

# Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*

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Sexual signals consist of multiple components, each of which can contribute to mating decisions. Male field crickets use 2 acoustic signals in the context of mating: a calling song that attracts females from a distance and a courtship song that follows once a female makes physical contact with a male. The function of courtship is unclear because females have already chosen and approached a particular male based on his calling song. Using the field cricket *Teleogryllus oceanicus*, we tested whether females evaluate the attractiveness of males based on their courtship song irrespective of other male qualities. In a tournament-style assay, females chose males based on elements of their courtship song and preferred males with longer courtship songs that had a higher duty cycle (more sound per unit time). Courtship song was also highly repeatable not only within a bout but also between days in the presence of different females. Additionally, we manipulated the attractiveness of males to females by surgically silencing males and broadcasting playbacks of artificially constructed courtship songs. When we broadcast a preferred song model during courtship by previously nonpreferred males, females showed greater attraction, and vice versa. Courtship song may be particularly important in systems with satellite males that do not produce long-range signals because it gives females their only opportunity to evaluate potential mates. *Key words*: courtship song, mate choice, multiple signals, repeatability, *Teleogryllus oceanicus*. [*Behav Ecol* 20:1307–1314 (2009)]

Sexual signals consist of multiple signals or components (Brooks and Caithness 1995; Dale and Slagsvold 1996; Møller et al. 1998; Wollerman 1998; Kodrick-Brown and Nicoletto 2001; Scheuber et al. 2004), each of which can contribute to mating decisions. Female choice for these signals can occur at different times (e.g., long range vs. short range), and which signal is most important will vary based on other factors (Candolin 2003), such as if a male is using a satellite strategy or not. Given the direct costs associated with signaling, such as increased energetic expenditure and predation risk (Hack 1998; Zuk and Kolluru 1998), why would an animal produce more than one signal? Field crickets are a useful model to explore the role of multiple signals in sexual displays. Males produce 2 acoustic signals sequentially during mating. The first, calling song, attracts females from a distance, whereas the second, courtship song, proceeds only after a female makes physical contact with the singing male (Alexander 1961). Much research has investigated the function of the calling song (Zuk and Simmons 1997). Although it has been demonstrated to convey information about certain aspects of male condition (Scheuber et al. 2003a, 2003b), it also appears to have an important role as a prezygotic mating barrier (Gray and Cade 2000; Honda-Sumi 2005), suggesting its importance in species recognition.

The significance of courtship song is more enigmatic. Its role in eliciting a proper female mounting response has been documented in *Teleogryllus oceanicus* (Burk 1983; Libersat et al. 1994; Balakrishnan and Pollack 1996; Bailey et al. 2008) and other cricket species (Crankshaw 1979; Adamo and Hoy 1994; Nelson and Nolen 1997). However, most studies only test whether the presence or absence of courtship song releases female mounting behavior and have not established whether

variation in courtship song parameters influences female mounting (Nelson and Nolen 1997). Unlike the calling song, courtship song does not appear to be a strong prezygotic isolating mechanism (Fitzpatrick and Gray 2001) but varies in its condition dependence (Gray and Eckhardt 2001, Rantala and Kortet 2003). Zuk et al. (2008) demonstrated that the courtship song is more variable than the calling song in the field cricket *T. oceanicus*, consistent with the idea that calling song is under directional selection for species recognition whereas courtship song is more likely to contain information about individual male quality. Additionally, females can simultaneously attend signals in other modalities, such as cuticular hydrocarbons (Tregenza and Wedell 1997; Kortet and Hedrick 2005; Thomas and Simmons 2008), antennal contact (Balakrishnan and Pollack 1997), and other tactile cues (Balakrishnan and Pollack 1997) once they have made contact with a courting male.

Courtship song might have particularly important effects on the dynamics of female mate choice when males use alternative mating strategies to obtain mates. For example, male field crickets sometimes use satellite behavior to locate females for mating (Cade 1979, 1980). Instead of calling themselves, satellite males aggregate around calling males and intercept females that are attracted to the callers (Cade 1980). As a result, intercepted females can only evaluate the courtship song of these satellite males. In such a situation, courtship song might play a more important role in female mate choice than calling song.

Female preferences for male courtship song parameters have been examined in *T. oceanicus* (Tregenza et al. 2006) as well as other cricket species (Wagner and Reiser 2000; Rantala and Kortet 2003). However, inferences about female preferences for courtship song have been made using phonotaxis experiments even though phonotaxis is not involved during female responses to courtship song; courtship song has been extensively documented to elicit female mounting in close proximity and not attraction from long distances (Crankshaw

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1979; Burk 1983; Adamo and Hoy 1994; Libersat et al. 1994; Balakrishnan and Pollack 1996; Nelson and Nolen 1997; Bailey et al. 2008). No study to our knowledge has directly demonstrated how courtship song variation influences female mounting behavior (but see Nelson and Nolen 1997).

