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Efficacy constraints on female directional preference stabilize a male call component in a multimodal cricket duet

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Mate advertisement signals can vary in their variability, with some signals or signal components being highly stereotyped and others highly variable. One hypothesis for differences in variability suggests that receivers provide stabilizing selection for signal components important for mate recognition, resulting in low variability, while also providing directional selection for signal components indicating mate quality, correlating with greater variability. Factors that affect signal efficacy, however, can also influence signal design – and might interact with receiver preferences to influence variability. We investigated these hypotheses in the duetting cricket species *Lebinthus bitaeniatus* Stål. Like many other insects, these crickets use call-and-response signalling to find mates. Males produce an acoustic signal, females reply with a vibrational signal, and males use these vibrations to find females. Male calls have two components: a series of isolated syllables (ticks) followed by a rapid series of syllables (a trill). The number of ticks per call is highly variable, whereas the number of pulses in the trill is stereotyped. Male size was correlated positively with the number of trill pulses per call, but not with the number of ticks. Female response amplitude was not related to the number of ticks in the male call (the variable component) and it increased with trill duration (the stereotyped signal component), contrary to the stabilizing directional selection hypothesis. We also found, however, that the latency of the female vibrational reply became erratic as the trill increased beyond the typical duration. Since males in duetting insect species rely on the species-specific latency of the female reply to recognize the signal, this reduces the efficacy of the female reply for communication despite the females' greater amplitude response. Given the prevalence of call-and-response signalling in insects, this relationship between female response amplitude and signal efficacy could be a common factor influencing signal variability across many species.

Keywords:

Eneopterinae

multicomponent signal

multimodal communication

signal efficacy

vibrational signal

Most animals produce species-specific communication signals to attract mates and reproduce. This simple imperative has given rise to diverse and complex signals that comprise multiple components or can be perceived across multiple sensory modalities (Andersson, 1994). Various hypotheses have been proposed to explain the evolution of multimodal and multicomponent signals (Candolin, 2003; Hebets & Papaj, 2005; Johnstone, 1996). Across taxa, there is empirical support for both content-based hypotheses, where each signal component provides a different type of information (e.g. Nelson & Poesel, 2007), and efficacy-based hypotheses, which concern the recognition of a signal by its intended receiver despite environmental noise (e.g. Grafe et al., 2012). In addition to the presence of multiple components, a notable aspect of some complex signals is the variability of each component. Some signal components are highly variable within and between individuals, while others are more stereotyped. For mate advertisement signals, variation is both predicted and observed to correspond to open-ended receiver preferences, meaning that potential mates prefer more extreme signal properties; conversely, stereotypy corresponds to unimodal receiver preferences, meaning that potential mates prefer a narrow range of signal properties (Gerhardt, 1991; Ritchie, 1996; Shaw & Herlihy, 2000; but see also Millan et al., 2020). One common hypothesis for differing levels of variability in different advertisement signal components is that different information is being conveyed by each component (Gerhardt, 1991; Hebets & Papaj, 2005), potentially to different receivers (Zambre & Thaker, 2017). Information for mate recognition, where similarity to conspecifics is necessary, can then be transmitted in parallel (or at least in close proximity; see Gray, 2022) to information about individual quality, where distinction from conspecifics is important (Pfennig, 1998).

Studies on mate-finding behaviour have largely focused on unidirectional male signals towards which female searchers orient. Many animals across diverse taxa, however, use bidirectional, call-and-

response duetting to locate mates (Bailey, 2003; Pika et al., 2018). In these dyadic interactions, both individuals produce signals, and males take on some or all of the searching role (Heller, 1992; Heller et al., 2021; Scherberich et al., 2017). To understand mate-finding interactions in these species, it is therefore necessary to consider the signals produced by both animals. When these signals contain multiple components, each component has the potential to influence the behavioural response of the other animal (Kuhelj, 2016; Rodríguez & Barbosa, 2014). Even when signals are relatively simple, multiple signal features often influence successful pair formation because of the need for precise temporal coordination between the initiating caller and the replier. In many orthopteran duets, for example, calling males insert a ‘trigger’ component into their call to prompt correctly timed replies from answering females (Heller et al., 2015; Heller & von Helversen, 1986). Conversely, whether a calling male recognizes and responds to a replying female depends on two efficacy-related features of her reply: amplitude and timing. Amplitude affects whether the reply is above the hearing threshold of the male (van Staaden & Römer, 1997). Timing of the reply relative to the male call affects whether the reply falls within a species-specific window of recognition, outside of which the male will often not keep calling or perform phonotaxis (Forrest et al., 2006; Heller & von Helversen, 1986; Zimmermann et al., 1989). In at least some duetting katydid species, females adjust the timing and intensity of their reply based on cues within the male call (Villarreal & Gilbert, 2013) as well as their own motivational level (Bailey & Hammond, 2004). Duetting species therefore present an opportunity to examine how different features of two interacting signals shape each other. If female replies vary in intensity and latency, and particular call features of males predictably elicit greater reply amplitudes or less variable reply latencies, then males with those call features will be favoured by selection because they should locate replying females more easily or more often (Bailey, 2003).

A cricket species with a particularly interesting communication system is *Lebinthus bitaeniatus*

Stål (Gryllidae; Eneopterinae), a member of the tribe Lebinthini. Unusually for crickets, Lebinthini use duets, rather than unidirectional calling, to locate mates (ter Hofstede et al., 2015). Their communication system is one of the only examples of multimodal acoustic–vibratory mate-finding duets currently known, with another described in a pseudophylline katydid (Rajaraman et al., 2015). These duets are distinct from exchanges of acoustic and vibrational signals by crickets during close-range courtship (e.g. Dambach & Beck, 1990; deCarvalho & Shaw, 2010) in that they occur prior to antennal or other physical or even visual contact, with a specific temporal relationship between the initiating acoustic call and the vibrational reply. *Lebinthus bitaeniatus* males produce high-frequency and temporally complex airborne calls made up of a series of longer syllables produced at long intervals (referred to here as individual ‘ticks’) followed by a series of shorter syllables produced at short intervals (referred to collectively as a ‘trill’) (Fig. 1). The different components of the male call exhibit different levels of variability, with the number of ticks varying more than the number of trill syllables (Tan et al., 2021; this study). Female crickets respond to this call with a short vibrational signal that travels through the plant substrate, and the male searches for her following these signals (ter Hofstede et al., 2015). We have also observed that the amplitude of the female reply can vary under some circumstances, whereas the latency of the reply remains relatively constant (ter Hofstede, personal communication). These observations raised the question: has selection by female crickets shaped male call structure in *L. bitaeniatus*? Specifically, we asked the following questions. (1) Do the numbers of syllables in each call component vary with male size? (2) Does the shape of female preference functions differ for variable compared to stereotyped signal components?

To answer these questions, we first quantified variation in the tick and trill components of the male *L. bitaeniatus* call and tested whether the average number of syllables in each call component was related to male size. Female crickets across diverse species typically prefer larger males (e.g. Forrest et

al., 1991; Stoffer & Walker, 2012) and the songs of larger males (e.g. Gray, 1997; Ponce-Wainer & del Castillo, 2008). Then, by playing back synthetic male calls and recording female vibrational replies, we tested whether and how female crickets vary the presence, amplitude and timing of their replies in response to male calls with different acoustic properties. Having observed that the number of trill syllables was much less variable within and between males than was the number of ticks, we assessed whether the two call components provide different types of information to the female. Specifically, we tested the hypothesis that the less variable component (trill) functions in mate recognition, predicting that the trill would be both necessary and sufficient to elicit a reply and that females would have a unimodal preference for the stereotypical trill, as measured by the intensity and latency of their replies. Concurrently, we tested the hypothesis that the more variable component (ticks) functions in mate discrimination. Because it is common for female orthopterans to prefer, or respond more strongly to, more frequent or longer-duration male calls (Gerhardt & Huber, 2002), we predicted that females would have an open-ended preference for more ticks in a call, producing greater amplitude replies to higher-tick calls. Finally, we considered an alternative hypothesis that the stereotyped duration of the trill is a compromise between a female preference for longer trills and limits on the efficacious timing of communication signals in a duet. In this scenario, the stereotyped trill would act to stabilize the latency of the female reply, improving the male's ability to recognize her signal, even if the number of ticks in male calls is highly variable.

<H1>METHODS

<H2>Study Animals

A colony of *L. bitaeniatus* crickets was established at Dartmouth College (Hanover, NH, U.S.A.) in 2013 from eggs laid by wild-caught females from the Philippines. Crickets were housed in plastic terraria (37 × 22 × 24 cm) and provided with water, food and egg carton shelters. Crickets that were used in experiments were isolated from the opposite sex as juveniles to ensure that all crickets were unmated at the time of initial testing. Newly emerged adults were housed in small containers with no more than two other individuals of the same sex. We recorded adult age as the number of days since the cricket's final moult.

<H2>Male Calling Song

To test for correlations between the amount of calling and male size, we recorded individual *L. bitaeniatus* male crickets ($N=15$) for 24 h under a 12:12 h light:dark cycle. Crickets were placed in a box (50 × 33 × 45 inches, 127 × 84 × 114 cm) lined with acoustic foam and with a mesh top, and food and water were provided ad libitum. Calls were recorded with a ¼-inch microphone (Type 4939-A-011; Brüel & Kjær, Nærum, Denmark) with a flat frequency response (± 2 dB from 4 Hz – 100 kHz) and digitized with an Avisoft single-channel data acquisition board (UltraSoundGate 116H) and Avisoft Recorder software (Avisoft Bioacoustics, Glienicke, Germany). Each cricket was weighed before and after recording on a digital scale (Mettler Toledo XS204, 0.1 mg readability). Crickets were euthanized by freezing after the end of recording. We later measured pronotum width, anterior–posterior pronotum length and left and right hind femur lengths using a digital calliper (Ares 70019, 0.01 mm resolution). We counted the total number of calls, the number of ticks in each call and the number of trill syllables in a randomly selected subset of 20 calls per individual using the pulse train analysis function in Avisoft SASLab Pro (v.5.2.12). Calls were counted as separate events if they were separated by more than 1 s (i.e.

almost three times the length of the average silent time between ticks).

To quantify acoustic parameters of the male call in more detail, we made additional measurements on 48 randomly selected calls from 10 individuals (6 individuals from the 24 h recordings described above and 4 from an additional set of males that was recorded in a cylindrical mesh cage (9 cm diameter \times 17 cm height) in 2016. Using the Automatic Parameter Measurement tool in Avisoft SASLab Pro, we measured the following variables for each syllable in a call: duration (time from the start to the end of the syllable, ms), period (time from the start of one syllable to the start of the next, ms) and peak frequency (frequency with the most energy, kHz). We also measured the gap duration (silence) between the last tick and first trill syllable (Fig. 1). The mean values calculated from these measurements are provided in Table 1.

