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Experience Affects Female Responses to Male Song in the Variable Field Cricket *Gryllus lineaticeps* (Orthoptera, Gryllidae)

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Abstract

Search theory predicts that females will use information on search costs and the characteristics of potential mates to adjust their search behavior and mate choices. We examined the effect of previous acoustic experience on female mating responses in the variable field cricket *Gryllus lineaticeps*. Females of this species prefer calling songs with higher chirp rates to those with lower chirp rates. In this study we examined how female responses to male calling songs change with experience by measuring the responses of females to male calls over a sequence of three trials. Females in one group (group I) were exposed to a sequence of three identical low chirp rate songs and females in a second group (group II) were exposed to two identical low chirp rate songs interspersed by a high chirp rate song. Females in group I did not show a significant difference in their responses to the initial and final low chirp rate presentations, whereas females in group II showed a significantly reduced response to the final low chirp rate song. In addition, the degree to which female responses to the initial and final low chirp rate song changed differed significantly between the treatment groups. Thus acoustic experience appears to affect female mating preferences in this species; exposure to either more attractive songs or more variable songs makes normally unattractive songs even less attractive. These results suggest that females do not use a fixed-threshold search rule in which they mate with any male with a phenotype that exceeds a given threshold. Instead, *G. lineaticeps* females appear to use a more complex search rule in

which they adjust their searching behavior based on the local distribution of male phenotypes.

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Introduction

Information on the decisions that females make while in search of a mate is central to the study of sexual selection, because these decisions directly influence how selection acts on male phenotypic characters (Janetos 1980; Real 1990; see also Lande 1981; Seger 1985). Such information is also necessary to understand how selection acts on female mating preferences, because the expression of mating preferences will depend on the tactics that females use to sample males. For example, females that adopt sampling tactics which result in less extensive sampling will exhibit different mate choices from females that sample more extensively. Several plausible models of search behavior have been proposed, each of which requires that females have different cognitive abilities (Janetos 1980; Brown 1981; Wittenberger 1983; Reeve 1989; Real 1990; Dale & Slagsvold 1994; Dombrovsky & Perrin 1994; Getty 1995; Luttbeg 1996). Females are typically presumed either to assess the mean and variance of a male trait prior to search (Janetos 1980; Real 1990), or to acquire this information during the actual search process (Dombrovsky & Perrin 1994; Luttbeg 1996). Female search behavior and the strength of preference for a particular male trait are then expected to depend on their assessment of the distribution of male phenotypes (see Palokangas et al. 1992; Reid & Stamps 1997; Jirotkul 1999).

A number of models of search behavior allow females to adjust their behavior in response to changes in search costs or the distribution of male phenotypes (see Real 1990; Wiegmann et al. 1996). These tactical search rules are predicted to generate higher fitness returns to searchers than random mating or inflexible search rules, like a one-step decision rule or a fixed threshold criterion (Janetos 1980; Wittenberger 1983; Real 1990). Experience is known to modify the mating responses of female vertebrates, which suggests that females of these species use a flexible decision criterion to evaluate males (Bakker & Milinski 1991; Downhower & Lank 1994; Collins 1995). In contrast to female vertebrates, Alexander et al. (1997) suggested that female insects often use a fixed threshold criterion to evaluate potential mates. This conclusion is based, in part, on the absence of evidence that experience affects female mating responses in any insect. Few studies of insects, however, have experimentally tested the hypothesis that prior exposure to male traits affects female mating responses. Indirect evidence nevertheless suggests that the mate choices of some insects may be influenced by experience with potential mates (Reid & Stamps 1997). Furthermore, the responses of crickets to the auditory stimuli of predators can be modified by

experience (Engel & Hoy 1999). Thus, it seems likely that many insects possess the cognitive abilities necessary to adjust their searching behavior and mate choices in response to changes in social conditions.

In this study, we tested the hypothesis that acoustic experience affects the mating responses of female variable field crickets, *Gryllus lineaticeps*. Male field crickets produce calling songs to attract females at a distance and females select mates, in part, based on variation in male calling song (Popov & Shuvalov 1977; Hedrick 1986; Simmons 1988; Stout & McGhee 1988). In *G. lineaticeps*, the chirp rate of the male calling song ranges between 0.83 chirps/s and 4.65 chirps/s and virgin females prefer calling songs with high chirp rates when given a choice in a two-speaker, simultaneous choice test (Wagner 1996; Wagner & Reiser 2000). We show that female responses to male calling song are flexible; exposure to a sequence of songs that has a higher mean and variance of chirp rate than an alternative sequence of songs reduces female responses to songs with lower chirp rates. These results are consistent with tactical models of search behavior in which females adjust their behavior to the distribution of male phenotypes.

