



Relative Amplitude of Courtship Song Chirp and Trill Components Does Not Alter Female *Teleogryllus oceanicus* Mating Behavior

Susan L. Balenger¹, Laura M. Lara & Marlene Zuk

Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, USA

Correspondence

Susan L. Balenger, Department of Biology, University of Mississippi, 214 Shoemaker Hall, University, MS 38677, USA.
E-mail: balenger@olemiss.edu

¹Present address:

Department of Biology, University of Mississippi, University, MS, USA

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Abstract

Low-amplitude acoustic signals intended for short-range communication, often called soft songs, remain poorly studied, especially among acoustically communicating invertebrates. Some insects do employ low-amplitude acoustic signals, but it remains unclear what the specific function, if any, is of quietness *per se*. Male *Teleogryllus oceanicus*, or Pacific field crickets, produce a two component, short-distance courtship song consisting of a high-amplitude series of chirps followed by a lower-amplitude trill. We investigated whether female *T. oceanicus* prefer to mate with males that sing courtship songs containing trill components that are equally as loud as (−0 dB) or quieter than (−3 dB and −10 dB) the loudest chirp (90 dB). We found no evidence that modifying trill amplitude affects female mate preference. We did, however, find that previously unmated females were faster to mount males than were females that had mated once before. Previous mating status showed no significant interaction with trill amplitude. What, if any, function of low-amplitude components of field cricket courtship song remains to be determined.

Introduction

The amplitude of animal acoustic signals varies widely; in many cases, this variation occurs within individuals and populations. More and more, special attention is being paid to signals containing or consisting solely of low-amplitude acoustic components that have traditionally been overlooked (Reichard & Anderson 2015). In particular, researchers are recognizing that the quiet signals produced by some insects may have important functional roles with respect to fitness (Sueur & Aubin 2004; Nakano et al. 2006; Balenger 2015). For example, the production of extremely quiet, high-frequency sound by male Asian corn borer moths (*Ostrina furnalcalis*) causes a freeze response by females, allowing males to mate unimpeded (Nakano et al. 2013).

Low-amplitude signals are defined according to a signal's active space such that they have evolved to function specifically in short-range communication (Reichard & Anderson 2015). Several authors have recently suggested that field cricket courtship song may be a good candidate for studying the function of

low-amplitude acoustic signals (Reichard & Welkin 2014; Balenger 2015; Reichard & Anderson 2015). Male field crickets typically exhibit a repertoire of two distinct songs primarily directed at communicating with females—a long-distance calling song and a short-distance courtship song—and a third song used during short-distance male–male aggressive interactions (Alexander 1961). The courtship song is only produced when a male is in close proximity with a female (Burk 1983) and therefore has likely evolved in the context of short-range communication.

Across short distances, signalers can communicate with intended receivers, including potential mates, while concurrently avoiding eavesdropping from conspecific competitors and predators (Reichard & Anderson 2015). A typical field cricket courtship song, however, contains both relatively high- and low-amplitude components. When short-distance courtship signals contain both high- and low-amplitude components, the change in amplitude itself may be important for mating success.

Behaviors that are consistent within individuals and variable between individuals are capable of

responding to selection (Boake 1989). While variability in absolute amplitude of high- and low-amplitude components of courtship song has rarely been studied (Nandi & Balakrishnan 2013), relative amplitude has been shown to be extremely variable among individuals in *Gryllus bimaculatus* and *G. assimilis* (Vedenina & Pollack 2012; Shestakov & Vedenina 2015), but not among *G. texensis* and *G. rubens* (Fitzpatrick & Gray 2001). In *G. bimaculatus*, Shestakov & Vedenina (2015) found relative amplitude within individuals to be essentially as variable as between individuals, while Vedenina & Pollack (2012) found it to be less variable within than among individuals in *G. assimilis* (58% vs. 93% coefficient of variation, respectively). Zuk et al. (2008) explored variation in temporal characteristics of both calling and courtship song of *Teleogryllus oceanicus*, but unfortunately did not include among or within individual variation information with respect to either absolute or relative amplitude of the chirp and trill portions of each song.