<H2>Playback Experiments to Females

<H3>General approach

Artificial call stimuli were synthesized using Avisoft SAS Lab Pro using the average call parameters from the male recordings as a guide (Table 1). For all playback stimuli, calls had a frequency of 20 kHz, a tick syllable duration of 45 ms, tick period of 400 ms, trill syllable duration of 23 ms, trill syllable period of 43 ms and a gap between the ticks and the beginning of the trill of 200 ms. The number of ticks, the amplitude of the calls and the duration of the trill (i.e. the number of trill syllables) varied depending on the experiment (details below). Experiments were conducted in an attenuating and anechoic acoustic chamber under infrared light. This species calls both during the day and at night (Tan & Robillard, 2021), and we conducted experiments during the day but under dark conditions to avoid potential visual disturbance. Female crickets were placed inside a cylindrical fibreglass mesh cage (9 cm

diameter × 17 cm height) and allowed 5 min to acclimate before playback of stimuli began. Stimuli were broadcast to crickets using an Avisoft ultrasonic speaker (Vifa model) and amplifier (USG216H) connected to a computer (HP Compaq Elite 8300), and the female vibrational reply was recorded using an accelerometer (Brüel & Kjær DeltaTron, Type 4517) or laser Doppler vibrometer (Polytec PDV-100, Waldbronn, Germany) positioned on the side of the cage. The cricket's position was monitored using a video camera (ICD-879 Type G, Ikegami, Tokyo, Japan) and external monitor outside of the sound chamber. Playbacks of stimuli were initiated only if the female was within 1 cm of the accelerometer or laser point; if she moved between treatments, she was repositioned and allowed another 5 min acclimation period before continuing. For all experiments, treatments consisted of five repetitions of each stimulus (at periods specified below), and treatments were presented in random order, with 5 min of silence between each treatment. In experiment 1, female crickets were tested for responsiveness to average synthetic male calls (10 tick syllables plus a 40-syllable trill, other parameters as reported in Table 1) at 80 dB peak equivalent SPL (peSPL; Burkard, 2006) every 3–4 days after their final moult and participated in the playback experiment about 3 days after becoming responsive. The average age at the time of the experiment for the experiment 1 females was 19 days after the final moult (range 12–27 days). For experiments 2, 3 and 4, we tested responsiveness at about 20 days and performed experiments either on the same day or within 5 days. The average age of experiment 2 females was 25 days (range 22–28) and for experiments 3 and 4 (same crickets) was 23 days (range 21–32).

For the female playback experiments, we measured duration, latency and peak-to-peak amplitude of each vibrational reply (Fig. 1). Amplitude and duration can each influence probability of signal detection, decision making and accuracy in locating the signal source in vibrationally communicating insects (Gibson & Cocroft, 2018; Polajnar et al., 2014). Latency can affect mate recognition in duetting orthopterans (Bailey, 2003; Heller et al., 2018; Heller & von Helversen, 1986; Zimmermann et al., 1989)

and other insects (Kuhelj et al., 2015; Rohde et al., 2013). We measured latency as the time between the end of the male call or playback stimulus and the beginning of the female reply. Duration and latency were measured directly from the oscillogram in Avisoft SASLab Pro, and amplitude was measured as the peak-to-peak difference of the signal in mV using the ‘time signal’ function. We did not calibrate these recordings, so they cannot be translated into absolute values in terms of displacement or velocity, but these values can be compared across all crickets in the same experiment that experienced the same recording conditions. In experiment 2, six stimulus presentations cut out during playback, and responses to these were excluded from analyses. For atypical replies that contained more than one distinct vibrational pulse, we measured the largest – usually the last – pulse in a series of premature replies occurring before the end of the playback. Atypical responses were uncommon in all experiments except experiment 3 and 4 (below), which mostly consisted of stimuli that were outside of the natural range of male call variation. For experiment 4, some individuals were excluded from statistical analyses because they produced atypical vibrational responses that could not be classified as discrete signals (see Appendix for examples).

<H2>Experiment 1: Male Call Components Required for Female Replies

To test whether the presence of each component was necessary and sufficient to elicit a vibrational reply, we played the individual components of the male call separately and together to female crickets ($N=40$). The three treatments were: (1) 10 ticks with no trill; (2) a trill of average duration (1576 ms, 38 syllables) with no ticks; (3) the complete call (10 ticks followed by a trill of average duration and syllable number). Each treatment consisted of five repeated stimuli with a call period (time from the start of one call to the start of the next) of 15 s and an amplitude of 80 dB peSPL at the cricket. Treatments

were presented in random order and female replies were recorded using the accelerometer. The same females used in this experiment were subsequently used in a pilot experiment for varying the number of ticks in the call and for testing whether a greater number of ticks might lower the amplitude threshold of the female reply by playing calls with varying numbers of ticks. These females were then retested several weeks later after either being housed with a male or being isolated to address the possibility that age or mating status might affect responses to male signals (details in the Appendix).

<H2>Experiment 2: Effect of the Number of Ticks per Call on Female Replies

To test whether varying the number of ticks in the male call affected the female reply, we played a second group of female crickets ($N=17$) stimuli of 0, 10, 20, 30, 40 or 50 ticks plus a trill of average syllable number and duration. The treatment with 50 ticks represents the 95th percentile observed in our male recordings; the average number of ticks we observed was 14. Each treatment consisted of five repeated stimuli at 80 dB peSPL. The call period for all treatments was 40 s, which differs from the other playback experiments to avoid temporal overlap of calls. The remainder of the experimental set-up was the same as in experiment 1, except that the female vibrational replies were recorded with the laser Doppler vibrometer rather than the accelerometer.

<H2>Experiment 3: Effect of the Number of Trill Syllables per Call on Female Replies

To examine female cricket responses to variation in the trill component of the call, we played two additional series of stimuli to a third set of female crickets ($N=15$). Playback of stimuli were conducted using the same set-up as the experiments previously described; female vibrational replies were recorded

with the laser Doppler vibrometer. All stimuli were played back at an amplitude of 80 dB peSPL with a period of 15 s and all consisted of 10 ticks followed by a trill. In the first series, we manipulated the number of syllables in the trill (and therefore the trill duration) but kept the call period and individual syllable duration constant. The treatments were 20, 30, 40, 50 and 60 trill syllables, with 40 being the average for male *L. bitaeniatus* crickets, 30 and 50 representing the 5th and 95th percentiles observed in males, and 20 and 60 representing syllable numbers that we almost never observed in our male crickets.

<H2>*Experiment 4: Effect of Trill Acoustic Parameters on Female Replies*

Temporal properties of a call are not limited to the number of syllables it contains, and other factors like syllable period and duty cycle can be essential for mate recognition in crickets (Clemens et al., 2021; Schöneich, 2020; Schöneich et al., 2015). In a second playback experiment with the same group of crickets that were used in experiment 3 ($N = 15$), we manipulated other parameters of the trill while keeping the tick component of the call constant (10 ticks). The treatments in this playback series manipulated syllable duration, syllable period and duty cycle and are summarized in Table 2. Eight of the crickets underwent experiment 3 first and the other seven underwent the experiment 4 first.

<H2>*Ethical Note*

All experiments with the crickets were noninvasive, consisting only of audio and vibrational recording and audio playback. For the male recordings, 16 crickets were euthanized by freezing. Crickets were maintained in a temperature-controlled room with a regular light/dark cycle, and they always had food and water ad libitum.

<H2>Statistical Analysis

All statistical analyses were conducted in R (v.4.0.5). Model assumptions for each statistical test were examined using residual quantile plots to test for normality of the data and the Levene test to test for homoscedasticity. In experiments 1 and 2, log transformation of the amplitude data achieved normal distributions of residuals. In some cases, latency data showed significant heteroscedasticity but could not be transformed to achieve homoscedasticity. Linear mixed models are highly robust to deviations from the assumptions of normally distributed data with equal variances across treatments (Schielzeth et al., 2020), so we report the results of these tests here with the acknowledgment that they did not meet the strict assumptions of the test.

To calculate the individual repeatability of the number of syllables of each call component, we used the function 'rpt' from the package 'rptR'. We calculated repeatability (R = ratio of the intergroup variance to the sum of the inter- and intragroup variance: Nakagawa & Schielzeth, 2010) and used 1000 parametric bootstraps to obtain 95% confidence intervals. Likelihood ratio tests are used by 'rpt' to obtain P values. We used a principal components (PC) analysis to obtain a multivariate measure of body size from the measurements of pre-recording mass, pronotum width and length, and left and right femur lengths. PC1 accounted for 78% of the variation in these measurements, with loadings for each original variable except pronotum width being approximately equal (~20%). To test whether male calling behaviour is related to body size, we used linear regression models with the average number of ticks or trills produced by a male over 24 h as the response variable and PC1 (with lower values representing smaller males) as the predictor variable.

To test whether acoustic treatment influenced females' responsiveness to playback stimuli in each of the four experiments, we used generalized linear mixed models with a Poisson link function ('glmer' function from 'lme4' package), which is appropriate for count data. In each case, the number of female replies elicited in each playback treatment (out of the five repetitions within a treatment) was the response variable, acoustic treatment was a fixed effect and individual cricket was included as a random effect due to repeated measurements on the same individual across treatments. We used the 'mixed' function from the 'afex' package in R to calculate *P* values for generalized linear mixed models using the likelihood ratio test method. For experiments in which there was an increasing number of syllables across treatments (experiments 1–3), acoustic treatment was an ordinal variable, whereas it was an unordered categorical variable for experiment 4.

We also tested whether acoustic treatment affected the properties of the female reply, specifically amplitude and latency. Although we also measured duration, we found a significant correlation between the two variables measuring the intensity of the female reply, amplitude and duration (Appendix). Reasoning that longer-duration female replies could necessarily result from higher-amplitude body movements during replies, but not vice versa, we included only female reply amplitude and latency as response variables in our models. For experiments in which variation in the timing or intensity of the female reply was of interest (experiment 2, experiment 3), we used linear mixed models to test whether acoustic treatment influenced amplitude or latency. We used the 'mixed' function from the 'afex' package in R to calculate *P* values for linear mixed models using the likelihood ratio test method. In our initial models, we included an interaction term between acoustic treatment and repetition; because the interaction term was not significant in any of the models, we dropped it from our final models. Final models included either female reply amplitude (mV output from the LDV or accelerometer) or latency (ms) as the response variable, acoustic treatment and repetition number as fixed effects and individual

cricket as a random effect. Because we were interested in whether specific pairs of acoustic treatments elicited different intensities of female replies, we used the ‘emmeans’ package to conduct post hoc tests (estimated marginal means, pairwise method) on models with significant main effects to see which treatments or repetitions differed from each other.