Methods

The females used in our experiment were the third generation offspring of 32 field-inseminated females that were collected from Tucker's Grove County Park, Santa Barbara, California. In the laboratory stocks, we actively managed the matings to reduce inbreeding. We raised the experimental females in family containers until they neared adult eclosion. The containers were provisioned with water, ad libitum Purina cat chow, cardboard shelters, and a paper towel substrate. Prior to adult eclosion, we transferred the females to individual containers in a room where they were acoustically isolated from males. These individual containers were provisioned with water, ad libitum Purina cat chow, cardboard shelters, and a paper towel substrate. We tested the preferences of the females between 7 and 30 d after adult eclosion. Because females were isolated from males prior to reaching sexual maturity, all were known to be virgins.

Table 1: Song stimuli presented to females in the two treatment groups and comparison of time spent in association with the speaker with the random expectation of 26.4 s (one-sample t-test, $df = 14$ for all comparisons)

Group	Trial	Chirp rate (chirps/s)	t	p
I	1	1.75	5.25	< 0.001
	2	1.75	4.20	< 0.001
	3	1.75	4.63	< 0.001
II	1	1.75	6.56	< 0.001
	2	4.00	8.19	< 0.001
	3	1.75	2.60	0.021

We tested the mating responses of females using one of two chirp rate sequences (Table 1). Females in group I were presented with a sequence of three low chirp rate songs, whereas females in group II were presented with a low chirp rate song followed by a high chirp rate song, followed by a low chirp rate song. Songs were constructed from a digital recording of a natural calling song chirp using SoundEdit 16 software and a Macintosh G3 computer. We selected a 14-ms pulse from this chirp and duplicated it to construct a synthetic 100-ms chirp composed of seven pulses. We then used this synthetic chirp to construct the two chirp rate stimuli used in the experiment: a low chirp rate of 1.75 chirps/s and a high chirp rate of 4.0 chirps/s (Table 1). The stimulus songs were otherwise identical.

We tested female preferences in a $2.2 \times 2.2 \times 2.7$ m chamber which was lined with foam to reduce echoes. The chamber was equipped with dim red lights so that subjects could be observed via a Panasonic WV-BP100 video camera mounted in the ceiling of the chamber and a Panasonic CT-1384Y monitor located outside the chamber. We placed a female in the center of the chamber under a box and allowed her to acclimatize for 5 min. In the final minute of the acclimatization period, we broadcast a song at 75 dB SPL (re: 20 μ Pa) from a Macintosh Quadra 840AV computer and Optimus SA-155 amplifier connected to a KLH 970 A speaker. The speaker was positioned in a corner of the chamber in the center of a cardboard circle with an arbitrary radius of 0.26 m. The circle comprised 4.4% of the total area of the arena. After the 5-min acclimatization period, we removed the box and recorded the time that females spent inside the circle around the speaker over a 10-min interval. The song broadcast continued during the 10-min test period. We tested each subject in three sequential trials, each trial separated by a 20-min rest period (Table 1). We calibrated the sound pressure level of the broadcast using a Brüel and Kjær 2236 sound level meter (fast RMS) prior to each set of trials. We conducted all tests between 22°C and 24°C and all of the song stimuli used were within the natural range of chirp rate variation observed at these temperatures (Wagner & Reiser 2000).

Females that did not move from the center of the arena within 10 min of the start of a trial were removed from the experiment and excluded from analyses. We compared the responses of individual females across trials using paired t-tests and the responses of females in the two treatment groups using ordinary t-tests (Sokal & Rohlf 1981).

Results

In the first trial, females of both treatment groups were presented with the low chirp rate calling song. Females in the two groups did not significantly differ in their responses to this initial low chirp rate song (Fig. 1; Student's t-test: $t_{28} = 0.402$, $p = 0.691$). In the second trial, females of group I were presented with the low chirp rate calling song, whereas females of group II were presented with the high chirp rate calling song. Females presented with the high chirp rate song

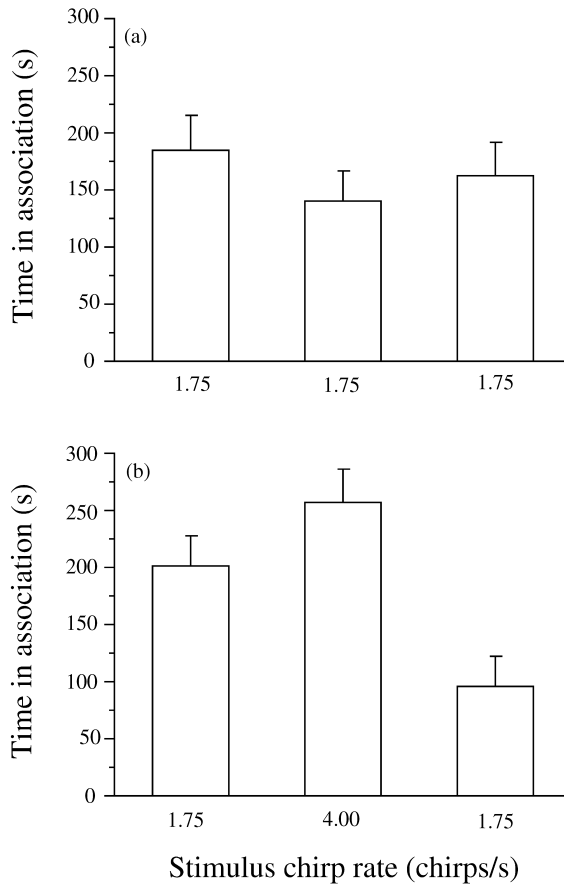


Fig. 1: Time in association ($\bar{x} + SE$) with the speaker in the three trials by females in (a) group I and (b) group II

spent significantly more time in association with the speaker than did females presented with the low chirp rate song (Fig. 1; $t_{28} = 3.016$, $p = 0.005$).