To examine this question, we carried out 2 experiments with *T. oceanicus*, a field cricket distributed throughout northern Australia and the Pacific (Otte and Alexander 1983). The first examined which components of the courtship song are associated with male attractiveness, measured as the amount of time a male produced courtship song before a female mounted. We hypothesized that females used properties of a male's courtship song in making mate choice decisions. As in previous studies of female preferences for courtship song (Wagner and Reiser 2000; Rantala and Kortet 2003; Tregenza et al. 2006), we predicted that females would prefer songs with a higher duty cycle (more sound per unit time). The second experiment examined the extent to which the courtship song itself, as opposed to other information that females might receive when in physical contact with males, was essential to the female's decision. We tested the hypothesis that acoustic information provided by male courtship song overrides information from other modalities, such as chemical or tactile cues. We therefore predicted that females would mount males, regardless of their actual attractiveness, faster if the male was associated with a preferred courtship song.

## MATERIALS AND METHODS

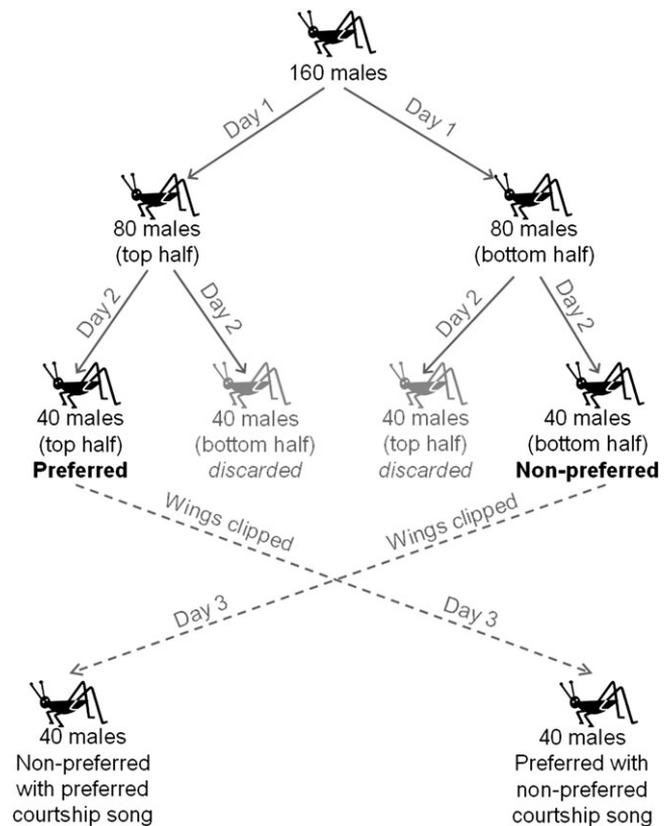
### Experimental animals

We used *T. oceanicus* from a laboratory colony derived from a population originally collected on Oahu at the University of Hawaii, Manoa (Zuk et al. 1995, 1998). Collection site details are given in Zuk et al. (1993, 2001). This colony has been supplemented with eggs from field-caught females approximately annually since establishment in 1993. The crickets were reared in incubators at 25 °C on a 12:12 h light:dark cycle. The population contains more than 100 individuals at any one time to reduce the potential effects of inbreeding. Up to 30 crickets were stored in plastic containers (27 × 38 × 15 cm) with egg cartons for cover, and Fluker's Cricket Chow and water were available ad libitum. Late instar nymphs were separated into single-sex containers. On eclosion, adult males and females were placed in individual plastic containers (6.5 × 4.5 cm) with a piece of egg carton for cover, and rabbit chow and water were available ad libitum. Individuals were checked daily and used after 5 days of age to ensure that they were sexually mature (Bailey and Zuk 2008) but not more than 8 days after eclosion to control for age effects.

### Experiment 1: female preferences for male courtship song

#### Male attractiveness assay

To determine female preferences for male courtship song components, we performed a 2-round tournament modeled after Bussière et al. (2006) and Ivy and Sakaluk (2007) that ranked each male based on the total time that he spent courting a female before she mounted him. In a closely related species, *Teleogryllus commodus*, a female's latency to mount in no-choice designs is a reliable indicator of male mating success (Shackleton et al. 2005). Sometimes males would court for only a few seconds as the female walked away, but on regaining antennal contact (seconds to minutes later), the male would resume courtship, potentially enticing the female to mount. A female's latency to mount was therefore not a true measure of her preference because the pair was not continuously interacting. Timing began as soon as the female was