While limited information is available to establish the extent of field cricket courtship song amplitude variation in the wild or laboratory, even less is known about the adaptive function, if any, of the change in relative amplitude within these songs. *T. oceanicus* courtship song consists of a sequence of chirps that increase in amplitude, followed by a longer, substantially quieter series of trills. Balakrishnan & Pollack (1996) proposed that the components of *T. oceanicus*' courtship song may in fact be intended for different receivers, with one part a signal to male competitors and the other a signal to receptive females. If true, it remains unclear which part of the song might be meant for which sex. In fact, while Balakrishnan & Pollack (1996) found that the chirp component on its own was sufficient to induce female mounting and that the trill component was unnecessary for mating success, Libersat et al. (1994) came to essentially the opposite conclusion—that the trill component was sufficient to induce female mounting and that the chirp was unnecessary for mating success. Burk (1983), on the other hand, observed *T. oceanicus* females mounting only males that produced both chirps and trills, suggesting that both components are necessary. It remains likely that the trill is important in courtship because its length increases with the length of courtship even although it is likely to be energetically costly (Prestwich & Walker 1981; Libersat et al. 1994; Hack 1997).

The role of amplitude modulation in the courtship song of *T. oceanicus* was most thoroughly examined by Balakrishnan & Pollack (1996), who found that removal of amplitude modulation from the chirp did

not affect mating success. This study, however, did not consider courtship song within a low-amplitude signaling paradigm. One possibility, therefore, is that the change in amplitude is itself attractive to females. Here, we ask whether the relative amplitude between the last, loudest chirp, and the trill of the *T. oceanicus* courtship song affects female mating behavior. Specifically, we test whether females exposed to synthetic courtship songs identical except for the amplitude of the trill are faster to mount when the trill portion is -0 dB, -3 dB, or -10 dB SPL (loud, medium, and soft, respectively) quieter than the loudest chirp (90 dB SPL) at a distance of 10 cm from the speaker. Courtship songs with medium and soft trill components fall within the normal range of chirp: trill relative amplitudes (S. Balenger, unpub. data). The relative amplitudes of the song designated as having a loud trill, however, do not fall within the normal range; the trill amplitude of the *T. oceanicus* courtship song is naturally lower than that of the loudest chirp.

Finally, we examine whether mating history affects female latency to mount. Female field crickets are faster to mate (*G. pennsylvanicus*, Judge et al. 2010) and more phonotactic (*G. integer*, Lickman et al. 1998) when virgins. However, as gryllid females typically mate repeatedly over their lifetime (Alexander & Otte 1967), they are more likely to make most of their mating choices in the wild as non-virgins. All females used in this study, therefore, were given the opportunity to mate with a single male 2 days before experimental trials were conducted (see Materials and Methods below). A subset of females, however, did not mate in this pre-trial mating exposure. We used this as an opportunity to assess whether virgin or mated females differed in their responsiveness to the treatment songs.

Materials and Methods

Colony Origin and Rearing

Subjects were the descendants of eggs laid by approximately 20 females collected in 2009 in Tahiti. We reared them in Caron insect growth control chambers (model 6025) at 26°C, 75% humidity and 12-h:12-h photoreversed light: dark cycle. Breeding populations of the laboratory colony consist of 100 or more individuals at all times to maintain genetic diversity. Adult crickets are housed in groups of 20–30 individuals within plastic containers (42 × 27 × 16 cm; l × w × h) each containing egg cartons for substrate and *ad libitum* access to Harlan high fiber rabbit diet and water. We transferred crickets to single-sex

containers upon development of external sexual characteristics and isolated individuals in 118-ml containers (4.5 cm high, 7 cm diameter) on the day of adult eclosion. All crickets were exposed to calling adults throughout development.