<H1>RESULTS

<H2>Male Calling Song

We recorded 2643 calls from 15 males calling over 24 h. Males called 176 ± 147 (mean \pm SD) times per 24 h, with a mean of 21 ± 11 ticks and 39 ± 5 trill syllables per call. The number of tick syllables per call was highly repeatable for individual males ($R = 0.245$, $CI = [0.111, 0.384]$, $P \leq 0.001$), and repeatability was the same as a previously published estimate for a different population of the same species ($R = 0.24$, Tan et al. 2021). Repeatability for the number of trill syllables per call was significant but low ($R = 0.091$, $CI = [0.007, 0.19]$, $P \leq 0.001$). The low R value, however, was not due to an inconsistent number of trill syllables within individuals but rather to low variation both within and between individuals (Table 1; Nakagawa & Schielzeth, 2010). The relationship between male body size and the mean number of ticks per call was not significant, but the P value was marginal (Fig. 2a). There was a significant positive relationship between body size and the mean number of trill syllables per call (Fig. 2b). The call parameters for a second set of males (see Methods, Male Calling Song) are summarized in Table 1. In general, the variation across males was greater for the tick component than for the trill for each measured parameter except syllable duration, more so for number of syllables and syllable period than for peak frequency.

<H2>Female Responses to Calls

Across experiments, we found that females produced vibrational replies to almost all calls containing a typical trill, and that both acoustic treatment and the repetition number of the playback stimulus within a treatment influenced the amplitude and latency of these replies. Results for acoustic treatments in each experiment are summarized below. Repetition number influenced reply amplitude in all experiments in which it was measured (experiments 1–3), with the first repetition of the five eliciting lower-amplitude replies than subsequent repetitions. Repetition number affected the reply latency in experiments 1 and 2 but not in experiment 3. In experiment 1, the first repetition elicited shorter reply latencies (mean latency of replies to first repetition = 82 ms; mean latencies of subsequent repetitions = 141–160 ms). In experiment 2, the first repetition elicited slightly longer reply latencies (mean latency of replies to first repetition = 90 ms; mean latencies of subsequent repetitions = 80–85 ms). Additional details are provided in the Appendix.

<H2>Experiment 1: Male Call Components Required for Female Replies

No females responded to the tick component of the male call without a trill component, either immediately after the playback or at other points during the playback treatment (Fig. 3). All but one of the females responded to at least one repetition of the trill component of the male call without the ticks. All females responded to at least three repetitions of the complete call, with no significant difference in the proportion of replies elicited by the trill alone and the complete call (Fig. 3). The ticks-only treatment could not be included in statistical tests because no females replied to this treatment, preventing parameter

estimates due to complete separation. Female replies had significantly higher amplitude in response to the complete call than to the trill alone ($\chi^2_1 = 41.87$, $P < 0.001$). Replies to the trill alone occurred at significantly longer latencies (102 ± 15 ms) than to the complete call (99 ± 17 ms; $\chi^2_1 = 7.61$, $P = 0.006$).

<H2>Experiment 2: Effect of the Number of Ticks per Call on Female Replies

Varying the number of ticks in the playback call did not affect whether a female replied to the acoustic treatment ($\chi^2_5 = 0.04$, $P > 0.999$; Fig. 4g), but higher numbers of ticks did elicit higher-amplitude replies ($\chi^2_5 = 79.2$, $P < 0.001$; Fig. 4c). Tukey post hoc tests for the amplitude of the female reply showed that this effect was due to a significant difference between zero ticks (i.e. a trill alone) versus all other tick numbers for acoustic treatment, with no significant differences between any other pairs of treatments ($P < 0.001$ for all contrasts with 0 tick treatment, see Appendix, Table A4). Varying the number of ticks in the playback also influenced the latency of the females' replies ($\chi^2_5 = 11.25$, $P = 0.047$; Fig. 4e). However, Tukey post hoc tests of this model found no significant pairwise contrasts (Appendix, Table A6). Five replies (out of 500 replies and 507 playbacks of stimuli) to 0, 10 and 30 tick treatments had negative latencies, occurring before the playback had ended.

<H2>Experiment 3: Effect of the Number of Trill Syllables per Call on Female Replies

Fewer female vibrational replies were elicited by the 20-syllable trill acoustic treatment compared to the treatments with more trill syllables, but this difference was not significant ($\chi^2_4 = 5.52$, $P = 0.238$; Fig. 4h). Varying the number of trill syllables significantly and positively affected the amplitude of the female replies ($\chi^2_4 = 197.8$, $P < 0.001$; Fig. 4d), and Tukey post hoc tests indicated that all pairs of

playback treatments except for the 50- and 60-syllable acoustic treatments were significantly different from each other ($P < 0.015$; see Appendix, Table A8). Latency of the female reply was also strongly influenced by the number of trill syllables in the call ($\chi^2_4 = 27.25$, $P < 0.001$; Fig. 4f). Tukey post hoc tests for pairs of acoustic treatments showed significant differences in latencies between the 20-syllable and 50-syllable treatments and between each treatment and the 60-syllable treatment (see Appendix, Table A10), with no significant differences between the other pairs. The spread of the latencies differed widely between playback treatments: the standard deviation for the 60-syllable treatment was 78, much greater than those of the 20-, 30-, 40- and 50-syllable treatments (with SDs of 9, 7, 9 and 25, respectively). For most individuals, within-individual variability also increased with longer trills (Fig. 5). Altogether we recorded 324 replies from 375 call playbacks during the experiments. Seventeen replies occurred before the playback call had ended, and all of these premature replies were in response to 50- and 60-syllable treatments.

<H2>Experiment 4: *Effect of Trill Parameters on Female Replies*

Each of the manipulated trills in this playback series elicited at least one response, including the playback of 10 ticks plus a long tone without any pulse structure (treatment e; Fig. 6). The acoustic treatments significantly affected a female's propensity to respond ($\chi^2_6 = 161.31$, $P < 0.001$): all females responded to all of the control (average call) stimuli, and Tukey post hoc comparisons of each treatment to the control showed significant differences in the number of replies for all treatments except treatment b and treatment c (Fig. 6; for details, see Appendix). Treatments b and c were the two in which the syllable period of the trill was the same as the control call (43 ms) but the syllable duration was either half the control duration (b) or 1.5 times the control duration (c). Treatment d had a normal syllable duration but

twice the control period. The previous treatments changed one parameter and held the other constant, but this necessarily changes the duty cycle of the call (proportion of time with sound). To control for duty cycle, treatments e and g had syllable durations that were twice (e) or half (g) the control duration with syllable periods maintained at a duty cycle close to the control. Finally, to test whether syllable structure is needed at all, treatment f was a continuous tone the duration of the control trill. Changes in other call acoustic parameters did not appear to influence female responsiveness, but not all possible combinations were tested.

This playback series also elicited more ‘atypical’ replies (39 out of 234 responses and 525 playbacks) than the other experiments. Examples of atypical replies, where the waveform did not closely resemble the female reply to actual (natural) male *L. bitaeniatus* calls, are provided in the Appendix. These atypical replies were not included in the above analyses. Because this experiment was conducted with several playback stimuli that were unrealistic representations of male calls, we were primarily interested in the first-order question of whether females would respond at all, and so only report the responsiveness data.

<H1>DISCUSSION

As predicted, *L. bitaeniatus* females varied the amplitude and other parameters of their vibrational reply depending on specific parameters of the male call, and they responded differently to each component of the call. The two components of the male call have different functions: females never replied to tick syllables alone, and our results demonstrate that the trill of the male call is both necessary and sufficient to elicit a reply. Adding ticks to the call resulted in a significant increase in the vibration amplitude of the female reply, but we found no significant difference in either reply amplitude or latency

to calls with few ticks (10) versus many ticks (50). Therefore, our results do not support the initial prediction of an open-ended preference for increasing syllable numbers in the more variable tick component of the call. This is surprising, as males can and often do produce upwards of 50 ticks when calling: one male produced 120 ticks in a single call. Our hypothesis that females would express a unimodal preference for the less variable component of the call was also not supported. We found that larger males produced longer trills (with greater numbers of syllables) and females produced higher-amplitude replies in response to longer trills. This pattern held even at the extreme range of the trills that males produce – i.e. an open-ended preference rather than a unimodal one. At these extremes, however, the average latency of the reply was shifted away from the typical reply latency, and replies sometimes occurred well before the end of the male call. This lends support to our alternative hypothesis that the trill functions at least in part to aid in the timing of the reply, similarly to ‘trigger’ pulses in some duetting katydid species (Bailey & Hammond, 2003; Stumpner & Meyer, 2001). A summary of these hypotheses, predictions, and findings is provided in the Appendix (Table A1). Our study shows that stereotyped communication signals can result from an interaction between the efficacy of different signal parameters, such as those influencing detection distance versus signal recognition, rather than unimodal selection of signal parameters by receivers.

The function of the tick syllables remains unclear. Our experiments were not designed to examine possible interactions where response to changes in one component is altered or enhanced by response to changes in the other (Candolin, 2003; Hebets & Papaj, 2005). We did find, however, that the addition of ticks to a trill altered the amplitude and latency of the reply compared to trills with no preceding ticks (experiment 1). Alerting components perceived prior to a more informative or species-specific signal can hasten a response or increase the overall probability of signal detection (Grafe et al., 2012; McLennan, 2003). In this study, the addition of more ticks to a call did not significantly affect the latency (or the

variation in latency) of the female reply, nor her propensity to reply. Other behaviours that increase the chances of successful mate finding are possible: for example, hearing ticks could prompt females to stop foraging or assume a better posture for producing a reply. We did not observe any postural changes in females during our playbacks, although these were not conducted under naturalistic conditions (females were perched within a mesh cylinder rather than on a plant stem or leaf). Furthermore, we found no evidence that adding ticks to a call decreases the threshold amplitude at which females will reply in a quiet laboratory setting (Appendix). It is possible, however, that calls with greater numbers of ticks would be more detectable to female crickets in acoustically noisy environments. Background noise can interfere with female responses to calls in *Gryllus bimaculatus* crickets (Schmidt et al., 2014) and can obliterate acoustically mediated mate preferences in crickets and other animals (Coss et al., 2021; Wollerman & Wiley, 2002). We note that an alerting function would not on its own account for the high variability in the number of ticks within and between *L. bitaeniatus* males recorded under consistent conditions.

The structure of the male call might be explained by the presence of multiple receivers (Hebets & Papaj, 2005; Zambre & Thaker, 2017), including receptive females of different ages (Tanner et al., 2019). The presence of ticks in a call appears to elicit replies more reliably from older females (Appendix), which could mean a larger pool of potential mates for calling males if individuals of different ages are present at the same time in a habitat. From the present study, it is not clear whether *L. bitaeniatus* females became choosier as they aged, or whether the changes we observed were due to senescence. It is also possible that ticks are involved in mediating male–male interactions, rather than male–female duets. Several taxa are known to produce signals where male and female audiences either perceive the same call in different modalities (Hill & Shadley, 2001), or use different components of a call to make decisions (Narins & Capranica, 1976). As signallers, males may emphasize different call components while in the presence of males versus females (Krobath et al., 2017). Multiple components or signals that target

different receivers are predicted to occur in situations where both types of receivers are likely to be encountered, such as in mate advertisement/competition. While there is little information available on male competition in *L. bitaeniatus*, there is some evidence that males of a related lebinthine species, *Ponca hebaridi* Robillard, engage in competitive masking and female reply mimicry in response to the calls of other males (Benavides-Lopez et al., 2020).