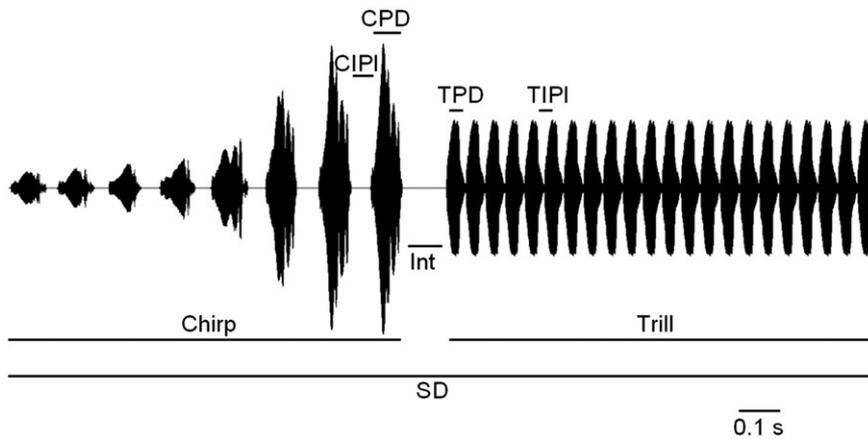
**Figure 1**

Diagram of the courtship song tournament used to establish the 2 groups of males used in subsequent song analyses. Male courtship time before female mounting determined the top and bottom half on each day.

placed in the container: Both the total amount of time that the male spent courting the female (i.e., time he spent producing courtship song) and the time until she mounted the male were recorded. We measured female preference using the total time the male actively courted a female so as to provide the most appropriate and relevant indication of female preference. All trials were conducted during the “night” portion of the animals' light:dark cycle at ambient room temperature (ca. 25.5 °C) under red light.

In the first round, all individuals were left in the room for at least 10 min to acclimate. Each of 160 virgin males was then placed in a clean, individual plastic container (12 × 17 × 10 cm) and paired with a randomly assigned virgin female. A mounting was considered successful if the female remained on top of the male for at least 3 s and the male attempted to transfer a spermatophore. On successful mounting, but before spermatophore transfer, the male and female were disrupted by lightly tapping the container. If a female did not mount the male within 5 min after the male began courting, the trial was stopped, and a female's latency to mount was recorded as the maximum (300 s). If the male failed to court altogether within 10 min, the trial was stopped, and both the male and female were removed from the experiment ( $n = 14$ ). In addition, females that mounted before the male produced courtship song were removed from the experiment ( $n = 11$ ).

Of the 160 males, the half that was mounted faster by females was designated as the “more preferred” group, whereas the 80 slower males became the “less preferred” group (Figure 1). The second round of trials was performed 24 h after the first, using the same protocol as above and the same crickets as the first round. Once again, females were randomly assigned, but

**Figure 2**

Waveform of an artificially constructed courtship song of *Teleogryllus oceanicus*. CPD: duration of the final pulse of the chirp; CIPI: interpulse interval preceding the final pulse of the chirp; Int: interval between the chirp and trill; TPD: trill pulse duration (first, fifth, and tenth pulse measured); TIPI: interval between pulses of trill (interval after first, fifth, and tenth pulse measured). Carrier frequency of the chirp and trill were measured also.

we ensured that no pairings were repeated. On successful mounting, the male and female were interrupted to prevent spermatophore transfer. From the originally designated more preferred males, the half that were mounted fastest ( $n = 40$ ) became preferred males, whereas the half that were mounted slowest ( $n = 40$ ) became “nonpreferred” males (Figure 1).

#### Song recording

Courtship song in *T. oceanicus* is defined as an amplitude-modulated chirp followed by a long trill of repeated pulses (Libersat et al. 1994; Balakrishnan and Pollack 1996; Figure 2). We recorded courtship songs from all males in both round 1 and round 2 of the tournament. Recordings were made during the trial with the female present. Songs were recorded using an AKG D9000 microphone and a Marantz PMD670 solid-state recorder for a minimum duration of 10 courtship songs, each song being defined as above. If the female mounted before the number of songs desired had been recorded, the trial was stopped, but the container was lightly tapped to disrupt the pair so that the male could court again until enough courtship song was recorded. Once 10 songs had been recorded, the crickets were returned to their individual containers.

#### Song analysis

Temporal components of the courtship song were measured using Adobe Audition 3.0 (Adobe, San Jose, CA). Sequences of 10 courtship songs were analyzed for each male. A band-pass filter was used (3500–6500 Hz) to remove background hiss and unwanted noise. The total song length and chirp length, trill length, and the interval between these 2 components were measured (Figure 2). Males would frequently trill, then pause, and begin trilling again before starting the next song. Trill length was therefore measured only to the first break in the trill (if applicable), and the total song length only incorporates the trill length to this first break. Additionally, in the chirp portion of the song, we measured the length of the last pulse, the interval preceding it, and its peak carrier frequency. In the trill portion, the duration of the first, fifth, and tenth pulses and the intervals after them along with the peak carrier frequency were all measured. Mean values from the 10 songs for each male were used in subsequent analyses. Files of the preferred and nonpreferred males were pooled and analyzed blind to the identity of the male’s group.

#### Statistical analysis

We compared song differences in the preferred and nonpreferred males using a discriminant function analysis (SYSTAT 12.0 for Windows, SYSTAT Software, Inc). All data were checked to assure normality and homogeneity of variances.