The sequence of male songs experienced by females produced different responses to the final low chirp rate song relative to a female's response to the initial presentation of the low chirp rate song. Females in group I did not show a significant difference in their response to the low chirp rate call broadcast in the first and third trials (Fig. 1a; paired-t-test: $t_{14} = 0.961$, $p = 0.353$). Females in group II, in contrast, showed a significant decrease in their response to the low chirp rate broadcast in the third trial compared with their response in the first trial (Fig. 1b; paired-t-test: $t_{14} = 3.478$, $p = 0.004$). Furthermore, there was a significant difference in the degree to which responses changed between females in each group; females in group II showed a significantly greater decrease in their response than did females in group I (Student's t-test: $t_{28} = 2.198$, $p = 0.037$).

The area of the circle around the speaker was 4.4% of the total area of the arena. If females moved randomly around the arena during a 10-min trial, they should spend, on average, 26.4 s in association with the speaker. In all trials, females spent more time in association with the speaker than expected by chance (Table 1).

Discussion

In two-speaker choice tests, female *G. lineaticeps* preferentially approach speakers broadcasting higher chirp rate songs as opposed to speakers broadcasting lower chirp rate songs (Wagner 1996; Wagner & Reiser 2000). Our results suggest that females make similar discriminations in single-speaker association tests. In trial 1, females in groups I and II were presented with a low chirp rate song. Females in the two groups did not differ in their responses to this song. In trial 2, females in group I were presented with a low chirp rate song, while females in group II were presented with a high chirp rate song. Females in group II spent more time in association with the speaker than females in group I. Because females of both groups were presented with a low chirp rate song in the first trial, this difference in response must be due entirely to the difference in chirp rate between the two songs presented in the second trial. This result suggests that time in association with a speaker provides a measure of female response to male song comparable to more traditional measures of first approach that are used in two-speaker choice tests; in both types of tests, females of this species prefer calling songs with higher chirp rates. The measure is analogous to measures of phonotaxis on a spherical treadmill (Weber & Thorson 1989; see also Wagner et al. 1995).

Acoustic experience had a significant effect on the mating responses of females. Females that were presented with a high chirp rate song between two presentations of a low chirp rate song showed a reduced response to the final presentation of the low chirp rate song. In contrast, females that were presented with a low chirp rate song between two presentations of a low chirp rate song showed no change of preference between trials. Exposure to either an attractive song or a variety of songs thus appears to make relatively unattractive songs even less attractive. Females showed significant phonotaxis in all trials. It could be argued that females exposed to the sequence containing the high chirp rate in trial two showed a reduced response to the low chirp rate in trial three because they gave up after failing to find a male around the speaker in trial two. Females exposed to the sequence containing only low chirp rate songs, however, continued to show significant phonotaxis throughout all three trials, suggesting that even relatively unattractive songs will continue to elicit phonotaxis for an extended period. Furthermore, female crickets tested on spherical treadmills respond to male calls for hours (reviewed by Weber & Thorson 1989). Because all aspects of the songs used in the experiments were held constant except for chirp rate, the generalizability of these results is not known (see Kroodsmas 1990). For example, it is possible that females might not exhibit similar responses if the synthetic songs

were of shorter or longer duration. The song characteristics that were held constant across trials, however, were near the mean value for males from the study population. Regardless of whether the results would be similar across the range of calling song variation, it is clear that acoustic experience can affect female responses to male song.

If female *G. lineaticeps* use a fixed threshold criterion to evaluate potential mates and mate with any male that possesses a trait exceeding the threshold criterion, we would not expect the mating responses of females to change as a result of acoustic experience. Because females exposed to a high chirp rate song showed a reduced response to a subsequent low chirp rate song compared to females that were not exposed to a high chirp rate song, our results suggest that female field crickets do not use a fixed threshold criterion to evaluate males. This finding is consistent with tactical models of female sampling behavior (Janetos 1980; Parker 1983; Real 1990; Dombrovsky & Perrin 1994; Luttbeg 1996). While few studies have demonstrated an effect of experience on mating preferences in insects (see Alexander et al. 1997), such an effect has been shown for a number of vertebrates (Bakker & Milinski 1991; Downhower & Lank 1994; Collins 1995). By adjusting their responses to males based on information about the distribution of male traits, females can potentially balance the benefits of mating with males with preferred phenotypes and the costs of additional sampling.

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