The trill appears to be critical to successful mate recognition by females, as it reliably elicits replies regardless of the presence of ticks. From the set of playback stimuli in experiment 4, we can conclude that it is the (species-specific) syllable rate of the trill, and not some other parameter, that most strongly determines whether females respond. Interestingly, the stimulus in experiment 4 that elicited the fewest responses (g) – even fewer than the single long tone without pulse structure – was one that resembles the call of a congeneric species, *Lebinthus luae* Robillard & Tan. *Lebinthus luae* have shorter trill syllables that are produced at a faster rate (mean call parameters for *L. luae*: number of syllables = 25, trill syllable duration = 15 ms, trill syllable period = 25 ms, trill duty cycle = 0.52; cf. Fig. 6; also see supplemental material in ter Hofstede et al., 2015). These species overlap slightly in geographical distribution (Baroga-Barbecho et al., 2020), and it could be that the lack of female replies to this experimental treatment are explained by selection against hybridization with *L. luae*.

We did observe that longer trills elicited more intense replies in the playback experiments. Larger males produced calls with more trill pulses, so females might prefer calls that reflect this measure of mate quality. It is possible that having a longer trill length helps females better sample the information in the trill and prepare their reply. From the occurrence of premature replies to elongated trills (Appendix, Fig. A4), we might conclude that the female reply is prompted by the number of pulses within the trill rather than the cessation of the trill. However, because the premature replies did not occur at a consistent time point (i.e. where the end of a natural-length trill would be; Fig. 4f), and because several females produced

a series of premature replies to long trills and then a reply at the typical latency (Appendix, Fig. A5), it seems more likely that both syllables within the trill and the cessation of the trill can affect reply timing. It is unlikely that females time their replies relative to the start of the trill since typical female reply latency was seen in response to unnaturally short trills (Fig. 4f). Regardless of the mechanism by which the females time their replies, consistent trill lengths within and across males appear to help maintain the timing of the duet, despite the possible advantages that eliciting a greater amplitude reply might confer to males that produced longer trills, such as detection of females at greater distances or more accurate directional information (Gibson & Cocroft, 2018).

The unusual multimodal nature of this duet, however, raises questions about how males respond behaviourally to the vibrational reply. The precise female reply latency to naturalistic male calls suggests that timing is important in this interaction, but we do not yet know how tolerant lebinthine males are to deviation from the typical latency. Duetting katydids using only airborne sound can have strict latency windows, particularly when replies occur with extremely short latency (Bailey, 2003). The latency for the female reply in *L. bitaeniatus* is short (ca. 100 ms) compared to many other insect species (Bailey, 2003), suggesting that males might have a strict latency window for recognition. Unlike airborne sound, however, substrate-borne vibrations can travel at variable (and relatively slow) velocities (Michelsen et al., 1982; Virant-Doberlet et al., 2006). Therefore, the use of vibrational signals in this duetting system might require more relaxed latency windows (Kuhelj et al., 2015), since males would benefit from recognizing replies produced on a variety of plant substrates and architectures. In insects with mixed airborne and vibrational duets (*Onomarchus uninotatus* Serville; Rajaraman et al., 2015) or purely vibrational duets (some Neuroptera, many Hemiptera; see Rodríguez & Barbosa, 2014 for a review), reply latencies occur with characteristic timing and in some cases differ between closely related and co-occurring species (Rodríguez & Cocroft, 2006). The tolerance of the male recognition window, however,

has only been tested in a few species (e.g. Kuhelj et al., 2015; Rohde et al., 2013). Furthermore, we do not know whether singing males are able to recognize vibrational replies that occur prior to the end of their call. We note that vibrational replies in *L. bitaeniatus* are typically repeated once per male call and are short in duration (122 ms in these experiments) compared to many other insect vibrational signals (Rodríguez & Barbosa, 2014). This, plus the observation that *Lebinthus* females do not appear to take on any of the searching role during duetting (ter Hofstede et al., 2015), could make the male's search an especially challenging task. Males are observed to track down the source of vibrations while females remain stationary, but it remains ambiguous exactly how the males are using the information in the reply. It is possible that they perform vibrotaxis by gleaning directional information about the position of the female relative to them, as seen in some other vibrationally duetting insects (Gibson & Cocroft, 2018; Virant-Doberlet et al., 2006), or they might simply search or walk more when they have detected replies.

We conclude that the potential for the female vibrational reply to have shaped features of the male call via sexual selection exists in this communication system. The stereotyped number of trill syllables in the male call is not the straightforward result of stabilizing selection but likely reflects an interaction between a female preference for longer trills and the efficacy of the female reply. Trills with more syllables elicit replies at greater amplitudes, but the atypical timing of these replies could interfere with effective male mate recognition. In this mate-searching duet, the modality of each signal likely plays an important role, and there is a need for further studies in this and other multimodal duet systems to understand the differences between these unimodal acoustic duets and unimodal vibrational duets. Comparisons of reply timing, latency windows and searching behaviour across insect duets could reveal important constraints placed upon communication by the particular modality in which signals are produced – and whether such constraints affect close-range courtship duets differently than those that function in longer-range mate searching. More broadly, our results suggest that interactions between mate

preferences and signal efficacy can influence signal design. While variable signals are often the result of open-ended receiver preferences and stereotyped signals of unimodal receiver preferences, other aspects of communication systems (e.g. competitors, environmental transmission, receiver constraints) can potentially disrupt this relationship. Studies in animal communication will benefit from experiments that simultaneously consider both mate preferences and signal efficacy in addressing proximate and ultimate questions about signal design.

Author Contributions

All authors conceptualized the study. C.E.K. and J.S.J. collected the data. C.E.K. and H.M.t.H. performed the statistical analyses. C.E.K. wrote the manuscript, with contributions by all authors.

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Appendix

<H2>*Effect of Ticks on Female Reply Threshold*

We tested the hypothesis that a greater number of ticks in a male call lowers the amplitude threshold at which a female will reply to the call, which would potentially allow males located farther away from females to elicit responses by adding ticks to their call. Using the same group of crickets that participated in experiment 1, we played calls with varying numbers of ticks (0, 5, 10, 15, 20) repeated five times, with each repetition increasing in amplitude (60–85 dB peSPL in 5 dB steps). We found the amplitude threshold of responses by noting the lowest-amplitude repetition in each treatment to which an individual responded, then tested for differences by comparing the mean response threshold of all the crickets for each treatment. As these data were not normally distributed, we used a Friedman rank test to examine potential differences in amplitude thresholds between acoustic treatments. To preserve equal blocking, 11 individuals who did not experience all five acoustic treatments were excluded from this analysis, resulting in a final sample size of 29 crickets.

We found that thresholds were not significantly different between acoustic treatments ($\chi^2_4 = 3.4764, P = 0.4815$). Across all treatments, only one female responded at 60 dB peSPL, approximately half responded first to 65 dB peSPL, approximately half responded first to 70 dB peSPL, and a few in most treatments first responded to 75 dB peSPL (Fig. A1).

<H2>*Age and Mating Effects on Female Responsiveness to Male Calls*

For the same group of 40 crickets that was used in experiment 1, we also investigated the effects

of age and mating history on the likelihood and amplitude of the female reply. Following the first round of experiments, conducted when the crickets became responsive to calls 2–3 weeks after their adult moult, each cricket was assigned to either a mating treatment or an isolated treatment. Females were each placed in a separate small terrarium and those in the mating treatment were paired with a male cricket. After 3 weeks, we repeated the experiment 1 playback series with the females that remained responsive to male calls.

Zero of 18 of the females in the mated treatment remained responsive to male calls at older ages, with two females in the mated treatment dying before their responsiveness could be retested. Most (18 out of 20) females in the nonmated treatment remained responsive to playbacks of male calls after 2 weeks, with two becoming nonresponsive. As in initial experiment 1 playback experiments, females never responded to the ticks-only stimulus. At older ages, females were somewhat less likely to respond to all five repetitions of the full call and the trill-only stimulus than at younger ages (Fig. A2). A generalized linear model with a Poisson link function (R package ‘lme4’) that included the number of replies out of five playbacks as the response, individual identity (ID) as a random effect, acoustic treatment and age (first or second test) as fixed effects, and an interaction term for age and acoustic treatment found no effect of age or acoustic treatment, nor any interaction between them (likelihood ratio test: age: $\chi^2_1 = 1.65$, $P = 0.199$; acoustic treatment: $\chi^2_1 = 0.06$, $P = 0.803$; age*acoustic treatment: $\chi^2_1 = 0.42$, $P = 0.516$).

<H2>Description of Female Vibrational Replies

Across experiments 1–4, we recorded female replies that fell into four categories: typical, premature, false-start and atypical. Most replies were ‘typical’ (Fig. A3) and occurred after the playback stimulus had ended. Some replies occurred before the playback had ended (Fig. A4) or contained multiple

pulses, usually smaller than the final pulse (Fig. A5). In experiments 3 and 4, which contained stimuli that did not closely resemble natural male calls, we observed unusual responses, some of which did not contain recognizable replies at all (Fig. A6).

<H2>Relationship between Female Reply Amplitude and Duration

Initially, we measured both the duration and amplitude of the female vibrational reply for experiments 1–3. Duration and amplitude might affect signal propagation and receiver sensory systems in different ways. For example, higher-amplitude vibrational signals might travel further through the substrate, although propagation of vibrational signals through plant stems (the preferred substrate for *Lebinthus* crickets) is not straightforward. However, we found that for the female vibrational replies across all of our experiments, duration and amplitude were highly correlated. Thus, we have included amplitude only in our statistical models, as we reason that longer durations are likely to be a by-product of higher-amplitude replies for this species, which has a simple, single pulse vibrational reply produced by a single jerk of the body.

Female reply amplitude and reply duration were significantly correlated across experiments (Pearson's product-moment correlation: $r = 0.49$, $t_{1195} = 19.6$, $P < 0.001$). Amplitude and duration of replies were more strongly correlated for experiment 1 ($r = 0.60$, $t_{371} = 14.3$, $P < 0.001$) and experiment 2 ($r = 0.66$, $t_{498} = 19.5$, $P < 0.001$) than for experiment 3 ($r = 0.45$, $t_{322} = 9.1$, $P < 0.001$). This difference could be due to differences in the playback stimuli of each experiment – unlike experiments 1 and 2, in which stimuli were all within a natural range for male calls, experiment 3 included stimuli with artificially high or low numbers of syllables. Experiments were conducted on different equipment with different gain settings, which might also explain differences in slope between the plots. We did not measure reply

parameters besides responsiveness for experiment 4.