Pre-Trial Mating Exposure

We provided females with a pre-trial mating exposure to allow them the opportunity to mate with one male prior to inclusion in experimental trials. On day six or eight post-eclosion, females were paired with a haphazardly selected male between the ages of 6–10 days post-eclosion. We silenced males within 24 h of eclosion by removing the scraper from each male's right wing by clipping it with surgical scissors. This procedure ensured that females only hear the acoustic treatment of interest when in the presence of a male. We allowed each pair a minimum of 15 min together in a 118-ml container in the presence of a speaker playing a courtship song with average temporal and amplitude properties at *T. oceanicus*' fundamental frequency (4.7 kHz; Libersat et al. 1994). Females were checked for spermatophores to determine whether successful copulation had occurred; females without spermatophores are hereafter described as 'virgins', while those with spermatophores are described as 'mated'. Males used in this pre-trial exposure were not used in courtship playback trials to avoid any effect of previous successful matings on male attractiveness.

Courtship Song Treatments

We constructed courtship songs using population specific temporal parameters derived from recordings of 10 seven-day-old laboratory-reared Tahitian *T. oceanicus* males. Songs were created by extracting and altering the number and spacing of pulses in the chirp and trill components of a previously recorded song. Relevant temporal parameters were selected based on Zuk et al. (2008). Average temporal values and pulse numbers are provided in Table 1. All song manipulation was performed using Raven 1.4 Interactive Sound Analysis Software (Cornell Lab of Ornithology, Ithaca, NY, USA).

Assays of female latency to mount in response to amplitude manipulated courtship songs were conducted 2 days following initial pre-mating exposure; therefore, females used in all subsequent trials were eight or ten days old. Females were haphazardly assigned to a courtship song treatment group (soft-, medium-, or loud-amplitude trill relative to chirp). As

Table 1: Average *Teleogryllus oceanicus* courtship song temporal parameters and pulse numbers

Song parameter	Mean value (s)
Chirp pulse duration (CPD)	0.044
Chirp pulse interval (CPII)	0.036
Trill pulse duration (TPD)	0.025
Trill pulse interval (TIPI)	0.010
Chirp duration (Chirp)	0.605
Chirp trill interval (Int)	0.152
Trill duration (Trill)	2.848
Song duration (Song)	3.605
Song parameter	Count
Chirp pulse number	8
Trill pulse number	82

with males from pre-trial mating exposures, all males used in trials had the scraper removed from their right forewing within 24 h of eclosion. We used six-, eight-, and ten-day-old males in experimental trials. Each male was used in up to three trials, but never more than once for any of the three treatments. Males used more than once were given at least 1 day of rest between trials.

We performed trials and matings in an anechoic room under red light at ambient temperature (22–24°C) between 15:00 and 20:00 h. Trials were conducted in a 10 × 10 × 7 cm (l × w × h) plastic container; one side of the container was removed and covered with an acoustically transparent mesh screen. We allowed females to acclimate alone in the container. After 150 s, a male was introduced to the container and a speaker located next to the mesh simultaneously began playing one of three male courtship songs. Female latency was measured as the number of seconds between start of playback and successful mounting of the male. Mating was interrupted prior to spermatophore transfer. Of the 118 females tested, six did not mount the male during the 300 s playback trial (n = 1 virgin female presented with the medium trill courtship song, n = 1, 2, and 2 previously mated females presented with the soft, medium and loud songs, respectively) and were excluded from the analysis.

Sound pressure level (SPL) was measured with an AZ Sound Meter (model 8922). The peak intensity of the courtship song of *T. oceanicus* is approximately 90 dB SPL at 10 cm (Balakrishnan & Pollack 1996), and trials were conducted using this as the maximum amplitude of the final chirp (see Fig. 1). Amplitude of trills for each treatment group was as follows: soft—80 dB SPL; medium—87 dB SPL; loud—90 dB SPL.

Analysis

We used a generalized linear model (GLM) to investigate whether the amplitude of the courtship trill (soft, medium, or loud), mating status of females (virgin or previously received a spermatophore), or their interaction predicted the latency with which females mounted courting males. Latency was log-normal-transformed to conform to normality (Kolmogorov's D, $p > 0.1$). Results were interpreted using likelihood ratio tests.