The variances of each measured component were acceptable. However, discriminant function analyses are highly sensitive to outliers, so we tested for univariate and multivariate outliers for preferred and nonpreferred males separately (Tabachnick and Fidell 2007). Removing the significant outliers did not qualitatively affect the results, and they were left in the data set.

We also performed 2 repeatability analyses. First, to assess within-day repeatability, courtship songs recorded on the second day of the tournament were analyzed. Randomly selecting 40 males from the pool of 160 males, repeatability was calculated as the intraclass correlation coefficient for each song component, such that  $s_A^2 / (s^2 + s_A^2)$ , where  $s^2 =$  error MS and  $s_A^2 = (\text{group MS} - \text{error MS}) / n$  and  $n$  is the number of measurements per song component per individual (Lessells and Boag 1987). The error MS and group MS were determined from a 1-way analysis of variance (ANOVA), and  $n$  was 10 for all individuals. Second, between-day repeatability was calculated from courtship song recordings of each male on the first and second day of the tournament. Randomly selecting 20 males from both the preferred and nonpreferred groups, we first calculated the Pearson correlation coefficient ( $r$ ) between the mean values for each component of the courtship song from the 2 days of the tournament. Because males interacted with different females for 2 days we treated each song measurement as a different trait. This allowed us to determine whether the female present had any influence on male courtship song. We then corrected this value for attenuation, defined as the bias toward zero due to either one or both days of a measured component exhibiting intraindividual variability (repeatability  $< 1.0$ ), by using formula 3 from Adolph and Hardin (2007), and report the corrected values as  $r_{\text{corr}}$ . Because we have multiple measures for each component of courtship song, ignoring the variation within these components when using the mean values to calculate the correlation can lead to error. Therefore, correcting for attenuation takes into account the within- and among-individual variation, which enabled us to better assess the repeatability of song components between the first and second trial and in the presence of different females. Although adjusting for attenuation is not necessary with a large number of samples per individual ( $n = 10$  here), traits with lower repeatability ( $r < 0.5$ ) benefit from this adjustment (Adolph and Hardin 2007). Because several of our traits fell into this category, we corrected for attenuation across all traits for consistency (Table 4).

#### Experiment 2: courtship song flip-flop tournament

##### Flip-flop assay

Using individuals from the same population as the earlier trials, we ran an extension of the previous male attractiveness

**Table 1**  
Song parameters for the representative preferred and nonpreferred courtship songs used in the flip-flop tournament

| Courtship song trait        | Preferred | Nonpreferred |
|-----------------------------|-----------|--------------|
| Song duration               | 3.289     | 2.730        |
| Chirp length                | 0.499     | 0.489        |
| Chirp fundamental frequency | 5023.4    | 5081.4       |
| Chirp pulse duration        | 0.038     | 0.034        |
| Chirp interpulse interval   | 0.029     | 0.033        |
| Chirp-trill interval        | 0.058     | 0.069        |
| Trill length to break       | 2.742     | 2.175        |
| Trill fundamental frequency | 4883.9    | 4915.3       |
| Trill pulse duration        | 0.017     | 0.014        |
| Trill interpulse interval   | 0.007     | 0.011        |

All times are in seconds and frequencies in hertz.

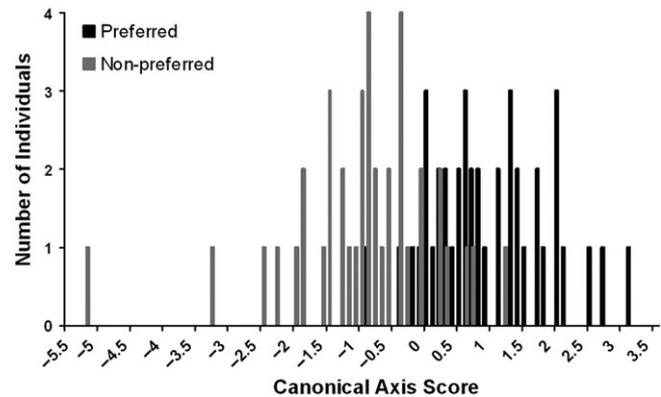
assay to further investigate the role of courtship song in female mate choice. This allowed us to test whether female evaluations of male attractiveness were primarily informed by the courtship song itself and not influenced by male traits from other modalities. The assay was performed as above ( $n = 160$  males), and all songs were recorded and analyzed in the same manner. Once the preferred and nonpreferred males were established after the second day, we muted all preferred and nonpreferred males by clipping their wings. We then performed playback experiments that separated the courtship song from the male that had produced it.