<H2>*Details of Statistical Tests for Experiments 1–3*

Here, we report the statistical effects that stimulus repetition number had on female reply amplitude and latency. We also summarize the post hoc tests that were conducted to examine differences in reply amplitude, latency or responsiveness between pairs of acoustic treatments (Tables A1, A3, A5, A7, A8, A9) or between the repetition number of each stimulus within an acoustic treatment (Tables A2, A4, A6). All stimuli were repeated five times within an acoustic treatment; see Methods in main text for details of timing. In general, the initial repetition of a stimulus elicited lower-amplitude replies than subsequent repetitions.

<H3>*Experiment 1: male call components required for female replies*

The amplitude of the replies was significantly and positively affected by the repetition number of the playback ($\chi^2_4 = 185.66, P < 0.001$; Table A2). The latency of the replies was also significantly affected by the repetition number of the playback ($\chi^2_4 = 129.79, P < 0.001$; Table A3).

<H3>*Experiment 2: effect of the number of ticks per call on female replies*

As in experiment 1, the repetition number of the playback significantly and positively affected the amplitude of the replies ($\chi^2_4 = 66.60, P < 0.001$). There were significant differences only between the first repetition of a playback and all subsequent repetitions (Table A5). The repetition number of the playback stimulus also significantly affected the latency of the replies, with later repetitions eliciting shorter latencies ($\chi^2_4 = 12.21, P = 0.016$). Post hoc tests showed that the latency of the reply was significantly

longer in the first than in the third and fifth repetitions (Table A7).

<H3>Experiment 3: effect of the number of trill syllables per call on female replies

In this experiment, repetition number significantly affected the amplitude of female replies ($\chi^2_4 = 10.82, P = 0.029$). Tukey post hoc tests showed a difference only between the first and fifth repetition (t ratio = -2.939, $P = 0.029$, all other contrasts not significant; Table A9). Latency of the replies was not affected by the repetition number of the playback ($\chi^2_4 = 5.14, P = 0.274$).

Table A1

Hypotheses, predictions and results for experiments on relationships between male signal variability, male quality, female preference and signal efficacy in the cricket *L. bitaeniatus*

Hypothesis	Prediction	Supported by results?
Highly variable male signal components correspond with male quality, whereas stereotyped signal components do not	(1) The multicomponent signal has both highly variable and highly stereotyped components (2) More variable signal component (ticks) correlates with male body size (3) Less variable component (trill pulses) does not correlate with male condition	Yes: number of ticks in male calls is more variable than number of trill pulses (Table 1, Fig. 4a, b) No: larger males do not produce more ticks than smaller males (Fig. 2a) No: larger males produce trills with more pulses than smaller males (Fig. 2b)
Stereotyped signals are required for mate recognition	(1) Trill component is necessary and sufficient to elicit female response	Yes: females only replied to calls with trills, and did not need ticks to reply
Highly variable male signal components correspond with open-ended female preferences	(1) Female responsiveness increases with number of ticks in male call (2) Female reply amplitude increases with number of ticks in male call (3) Female reply latency becomes more stereotyped with number of ticks in male call	No: no relationship between proportion of calls with female replies and number of ticks in male call (Fig. 3, Fig. 4g) No: no relationship between amplitude of female replies and number of ticks in male call, only calls with no ticks differed from calls with ticks (Fig. 4c) No: no relationship between reply latency and number of ticks in male call (Fig. 4e)
Stereotyped male signal components correspond with:	H1: unimodal female preferences	(1) Female responsiveness greatest for mean number of sound pulses in male trill (2) Female reply amplitude greatest for mean number of sound pulses in male trill
		No: no relationship between proportion of calls with female replies and number of trill pulses in male call (Fig. 4h) No: female reply amplitude increased with number of trill pulses in male call (Fig. 4d)

H2: opposing selection by female preference and signal efficacy	(3) Female reply latency most stereotyped for mean number of sound pulses in male trill	No: variability of female reply latency increased with the number of trill pulses in male call (Fig. 4f)
	(1) Female responsiveness is greatest at trill lengths for which reply latency is least predictable (2) Female reply amplitude is greatest at trill lengths for which reply latency is least predictable	No: female responsiveness did not vary with trill length, but reply latency did (Fig. 4f, h) Yes: female reply amplitude increased with number of trill pulses but reply latency became more variable (Fig. 4d, f)

Table A2

Results of post hoc tests comparing amplitude of the female reply for different repetition numbers of playback stimuli in experiment 1

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
1–2	-0.21	0.02	-8.734	<0.0001
1–3	-0.25	0.02	-10.532	<0.0001
1–4	-0.33	0.02	-13.526	<0.0001
1–5	-0.34	0.02	-14.049	<0.0001
2–3	-0.04	0.02	-1.809	0.370
2–4	-0.11	0.02	-4.941	<0.0001
2–5	-0.12	0.02	-5.457	<0.0001
3–4	-0.07	0.02	-3.182	0.0138
3–5	-0.08	0.02	-3.697	0.0024
4–5	-0.01	0.02	-0.507	0.987

‘Contrast’ refers to the specific repetition numbers being compared in each pair of contrasts. ‘Estimate’ shows the difference between treatment means. Results are averaged over the level of acoustic treatment.

Note: contrasts are still on the \log_{10} scale. Degrees-of-freedom method: Kenward–Roger. *P* value adjustment: Tukey method for comparing a family of five estimates. Significant *P* values are shown in bold.

Table A3

Results of post hoc tests comparing latency of the female reply for different repetition numbers of playback stimuli in experiment 1

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
1-2	11.68	1.79	6.540	<0.0001
1-3	15.19	1.77	8.571	<0.0001
1-4	18.58	1.77	10.487	<0.0001
1-5	19.93	1.77	11.286	<0.0001
2-3	3.50	1.69	2.068	0.237
2-4	6.90	1.69	4.072	0.0006
2-5	8.25	1.69	4.884	<0.0001
3-4	3.39	1.67	2.036	0.251
3-5	4.74	1.66	2.851	0.0371
4-5	1.35	1.66	0.810	0.928

Details as given in Table A2.

Table A4

Results of post hoc tests comparing amplitude of female reply for different acoustic treatments in experiment 2

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
0–10	-0.13	0.02	-7.16	< 0.0001
0–20	-0.13	0.02	-6.75	< 0.0001
0–30	-0.11	0.02	-5.92	< 0.0001
0–40	-0.13	0.02	-6.80	< 0.0001
0–50	-0.15	0.02	-8.04	< 0.0001
10–20	0.01	0.02	0.42	0.998
10–30	0.02	0.02	1.25	0.810
10–40	0.01	0.02	0.34	0.999
10–50	-0.02	0.02	-0.91	0.945
20–30	0.02	0.02	0.84	0.961
20–40	-0.00	0.02	-0.08	1.000
20–50	-0.02	0.02	-1.32	0.773
30–40	-0.02	0.02	-0.91	0.944
30–50	-0.04	0.02	-2.16	0.261
40–50	-0.02	0.02	-1.24	0.815

‘Contrast’ refers to the specific treatments (number of tick syllables) being compared in each pair of contrasts. ‘Estimate’ shows the difference between treatment means. Results are averaged over the level of repetition number. Note: contrasts are still on the \log_{10} scale. Degrees-of-freedom method: Kenward–Roger. *P* value adjustment: Tukey method for comparing a family of six estimates.

Table A5

Results of post hoc tests comparing amplitude of female reply for different repetition numbers of playback stimuli in experiment 2

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
1-2	-0.09	0.02	-5.08	<0.0001
1-3	-0.11	0.02	-6.25	<0.0001
1-4	-0.12	0.02	-7.23	<0.0001
1-5	-0.12	0.02	-6.93	<0.0001
2-3	-0.02	0.02	-1.17	0.767
2-4	-0.04	0.02	-2.13	0.210
2-5	-0.03	0.02	-1.87	0.337
3-4	-0.02	0.02	-0.95	0.877
3-5	-0.01	0.02	-0.70	0.957
4-5	0.00	0.02	0.25	0.999

Details as given in Table A2.

Table A6

Results of post hoc tests comparing the latency of the female reply for different acoustic treatments in experiment 2

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
0–10	2.79	3.66	0.76	0.974
0–20	-0.27	3.66	-0.08	1.000
0–30	3.62	3.66	0.99	0.921
0–40	-3.34	3.67	-0.91	0.944
0–50	-6.71	3.67	-1.83	0.450
10–20	-3.06	3.64	-0.84	0.960
10–30	0.83	3.64	0.23	0.999
10–40	-6.13	3.65	-1.68	0.547
10–50	-9.50	3.65	-2.60	0.099
20–30	3.90	3.64	1.07	0.893
20–40	-3.07	3.65	-0.84	0.960
20–50	-6.43	3.65	-1.76	0.491
30–40	-6.96	3.65	-1.91	0.399
30–50	-10.33	3.65	-2.83	0.055
40–50	-3.37	3.66	-0.92	0.941

Results are averaged over the level of acoustic treatment. All other details as given in Table A4.

Table A7

Results of post hoc tests comparing the latency of the female reply for different repetition numbers of playback stimuli in experiment 2

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
1-2	4.58	3.34	1.37	0.647
1-3	9.89	3.34	2.96	0.027
1-4	4.42	3.33	1.33	0.673
1-5	9.58	3.35	2.86	0.036
2-3	5.31	3.33	1.59	0.503
2-4	-0.16	3.32	-0.05	1.000
2-5	5.00	3.34	1.50	0.567
3-4	-5.47	3.32	-1.65	0.468
3-5	-0.31	3.34	-0.09	1.000
4-5	5.16	3.33	1.55	0.530

Details as given in Table A2.

Table A8

Results of post hoc tests comparing amplitude of female reply for different acoustic treatments in experiment 3

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
20–30	-404.27	67.78	-5.96	<0.0001
20–40	-600.92	68.00	-8.84	<0.0001
20–50	-883.18	67.89	-13.01	<0.0001
20–60	-974.98	66.71	-14.62	<0.0001
30–40	-196.65	62.38	-3.15	0.015
30–50	-478.91	62.28	-7.69	<0.0001
30–60	-570.71	61.04	-9.35	<0.0001
40–50	-282.26	60.77	-4.65	<0.0001
40–60	-374.06	60.24	-6.21	<0.0001
50–60	-91.80	60.22	-1.52	0.547

‘Contrast’ refers to the specific treatments (number of trill syllables) being compared in each pair of contrasts. ‘Estimate’ shows the difference between treatment means. Results are averaged over the level of repetition number. Degrees-of-freedom method: Kenward–Roger. *P* value adjustment: Tukey method for comparing a family of five estimates.