Results

The amplitude of the courtship song trill did not significantly affect the latency with which females mounted courting males (soft: $\bar{x} = 47.9$ s, $n = 3$, $SE = 9.29$; medium: $\bar{x} = 37.5$ s, $n = 36$, $SE = 3.24$; loud: $\bar{x} = 38.8$ s, $n = 38$, $SE = 3.59$; $\chi^2 = 1.16$, $df = 2$, $p = 0.56$; Fig. 2). Whether a female obtained a spermatophore the day prior to the trial or was a virgin (i.e., had spent time with a male, but spermatophore transfer did not take place) was a significant predictor of latency to mount. Specifically, virgin females mounted males, regardless of trill amplitude, faster than did mated females (virgin: $\bar{x} = 48.1$ s, $n = 36$, $SE = 10.1$; mated: $\bar{x} = 57.7$ s, $n = 76$, $SE = 7.8$; $\chi^2 = 3.86$, $df = 1$, $p = 0.05$; Fig. 2). Tukey's *post hoc* tests did not find a significant difference between virgin and mated females for any of the three treatment groups alone. Trill amplitude and mating history did

not show an interaction effect with regard to mounting latency ($\chi^2 = 0.74$, $df = 2$, $p = 0.69$).

Discussion

The function of chirp and trill relative amplitude during field cricket courtship song has heretofore been virtually unstudied. Here, we examined whether altering the amplitude of the trill component of *T. oceanicus* courtship song affects female mating behavior, which would suggest that relative amplitude per se might contain intraspecific signaling content. We found no significant differences between female latency to mount males when hearing courtship songs with a loud (-0 dB), medium (-3 dB), or soft (-10 dB) trill component. Our results strongly suggest that softening the trill is not important for female choice in this species.

While we found no support for our hypothesis that male *T. oceanicus* soften their trill relative to their chirps based on female preference, it is possible that we were unable to find an effect because we did not soften the trill enough for it to make a difference to females. If the amplitude measured in the laboratory is not consistent with what males produce or females perceive in the wild, then our treatments for medium and soft trills may not accurately reflect the conditions under which females choose in natural conditions. In the wild, field crickets are subject to eavesdropping by intrasexual competitors as well as acoustically orienting predators. Under such conditions, males may

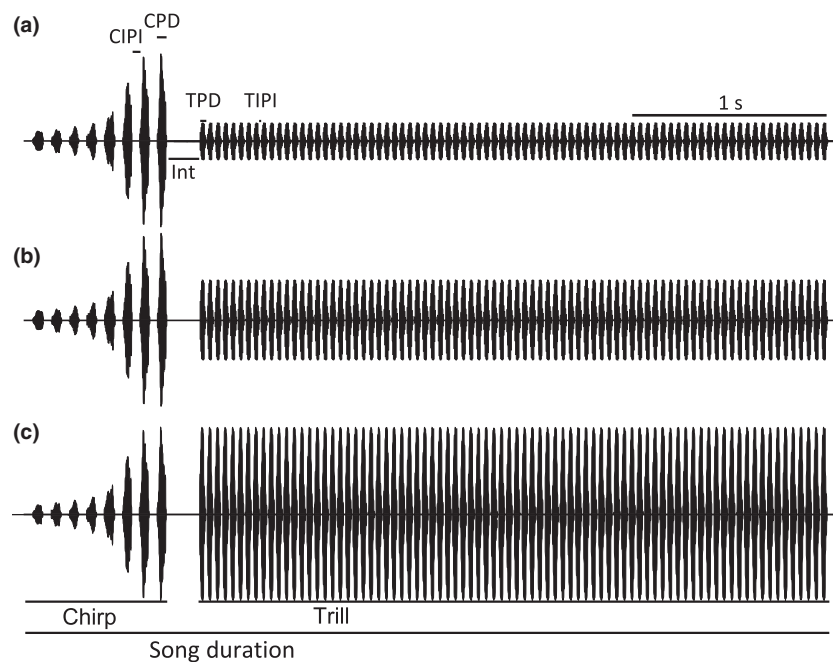


Fig. 1: Synthetic courtship songs of *Teleogryllus oceanicus* used to examine female latency to mount a male when played an average chirp followed by a soft (a), medium (b), and loud (c) trill. All temporal parameters were identical between courtship songs.