Two representative courtship songs were artificially constructed using the mean song parameters from preferred and nonpreferred males in Adobe Audition 3.0 (Adobe). Using pulses from a male in each respective group that matched the mean song parameters (in terms of length and frequency), we cut and pasted them into a new audio file and manually adjusted the interpulse intervals to reflect the mean interpulse intervals of the respective preferred or nonpreferred group's songs. Thus, the 2 artificially constructed song models reflected the mean parameters estimated from songs of preferred and nonpreferred males, whereas pulses and interpulse intervals were standardized within each respective song model (Figure 2). The resulting preferred song was longer in total time and had longer pulses at a lower fundamental frequency with shorter intervals in the chirp, trill, and the interval between the 2 parts than the nonpreferred song (Table 1).

After the second day of the tournament when the 2 groups of males (preferred and nonpreferred) had been established, the scraper of each male was removed, which rendered him mute. On the third day, males were again presented to the same pool of females, with either the preferred or nonpreferred courtship song broadcast through a Sony SRS-A27 speaker on top of the arena connected to a Sony D-EJ011 CD Walkman. The type of courtship song was reversed for the 2 groups of males: A nonpreferred courtship song was broadcast when a preferred male courted, and a preferred courtship song was broadcast when a nonpreferred male courted. We calibrated the sound pressure level to 90 dB at a distance of 10 cm from the speaker (the intensity of normal courtship song, Balakrishnan and Pollack 1996) using a Precision (type 2235) sound level meter. Courtship song was only broadcast when males stridulated, maintaining the same timing as in the previous 2 assays. Similar to Experiment 1, any male failing to court ( $n = 18$ ) or any female mounting before the male stridulated ( $n = 8$ ) on any day of the tournament resulted in the pair's removal.

#### Statistical analysis

Because a new pool of virgin males and females were used in the second tournament, courtship songs were recorded during



**Figure 3**  
Distribution of scores on the discriminant function axis from analysis of preferred and nonpreferred males' courtship songs recorded on the second day of the first tournament ( $F_{7,72} = 10.112$ ,  $P < 0.0001$ ). As the canonical axis score increases, duty cycle increases.

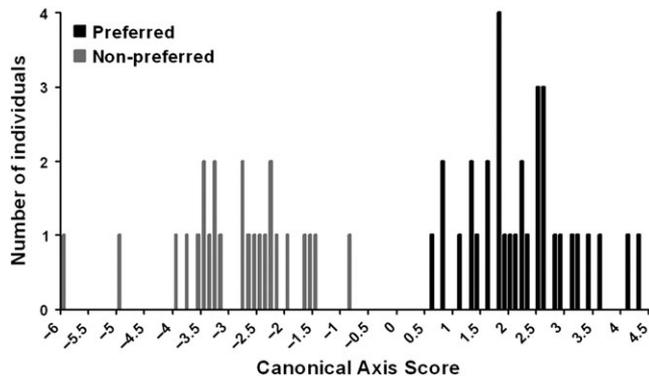
the second day of the tournament. Temporal components of the courtship songs for the preferred and nonpreferred males were analyzed as above, and a discriminant function analysis was performed to confirm the results of our initial female preference assay. To determine whether the courtship song itself mediated female preferences, as opposed to other nonacoustic information that females gained from males, we performed a 2-way ANOVA using the data on the second and third day of the assay with the attractiveness of the male (as determined by the tournament) as one factor and the trial in the tournament (day 2 or day 3) as the other factor. The interaction term was of particular interest because it indicates whether switching the courtship song changed the attractiveness of the male. The courtship songs were reversed for each male from the second to third trial. The data were skewed, so we performed a log transformation to establish normality and homogeneity of variances. As with the previous discriminant function analysis, the variances of each measured component were acceptable, so we tested for univariate and multivariate outliers for preferred and nonpreferred males separately (Tabachnick and Fidell 2007). As before, removal of significant outliers did not affect the results and have been retained in the present analysis.

We also performed post hoc *t*-tests on males with preferred courtship songs on days 2 and 3 and also on males with nonpreferred courtship songs on days 2 and 3 to test whether there was a full reversal of female response to the presence of the courtship song or not. If females' attraction to previously unattractive males in the presence of preferred song playbacks was lower than their attraction to attractive males, this would indicate that females use additional cues to evaluate males at close range.

## RESULTS

### Female preferences for male courtship song

Males preferred by females had songs that differed significantly from those of the nonpreferred males (discriminant function analysis—first tournament: Wilks's lambda = 0.504, approximate  $F_{7,72} = 10.112$ ,  $P < 0.0001$ , 85% correct classification for both groups of males, Figure 3; second tournament: Wilks's lambda = 0.130, approximate  $F_{7,50} = 47.597$ ,  $P < 0.0001$ , 100% correct classification for both groups of males, Figure 4). In both tournaments, preferred males' courtship songs, on average, had a higher duty cycle (more sound per unit time) than nonpreferred males' songs. Additionally,



**Figure 4**  
Discriminant function analysis on the measured components of preferred and nonpreferred males' courtship songs recorded on the second day of the courtship song flip-flop tournament ( $F_{7,50} = 47.597, P < 0.0001$ ). As the canonical axis score increases, duty cycle increases.

preferred males' songs were longer due to a longer chirp and trill composed of longer pulses and shorter intervals (Tables 2 and 3).