Table A9

Results of post hoc tests comparing the amplitude of the female reply for different repetition numbers of playback stimuli in experiment 3

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
1-2	-79.00	62.63	-1.26	0.715
1-3	-116.76	62.59	-1.87	0.338
1-4	-160.42	62.88	-2.55	0.082
1-5	-183.41	62.39	-2.94	0.029
2-3	-37.76	62.35	-0.61	0.974
2-4	-81.42	62.60	-1.30	0.691
2-5	-104.41	62.12	-1.68	0.447
3-4	-43.66	62.62	-0.70	0.957
3-5	-66.65	62.08	-1.07	0.820
4-5	-22.99	62.41	-0.37	0.996

Details as given in Table A2.

Table A10

Results of post hoc tests comparing the latency of the female reply for different acoustic treatments in experiment 3

Contrast	Estimate	SE	<i>t</i> ratio	<i>P</i>
20–30	10.98	7.10	1.548	0.532
20–40	15.25	7.09	2.151	0.201
20–50	27.13	7.08	3.833	0.001
20–60	62.73	6.97	8.995	<0.0001
30–40	4.26	6.51	0.655	0.966
30–50	16.15	6.50	2.483	0.097
30–60	51.75	6.39	8.094	<0.0001
40–50	11.89	6.36	1.868	0.337
40–60	47.48	6.31	7.531	<0.0001
50–60	35.60	6.30	5.647	<0.0001

Details as given in Table A8.

Table 1Male call parameters for 10 individuals of the cricket species *L. bitaeniatus*

Cricket	No. of calls measured	Number of syllables		Syllable duration (ms)		Syllable period (ms)		Peak frequency (kHz)		Gap duration (ms)
		Ticks	Trill	Ticks	Trill	Ticks	Trill	Ticks	Trill	
1	5	25.6±15.6	37.0±2.4	53.9±3.1	23.7±1.3	362.7±19.5	43.8±1.9	20.4±0.7	20.5±0.8	123.7±68.6
2	5	18.5±26.6	35.2±2.3	50.4±11.5	20.9±1.0	671.9±385.1	41.5±0.9	21.2±1.0	20.9±0.3	157.5±37.2
3	5	7.0±8.0	43.2±4.6	39.6±8.4	17.9±1.1	331.7±28.2	38.8±0.7	20.9±0.9	20.3±1.5	191.6±28.1
4	5	3.8±0.5	41.6±3.0	44.4±8.5	19.4±0.7	369.3±70.7	38.3±2.3	20.2±0.5	20.1±0.4	159.5±103.0
5	5	31.6±25	40.8±2.9	51.5±2.5	22.4±1.3	362.9±23.3	39.1±1.9	21.4±1.6	20.4±0.5	174.2±56.9
6	5	14.4±5.2	40.6±1.5	49.2±2.6	23.0±1.1	406.6±22.5	45.9±0.7	19.0±0.3	18.7±0.3	119.1±63.7
7	5	7.6±2.6	32.0±1.6	41.7±2.1	23.2±0.4	415.1±24.2	44.0±1.0	20.8±0.3	20.7±0.4	184.8±76.2
8	3	8.3±2.5	41.0±5.3	44.7±4.1	22.3±1.3	409.3±87.3	44.0±0.3	17.9±0.2	17.4±0.1	270.1±2.3
9	5	11.6±2.3	38.4±2.3	48.2±1.5	21.2±0.2	373.7±23.7	39.3±0.6	19.0±0.2	19.0±0.2	190.1±35.9
10	5	9.0±6.2	38.4±3.2	43.4±5.4	24.2±0.7	407.8±30.6	44.9±1.2	26.7±2.0	22.8±0.4	187.6±55.3
Mean		13.7±9.0	38.8±3.4	47.7±4.6	21.8±2.0	411.1±95.6	41.9±2.9	20.7±2.4	20.1±1.5	173.8±42.3
CV		1.04	0.11	0.15	0.10	0.25	0.14	0.12	0.07	0.38

Values are means ± SD. Mean = grand mean (± SD) of the individual means for each call parameter; CV = among-male coefficient of variation for each call parameter.

Table 2

Acoustic parameters for each treatment (a–g)

	Playback treatment						
	a	b	c	d	e	f	g
No. trill syllables	40	40	40	20	20	1	40
Trill syllable duration (ms)	23	11.5	34.5	23	46	1700	11.5
Trill syllable period (ms)	43	43	43	86	109	—	21.5
Trill duty cycle	0.53	0.27	0.80	0.27	0.42	1.00	0.53
Total trill duration (ms)	1700	1688.5	1711.5	1657	2117	1700	850

Values in bold were within $\pm 3\%$ of the control values (treatment a). Also see Fig. 6.

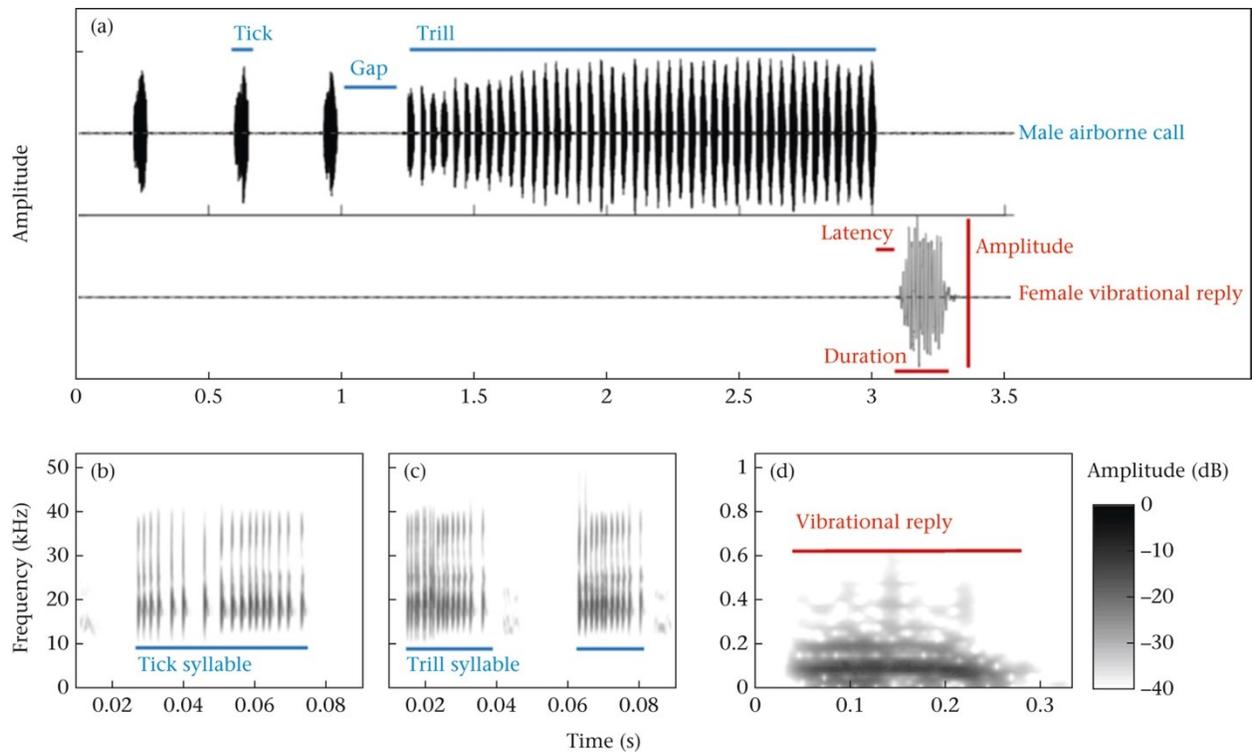


Figure 1. Oscillogram and spectrograms of each component of the *L. bitaeniatus* duet. This is a composite of a male call recorded in isolation and a reply from a female recorded during a playback experiment, placed at the appropriate (average) latency. Blue and red lines illustrate the measured acoustic and vibrational call parameters. (a) Oscillogram showing the last three ticks of a male call followed by a trill (top trace) and the female vibrational reply (bottom trace). Spectrograms on second row show a single tick syllable (b), two separate trill syllables on the same timescale as the tick (c), and the female reply (d).

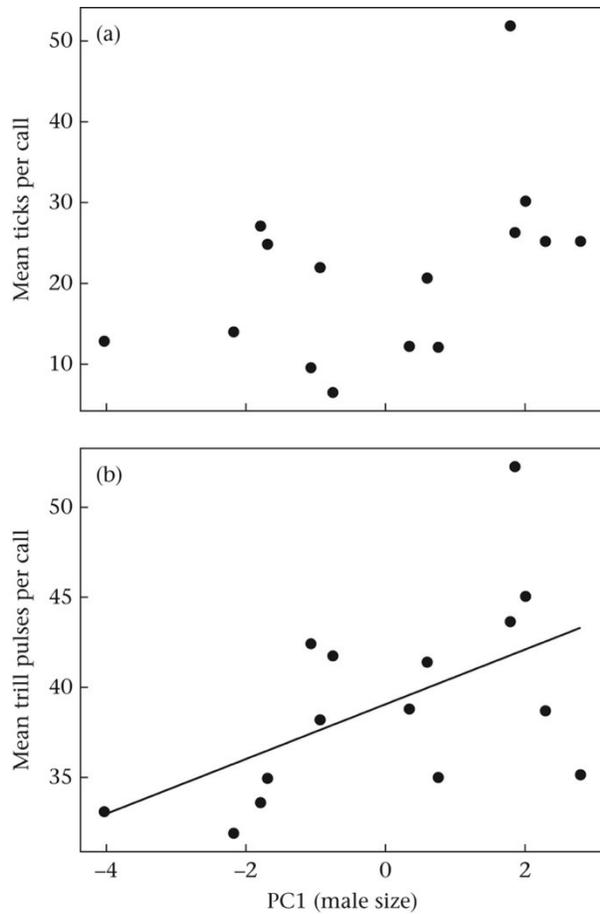


Figure 2. Relationship between (a) the number of ticks per call and PC1 ($F_{1,13} = 3.83$, $P = 0.072$) and (b) the number of trill syllables per call and male mass ($F_{1,13} = 5.53$, $P = 0.035$, $R^2 = 0.30$) in *L. bitaeniatus*. Points show mean number of pulses in each component for all the calls produced by an individual over 24 h.