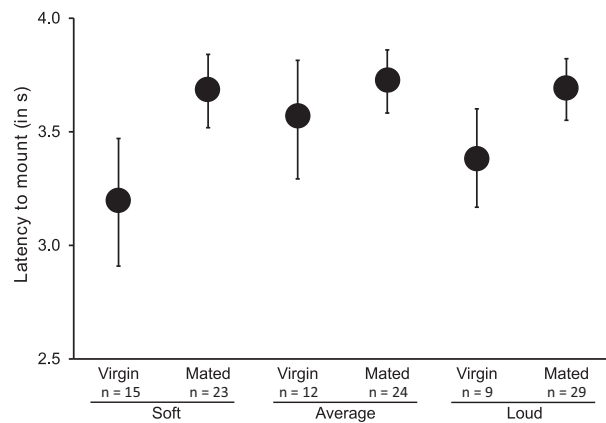


Fig. 2: The role of relative amplitude of the trill portion of *Teleogryllus oceanicus*' courtship song and prior mating history on female latency to mount. Larger latency values equate to a lower preference, while smaller latency equates to a greater preference. Latency to mount ($\bar{x} \pm SE$) is shown on a logarithmic scale, which was used to normalize the response variable.

produce quieter trills relative to chirps in an attempt to lessen eavesdropping while courting. Previous studies of *T. oceanicus* mating behavior have found conflicting evidence as to the necessity of the trill component of the courtship song for female mounting (Burk 1983; Libersat et al. 1994; Balakrishnan & Pollack 1996). In fact, Balakrishnan & Pollack (1996) speculated that the movement of a male's wings during courtship (i.e., absent any sound) is sufficient for female mounting.

Previous studies of female preference for the temporal properties of courtship song waited to start playback until males began stridulating (Balakrishnan & Pollack 1996; Rebar et al. 2009), but in this study, we began playing courtship song as soon as males were introduced to females. We also did not record when males began stridulating or engaging in other courtship associated behaviors and thus could not evaluate mounting latency subsequent to initiation of male courting behavior. We can reasonably expect that differences in latency are random with respect to treatment group, but the variability in female latency to mount may be less than that presented here if males differed widely in their latency to court.

The female subjects of this study were provided with the opportunity to mate 2 days prior to their experimental trial. A subset of females (32%) did not allow spermatophore transfer during this pre-trial mating exposure. Interestingly, females that did not mate during the pre-trial mating exposure were faster to mount males during the subsequent trials of interest. It has been demonstrated in a variety of field crickets that virgins are more phonotactic (*Acheta domesticus*, Koudele et al. 1987; *G. integer*, Lickman

et al. 1998; *G. bimaculatus*, Loher et al. 1993) and faster to copulate (*G. pennsylvanicus*, Judge et al. 2010) than are mated females. These studies, however, investigated females that were physically not allowed to mate—that is, the virgin females did not have a choice as to whether to mate or not prior to testing phonotaxis. Typically, when females are allowed to mate prior to experimental trials, those that do not successfully mate are removed from the study and their behaviors are not examined in comparison to those that mated (Swanger & Zuk 2015). Here, we not only found that nearly one-third of females did not (or would not) successfully copulate with the first male provided, we found that such females mounted the second male faster than females that mounted and received a spermatophore 2 days earlier. Although not significant, the biggest difference between virgin and mated female time to mounting was seen in the soft song treatment where virgin females mounted males on average 12 s sooner than did previously mated females.

This study experimentally examined the function of relatively loud and quiet components contained in field cricket courtship song. Our findings do not support a role for courtship amplitude per se in female pre-copulatory mating preference. It will be important in the future, however, to validate these findings under natural field conditions and to examine a possible role for courtship amplitude at the post-copulatory level. Rebar et al. (2011) found that the temporal quality of the courtship song a female was previously exposed to affect both her latency to mount a male and spermatophore retention. It will also be of interest to test the potential function of amplitude in other cricket species, particularly within the North American *Gryllus* genus in which courtship song typically consists of a single high-amplitude tick and a series of dramatically lower-amplitude chirps (see Fitzpatrick & Gray 2001 Fig. 2 for examples). While our results suggest that the change in amplitude during courtship song is not important for female choice in *T. oceanicus*, what if any function it does have remains to be determined.

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