Male courtship song was repeatable not only within a courting bout with a single female but also for a period of 2 days in the presence of different females (Table 4). All repeatability estimates were statistically significant except for the interval between the chirp and trill between days of the tournament (Table 4).

**Flip-flop tournament**

Overall male attractiveness, regardless of courtship song, was significantly different for preferred and nonpreferred males (2-way ANOVA,  $F_{1,126} = 30.941, P < 0.0001$ , Figure 5, Table 5). The day of the trial also had an effect on female response (2-way ANOVA,  $F_{1,126} = 8.245, P = 0.005$ , Figure 5, Table 5). However, female attraction to males could be manipulated simply by switching the courtship song so that previously unattractive males could be made attractive if the preferred song was played back during courtship, and vice versa (2-way ANOVA,  $F_{1,126} = 153.700, P < 0.0001$ , Figure 5, Table 5). The post hoc analyses indicated that preferred males with preferred courtship songs were more attractive than nonpreferred males with preferred courtship songs ( $t$ -test,  $t_{63} = -7.266, P < 0.001$ ). However, preferred males with nonpreferred courtship songs

were not significantly different than nonpreferred males with nonpreferred courtship songs ( $t$ -test,  $t_{63} = -1.649, P = 0.104$ ). In other words, the manipulation had a greater effect when looking at preferred songs as opposed to nonpreferred songs. Thus, it was easier to make a male unattractive by switching his song but less easy to make a male attractive.

**DISCUSSION**

Multiple signals exist in many species, and here, we have shown that the close-range courtship song as well as the long-range calling song are important in crickets. This suggests that mate choice can occur at several points during courtship and mating. A signal's importance in mate choice can vary based on other factors, such as whether a male acts as a satellite or not. Here, we show that the courtship songs of preferred and nonpreferred male field crickets are structurally different. Because females preferred the same courtship song characteristics in the 2 tournaments we performed, we can be confident that our results are not an artifact of the random pairings of females and males. Additionally, by artificially reversing the courtship songs of our males, and, as a result, reversing female preference, it appears that female mate choice in this species relies strongly on courtship song, even when other signals are available. The repeatability of courtship song components not only within the same courting bout but also over days and between females supports the idea that females could use courtship song as a reliable indicator of male characteristics.

In the field, calling male *T. oceanicus* have a mean nearest neighbor distance of 3.5 m, with the closest neighbor at 0.9 m (Cade 1981). Once a female makes contact with a male and he begins producing courtship song, the likelihood of another male courting the same female seems small, given this male spacing in aggregations. However, adoption of an alternative male mating strategy, satellite behavior, has been observed in field crickets, including *T. oceanicus* (Cade 1979, 1980). As such, females intercepted by males acting as satellites have only the courtship song and associated behaviors to use in evaluating a male before mounting. Therefore, the demonstrated female preference for males based on their courtship song is relevant in that close-range interactions may be the only opportunity for some females to evaluate a male, suggesting that a larger role of courtship song in female mate choice may be concealed unless alternative male strategies such as satellite behavior are taken into account. Recently, Hall et al. (2008) found support for nonlinear aspects of multivariate selection on courtship song in the closely related species

**Table 2**  
Means  $\pm$  standard errors of courtship song components of preferred and nonpreferred males from the 2 tournaments

| Courtship song trait        | Courtship song tournament |                       | Courtship song flip-flop tournament |                       |
|-----------------------------|---------------------------|-----------------------|-------------------------------------|-----------------------|
|                             | Preferred                 | Nonpreferred          | Preferred                           | Nonpreferred          |
| Song duration               | 3.289 $\pm$ 0.213         | 2.730 $\pm$ 0.177     | 3.147 $\pm$ 0.179                   | 2.617 $\pm$ 0.186     |
| Chirp length                | 0.499 $\pm$ 0.012         | 0.489 $\pm$ 0.012     | 0.514 $\pm$ 0.013                   | 0.475 $\pm$ 0.011     |
| Chirp fundamental frequency | 5023.379 $\pm$ 24.293     | 5081.350 $\pm$ 34.364 | 4922.117 $\pm$ 29.171               | 4927.773 $\pm$ 39.392 |
| Chirp pulse duration        | 0.038 $\pm$ 0.000         | 0.034 $\pm$ 0.001     | 0.041 $\pm$ 0.001                   | 0.035 $\pm$ 0.001     |
| Chirp interpulse interval   | 0.029 $\pm$ 0.001         | 0.033 $\pm$ 0.001     | 0.027 $\pm$ 0.001                   | 0.036 $\pm$ 0.001     |
| Chirp-trill interval        | 0.058 $\pm$ 0.003         | 0.069 $\pm$ 0.004     | 0.049 $\pm$ 0.004                   | 0.065 $\pm$ 0.004     |
| Trill length to break       | 2.742 $\pm$ 0.210         | 2.175 $\pm$ 0.171     | 2.584 $\pm$ 0.178                   | 2.077 $\pm$ 0.186     |
| Trill fundamental frequency | 4883.941 $\pm$ 28.005     | 4915.312 $\pm$ 32.872 | 4723.991 $\pm$ 22.087               | 4807.373 $\pm$ 44.319 |
| Trill pulse duration        | 0.017 $\pm$ 0.000         | 0.014 $\pm$ 0.000     | 0.018 $\pm$ 0.000                   | 0.015 $\pm$ 0.000     |
| Trill interpulse interval   | 0.007 $\pm$ 0.000         | 0.011 $\pm$ 0.000     | 0.007 $\pm$ 0.000                   | 0.011 $\pm$ 0.000     |

All times are in seconds.