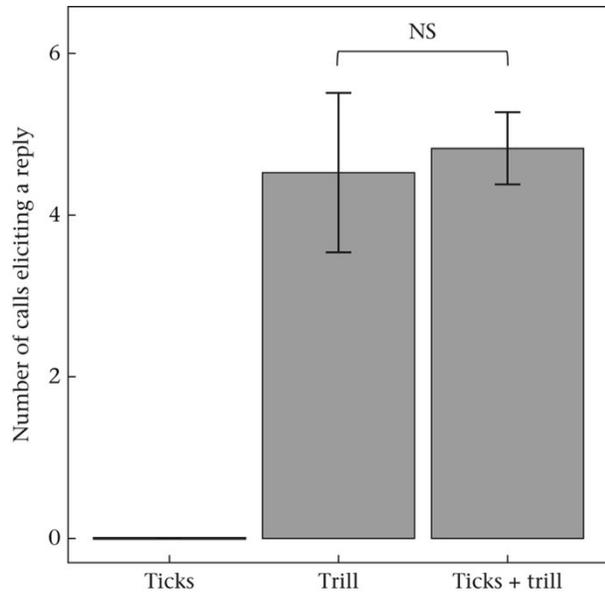


Figure 3. Mean \pm SD number of male calls out of five replicates per acoustic treatment that elicited female vibrational replies. Treatments contained one or both of the two main components of each male call: ticks and a trill. Treatments: a sequence of 10 ticks (Ticks), 10 ticks and a trill (Ticks + trill) and just the trill with no ticks (Trill). $\chi^2_1 = 0.39$, $P = 0.535$.

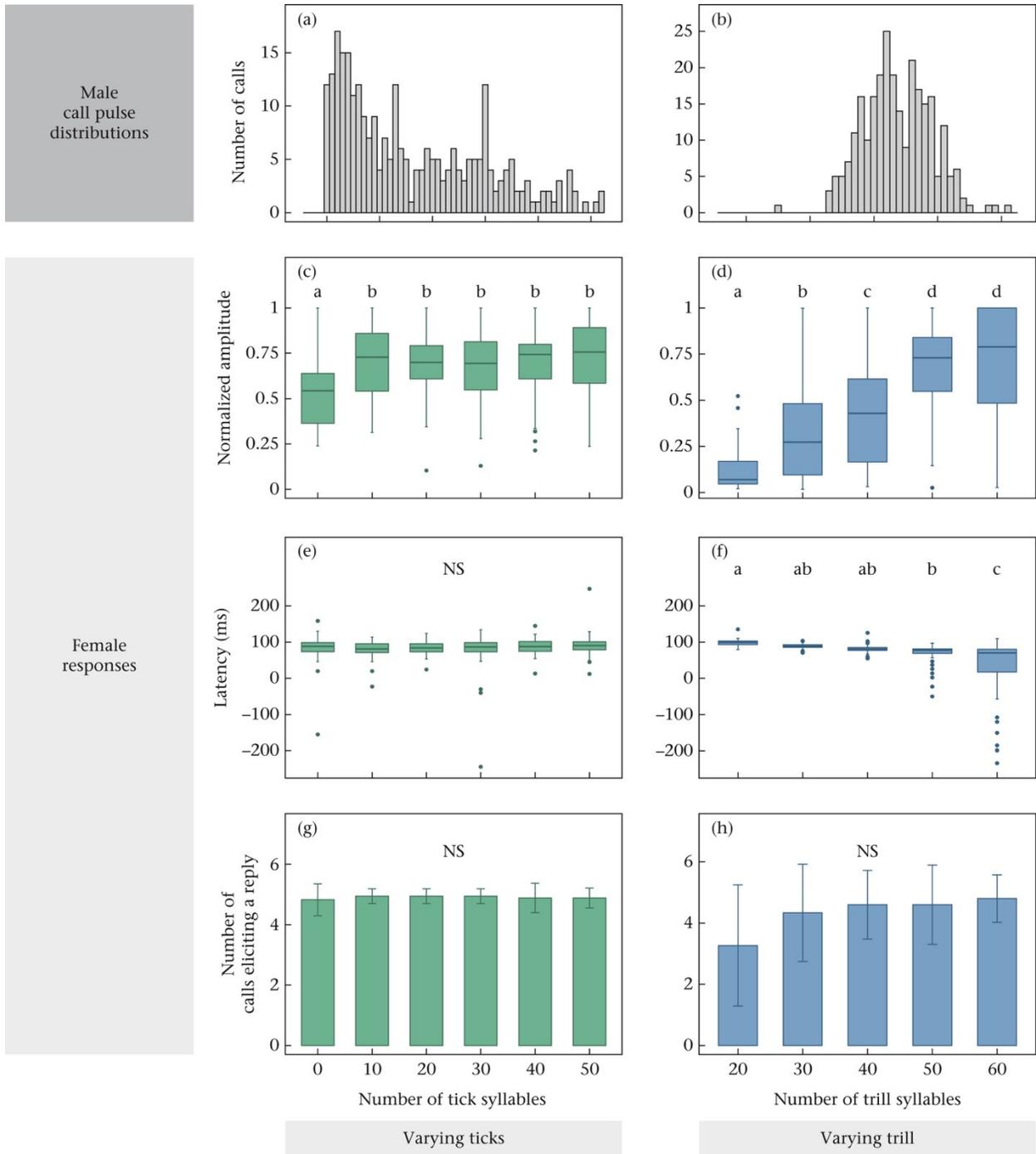


Figure 4. Distribution of syllable numbers in male calls and female responses to playbacks where the number of syllables in each component was altered. (a, b) Histogram of syllable numbers for the tick

component and the trill component, respectively, from the subset of 24 h calls that were scored for both the number of tick syllables and the number of trill syllables. (c, d) Amplitude of female vibrational replies (normalized by dividing the measured amplitude of each response by the maximum of each individual) for (c) varying ticks experiment and (d) varying trill experiment. (e, f) Latency of female response for (e) varying ticks experiment and (f) varying trill experiment. (g, h) Mean number of male calls out of five replicates per acoustic treatment that elicited female vibrational replies for (g) varying ticks experiment and (h) varying trill experiment. For box plots (c–f), boxes represent the 25th and 75th percentiles (ends) and the median (centre line); whiskers represent $Q1 - 1.5 \times IQR$ and $Q3 + 1.5 \times IQR$; and all outliers beyond these whiskers are shown. For bar plots (g, h), error bars show standard deviation. Lower case letters show significant differences between acoustic treatments determined by post hoc tests, with shared letters indicating no significant differences.

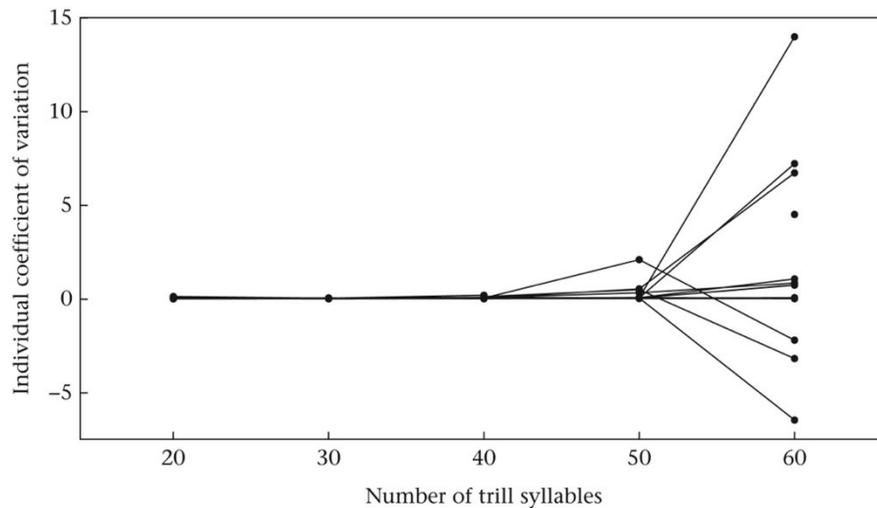


Figure 5. Individual coefficients of variation for female replies to male calls containing different numbers of trill syllables. Coefficients are the standard deviation divided by the mean reply latency of the five presentations of each treatment. Some coefficients are negative because the mean reply latency for that

individual occurred before the end of the male call.

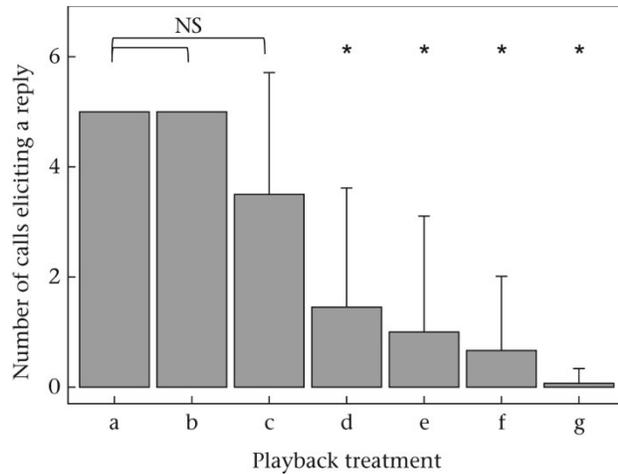


Figure 6. Mean number of five replicates that elicited a female reply in experiment 4. Acoustic parameters for each treatment are provided in Table 2. The treatments in this playback series were as follows: a = control with 40 trill syllables, similar to the average syllable duration, number and period of recorded males; b = 40 trill syllables with the average syllable period but half the average syllable duration; c = 40 trill syllables with the average syllable period but 50% longer syllable duration; d = 20 trill syllable with an average syllable duration but twice the average syllable period; e = 20 trill syllables with twice the average syllable duration; f = a single, long continuous tone rather than a series of syllables, with duration equivalent to total trill duration of control; g = 40 trill syllables with half the average syllable duration and period, but an equivalent duty cycle to the control. An asterisk denotes a significant difference between an acoustic treatment (b–g) and the control (a).

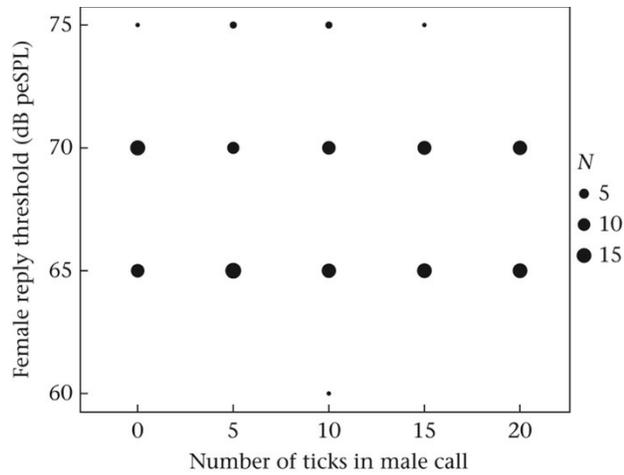


Figure A1. Relationship between the amplitude threshold at which a female cricket replies to a male call and the number of ticks in the call. Black points showing the amplitude at which females first responded are scaled to the number of crickets at each amplitude.

