5:1–6.
- Kasumovic MM, Hall MD, Try H, Brooks RC. 2011. The importance of listening: juvenile allocation shifts in response to acoustic cues of the social environment. *J Evol Biol*. 24:1325–1334.
- Kelly CD, Jennions MD. 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biol Rev*. 86:863–884.
- Kokko H, Jennions MD, Brooks R. 2006. Unifying and testing models of sexual selection. *Annu Rev Ecol Syst*. 37:43–66.
- Krebs JR. 1977. The significance of song repertoires: the Beau Geste hypothesis. *Anim Behav*. 25:475–478.
- Lessells CM, Boag PT. 1987. Unrepeatable repeatabilities: a common mistake. *Auk*. 104:116–121.
- Meyer K, Kirkpatrick M. 2005. Up hill, down dale: quantitative genetics of curvaceous traits. *Philos Trans R Soc B*. 360:1443–1455.
- Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev*. 82:591–605.
- Parker GA, Lessells CM, Simmons LW. 2012. Sperm competition games: a general model for precopulatory male–male competition. *Evolution*. 67:95–109.
- Parker GA, Pizzari T. 2010. Sperm competition and ejaculate economics. *Biol Rev*. 85:897–934.
- Party V, Brunel-Pons O, Greenfield MD. 2014. Priority of precedence: receiver psychology, female preference for leading calls and sexual selection in insect choruses. *Anim Behav*. 87:175–185.
- Quinn JS, Sakaluk SK. 1986. Prezygotic male reproductive effort in insects: why do males provide more than sperm? *Fl Entomol*. 69:86–94.
- Ratikainen II, Kokko H. 2010. Differential allocation and compensation: who deserves the silver spoon? *Behav Ecol*. 21:195–200.
- R Development Core Team. 2014. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.
- Rebar D, Rodríguez RL. 2013. Genetic variation in social influence on mate preferences. *Proc Biol Sci*. 280:20130803.
- Rebar D, Rodríguez RL. 2014. Genetic variation in host plants influences the mate preferences of a plant-feeding insect. *Am Nat*. 184:489–499.
- Rebar D, Zuk M, Bailey NW. 2011. Mating experience in field crickets modifies pre- and postcopulatory female choice in parallel. *Behav Ecol*. 22:303–309.
- Ritchie MG. 1991. Female preference for “song races” of *Ephippiger ephippiger* (Orthoptera: Tettigoniidae). *Anim Behav*. 42:518–520.
- Ritchie MG. 1992. Variation in male song and female preference within a population of *Ephippiger ephippiger* (Orthoptera: Tettigoniidae). *Anim Behav*. 43:845–855.
- Ritchie MG. 1996. The shape of female mating preferences. *Proc Natl Acad Sci USA*. 93:14628–14631.
- Rodríguez RL, Rebar D, Fowler-Finn KD. 2013. The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim Behav*. 85:1041–1047.
- Rössler W. 1992. Postembryonic development of the complex tibial organ in the foreleg of the bushcricket *Ephippiger ephippiger* (Orthoptera, Tettigoniidae). *Cell Tissue Res*. 269:505–514.
- Simmons LW, Thomas ML, Simmons FW, Zuk M. 2013. Female preferences for acoustic and olfactory signals during courtship: male crickets send multiple messages. *Behav Ecol*. 24:1099–1107.
- Spooner LJ, Ritchie MG. 2006. An unusual phylogeography in the bushcricket *Ephippiger ephippiger* from Southern France. *Heredity*. 97:398–408.
- Stearns SC. 1992. *The evolution of life histories*. Oxford: Oxford University Press.
- Stinchcombe JR, Kirkpatrick M. 2012. Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol Evol*. 27:637–647.
- Svensson EI, Runemark A, Verzijden MN, Wellenreuther M. 2014. Sex differences in developmental plasticity and canalization shape population divergence in mate preferences. *Proc Biol Sci*. 281:20141636.
- Thomas ML, Simmons LW. 2009. Sexual selection on cuticular hydrocarbons in the Australian field cricket, *Teleogryllus oceanicus*. *BMC Evol Biol*. 9:162.
- Verzijden MN, ten Cate C, Servedio MR, Kozak GM, Boughman JW, Svensson EI. 2012. The impact of learning on sexual selection and speciation. *Trends Ecol Evol*. 27:511–519.
- Verzijden MN, Korthof REM, ten Cate C. 2008. Females learn from mothers and males learn from others. The effect of mother and siblings on the development of female mate preferences and male aggression biases in Lake Victoria cichlids, genus *Mbipia*. *Behav Ecol Sociobiol*. 62:1359–1368.
- Wagner WE Jr, Smeds MR, Wiegmann DD. 2001. Experience affects female responses to male song in the variable field cricket *Gryllus lineaticeps* (Orthoptera, Gryllidae). *Ethology*. 77:769–776.
- Wedell N. 1994. Dual function of the bushcricket spermatophore. *Proc Biol Sci*. 258:181–185.
- West-Eberhard MJ. 1983. Sexual selection, social competition, and speciation. *Q Rev Biol*. 58:155–183.
- West-Eberhard MJ. 2014. Darwin's forgotten idea: the social essence of sexual selection. *Neurosci Biobehav Rev*. 46:501–508.
- Withers GS, Day NF, Talbot EF, Dobson HEM, Wallace CS. 2008. Experience-dependent plasticity in the mushroom bodies of the solitary bee *Osmia lignaria* (Megachilidae). *Dev Neurobiol*. 68:73–82.