**Table 3**  
Structure matrix (loadings) of the discriminant function analysis on male courtship song for the two tournaments

| Courtship song trait      | Courtship song tournament | Courtship song flip-flop tournament |
|---------------------------|---------------------------|-------------------------------------|
| Chirp length              | 0.066                     | 0.115                               |
| Chirp pulse duration      | 0.582                     | 0.275                               |
| Chirp interpulse interval | -0.347                    | -0.376                              |
| Chirp-trill interval      | -0.245                    | -0.140                              |
| Trill length to break     | 0.239                     | 0.101                               |
| Trill pulse duration      | 0.729                     | 0.401                               |
| Trill interpulse interval | -0.784                    | -0.663                              |

All numbers are normalized between -1 and 1.

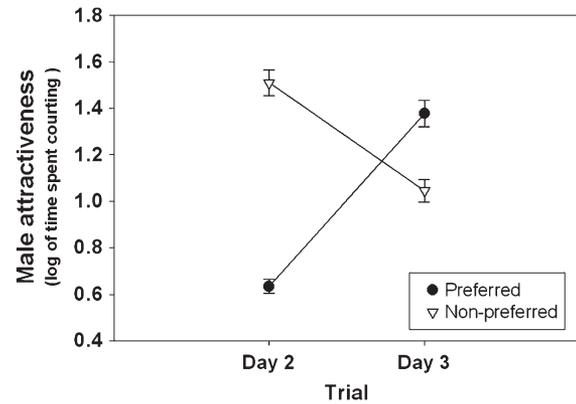
*T. commodus*. Although our data appear to support linear rather than nonlinear selection, our analysis could only discriminate between attractive and unattractive males based on their courtship songs, rather than address the nature of selection exerted by female choice on them. It would be worthwhile to further quantify the shape of selection on courtship song in this species.

Other signals still may play an important role in female mate choice. In some of the preference trials performed here, females mounted the males shortly after antennation but before the male stridulated (19 trials total; removed from study). This behavior has been noted before, but courtship song has often been assumed to be necessary to elicit a normal female response (Adamo and Hoy 1994; Libersat et al. 1994). However, recent work on *T. oceanicus* has shown that females will mate with obligately silent males (Bailey et al. 2008; Tinghitella and Zuk 2009). These males have a wing mutation that renders them mute (flatwing) but protects them from the acoustically orienting parasitoid fly *Ormia ochracea* (Zuk et al. 2006). Despite lacking the ability to call or court, this wing mutation occurs in more than 90% of male field crickets in certain populations on Hawaii (Zuk et al. 2006). Females from ancestral Australian and Pacific Island populations, where flatwing males do not occur, mated with these silent flatwing males, but they discriminated against them more than females from Hawaii, where these flatwing males are found (Tinghitella and Zuk 2009). This suggests that tactile and other behavioral cues may play a role in mate

**Table 4**  
Repeatability of the 10 courtship song components

| Courtship song trait      | <i>r</i>    | <i>F</i> <sub>9,399</sub> | <i>P</i> | <i>r</i> <sub>corr</sub> | <i>P</i>      |
|---------------------------|-------------|---------------------------|----------|--------------------------|---------------|
| Song duration             | <b>0.28</b> | 4.97                      | <0.0001  | <b>0.46</b>              | <b>0.0395</b> |
| Chirp length              | <b>0.34</b> | 6.05                      | <0.0001  | <b>0.57</b>              | <b>0.0093</b> |
| Chirp frequency           | <b>0.80</b> | 40.67                     | <0.0001  | <b>0.95</b>              | <0.0001       |
| Chirp pulse duration      | <b>0.45</b> | 9.11                      | <0.0001  | <b>0.61</b>              | <b>0.0015</b> |
| Chirp interpulse interval | <b>0.58</b> | 14.76                     | <0.0001  | <b>0.75</b>              | <0.0001       |
| Chirp-trill interval      | <b>0.17</b> | 3.09                      | <0.0001  | 0.25                     | 0.4044        |
| Trill length to break     | <b>0.27</b> | 4.78                      | <0.0001  | <b>0.46</b>              | <b>0.0422</b> |
| Trill frequency           | <b>0.81</b> | 42.79                     | <0.0001  | <b>0.97</b>              | <0.0001       |
| Trill pulse duration      | <b>0.33</b> | 5.98                      | <0.0001  | <b>0.67</b>              | <b>0.0024</b> |
| Trill interpulse interval | <b>0.38</b> | 7.11                      | <0.0001  | <b>0.83</b>              | <0.0001       |

Within-trial repeatability (*r*) was measured as the intraclass correlation coefficient (Lessells and Boag 1987). *P* values were obtained from an *F* test using a 1-way ANOVA. Between-trial repeatability (*r*<sub>corr</sub>) was calculated as a Pearson correlation corrected for attenuation following formula 3 of Adolph and Hardin (2007).