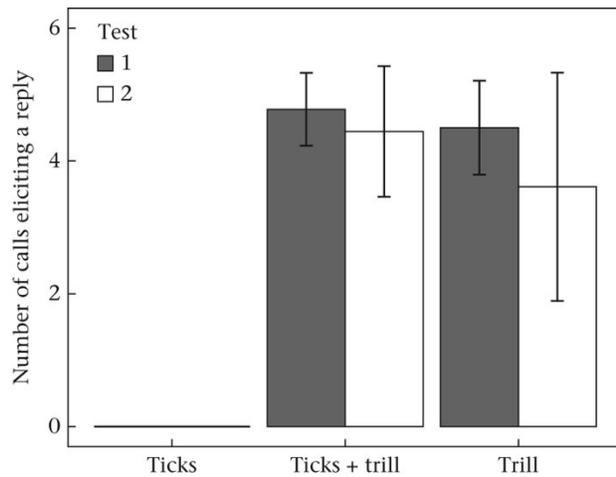


Figure A2. Mean \pm 1 SD number of male calls eliciting female replies out of five repetitions to each individual. Only crickets that were assigned to the unmated test and that remained responsive in the second round of playback tests are included here. Mean age of crickets: test 1 = 18.3 days after final moult; test 2 = 39.3 days after final moult.

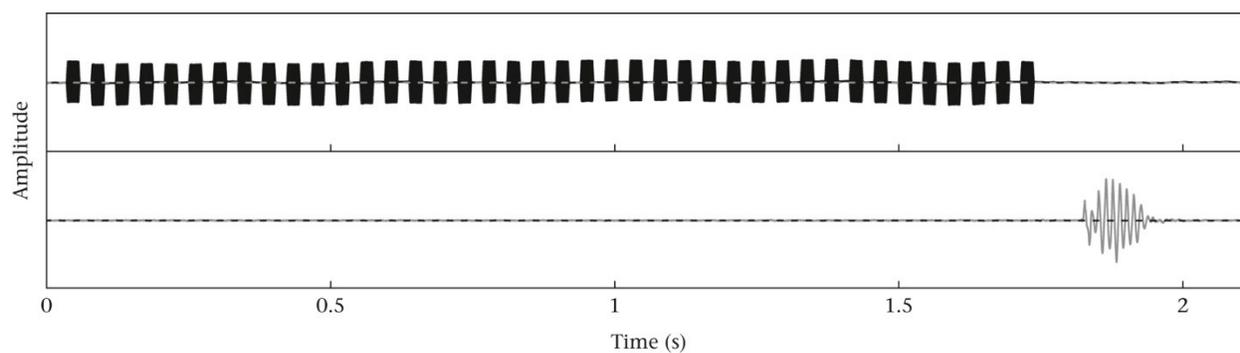


Figure A3. Oscillogram of a typical female vibrational reply, common across all experiments except experiments 3 and 4. The end (trill) of the playback stimulus (here, control 10 ticks and 40-syllable trill call) is shown on the top trace, and the female reply is shown on the bottom trace.

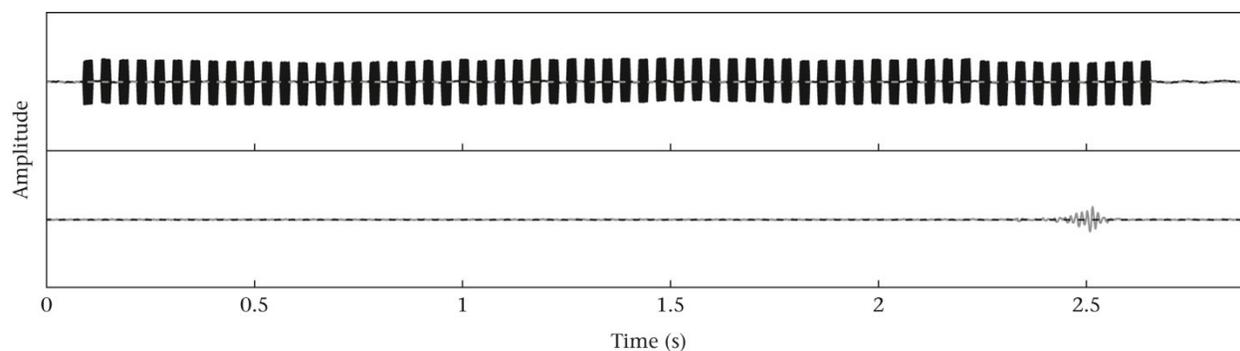


Figure A4. Oscillogram of a premature female vibrational reply, occurring before the end of the playback stimulus. The end (trill) of the playback stimulus (here, 10 ticks and 60-syllable trill call from experiment 3) is shown on the top trace, and the female reply is shown on the bottom trace.

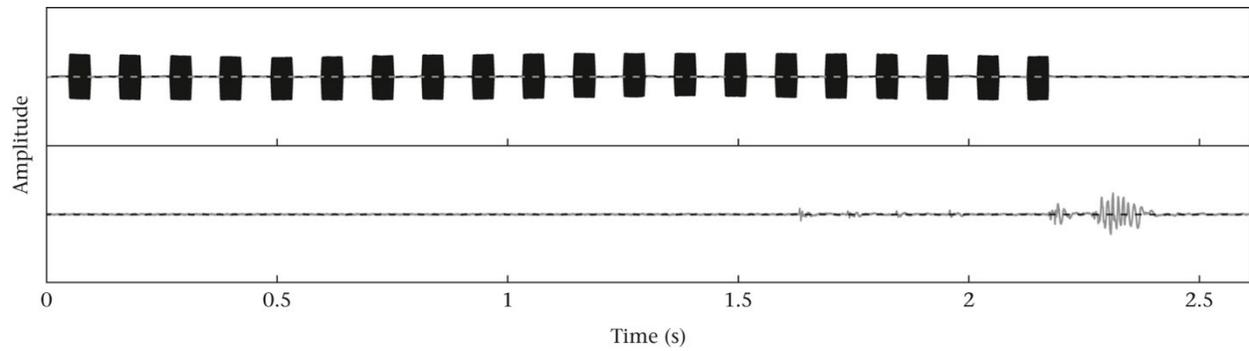


Figure A5. Oscillogram of a false-start female vibrational reply, where one or several smaller pulses occurred before a ‘typical’ response. This type of reply occurred throughout all playback experiments but was most common in experiments 3 and 4. For this type of call, parameter measurements (amplitude, duration, latency) were made on the largest pulse, which tended to occur last. The end (trill) of the playback stimulus (here, stimulus ‘e’ from experiment 4) is shown on the top trace, and the female reply is shown on the bottom trace.

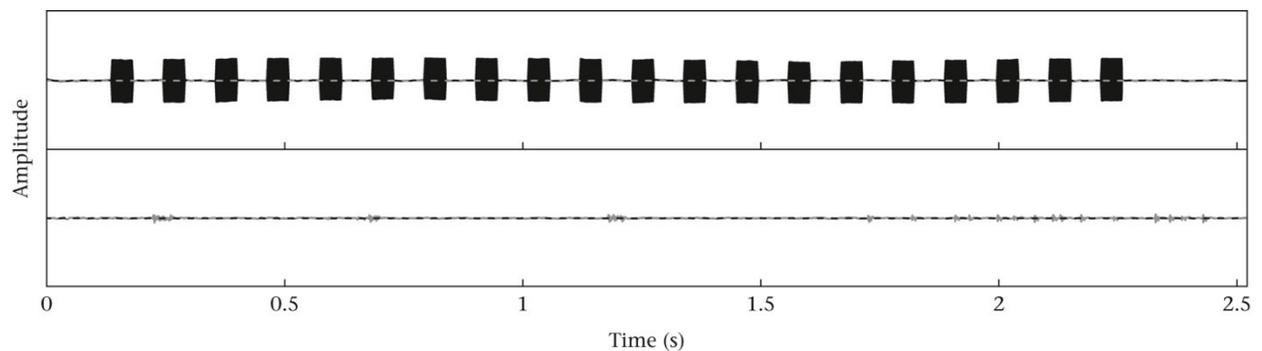


Figure A6. Oscillogram of an atypical female vibrational reply, which was not scored as a response and was not measured. This type of reply occurred mostly in experiment 4. The end (trill) of the playback stimulus (here, stimulus ‘e’ from experiment 4) is shown on the top trace, and the female reply is shown on the bottom trace. Atypical replies were generally of very low amplitude compared to typical, premature and false-start replies.

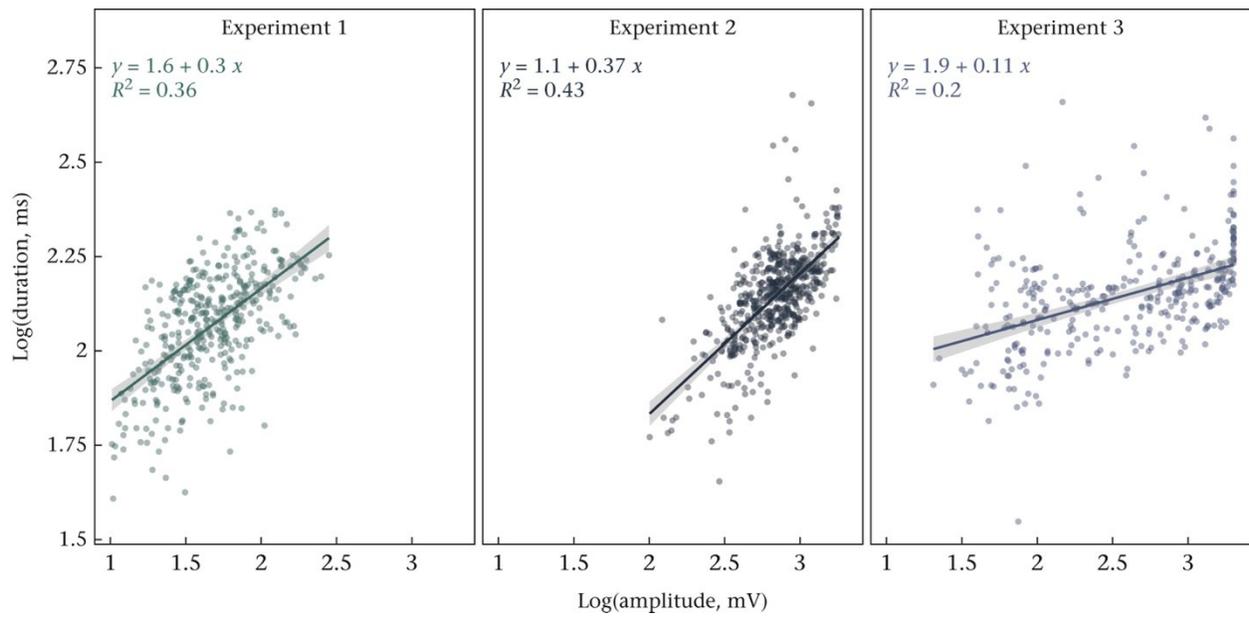


Figure A7. Relationship between log-transformed amplitude and duration across the three experiments in which we measured reply parameters.