**Figure 5**

Male attractiveness before and after manipulation of courtship song. Means and standard errors are shown for the amount of time a male courted before the female mounted. Day 2-preferred males ( $n = 37$ ) were given a nonpreferred courtship song on day 3, whereas day 2-nonpreferred males ( $n = 28$ ) were given a preferred courtship song on day 3.

choice but that their role is not as influential as courtship song.

Although females reversed their preferences after the reversal of courtship songs in the courtship flip-flop tournament, their response times changed. On day 3, the perceived preferred males were mounted more slowly than the preferred males on day 2, and the perceived nonpreferred males were mounted faster (although not significantly) than the nonpreferred males on day 2. One explanation for this result is that although courtship song has the most influential role in mediating females' attraction to males, other cues, such as cuticular hydrocarbons, might also play a role in female mate choice. Cuticular hydrocarbons have been identified as necessary for eliciting proper responses from both males and females as these signals are used in sex recognition of conspecifics, thus releasing these proper responses (e.g., courtship song to females, aggressive song to other males) (Hardy and Shaw 1983; Tregenza and Wedell 1997). If preferred males also have preferred cuticular hydrocarbon profiles and vice versa, then preferred males with a nonpreferred courtship song would be mounted faster than nonpreferred males but slower than they were originally mounted. Our study showed that preferred males were mounted faster than nonpreferred males even after the reversal of courtship songs performed in the flip-flop tournament, consistent with the idea that something other than courtship song itself plays a role in female mate choice. The work of Kortet and Hedrick (2005) work on male pheromones provides some evidence for their role in female mate choice, but whether males with attractive pheromone profiles

**Table 5**  
Two-way ANOVA examining male time spent courting before female mounting with actual male attractiveness (as determined by tournament) and trial (day of tournament) as factors

| Source                      | df  | Mean Square | <i>F</i> | <i>P</i>     |
|-----------------------------|-----|-------------|----------|--------------|
| Male attractiveness         | 1   | 2.349       | 30.941   | <0.0001      |
| Trial                       | 1   | 0.626       | 8.245    | <b>0.005</b> |
| Male attractiveness × trial | 1   | 11.668      | 153.700  | <0.0001      |
| Error                       | 126 | 0.076       |          |              |

also tend to produce attractive courtship songs remains unknown.

Overall, females had an increased latency to respond to male courtship song on day 3 of the courtship flip-flop tournament. However, they exhibited an increased latency to respond to the preferred courtship song and decreased latency to respond to the nonpreferred courtship song on day 3. Both age and previous experience may be responsible for the seen changes in female mounting response. Female responsiveness to above average songs appears to decline with age in the cricket *Allonemobius socius* (Olvido and Wagner 2004). In the cricket *Acheta domesticus*, young females were more selective than older females (Gray 1999; Mautz and Sakaluk 2008). In our study species, females previously exposed to calling song during rearing have been shown to be more discriminating of male song (increased latency), showing long-term effects of acoustic experience on female choosiness, and moreover, females may alter subsequent mating decisions based on the attractiveness of songs they perceive previously (Bailey and Zuk 2008, 2009). Although these studies used the calling rather than courtship song, they might explain the overall increased latency and the respective shifts in latency to respond to preferred and nonpreferred courtship songs. Specifically, females that had heard attractive songs would be less responsive to any subsequent song relative to other females, and all females would be less responsive overall due to previous song exposure.

Here, we show that a signal in addition to the calling song, the courtship song, plays a strong and previously underappreciated role in female mate choice in field crickets. Although we have limited information on female choice across different modalities for most species (Candolin 2003, Hebets and Papaj 2005), this study suggests that female choice occurs at different points and that one main signal at these points may be the most important in mediating this choice. Given the prominence of species that use close-range courtship (e.g., spiders, fruit flies, fish, lizards, birds), mating decisions may be more heavily based on a single signal as opposed to a composite of signals. This is not to say that other signals do not play an important role in mate choice, but the reversal of female preferences based on the reversal of courtship song here illustrates the importance of this single signal. The importance of such a signal could facilitate the evolution of alternative mating strategies, such as satellite males. Because of the direct costs associated with signaling (Hack 1998; Zuk and Kolluru 1998), limited strategic signaling, as is the case with some alternative mating strategies, may be advantageous.

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