ANIMAL BEHAVIOUR, 2006, **72**, 809–818
doi:10.1016/j.anbehav.2006.01.019

Female preference for male courtship song and its role as a signal of immune function and condition

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(Received 28 June 2005; initial acceptance 18 August 2005;
final acceptance 6 January 2006; published online 1 September 2006; MS. number: 8592)

The acoustic signals of insects are examples of sexually selected traits predominantly used by females to identify, locate and choose between potential mates. In addition to the conspicuous calling songs found in many groups, many male Orthopterans also produce a distinct courtship song when in close proximity to a female. It has been suggested that these courtship songs are indicators of male quality and may be used by females to discriminate between potential mates, but little evidence is available. We measured temporal characteristics of courtship song in the cricket *Teleogryllus oceanicus* and three different aspects of the immune response of the same males. The relations between courtship song structure and immunity appeared to be too weak to be biologically important and hence it seems unlikely that females could use only the song parameters we measured to discriminate in favour of mates with higher immunity. However, when we compared the propensity of males to sing and the structure of their courtship songs before and after they were forced to mount an immune response, we found strong effects of our immune challenge procedure. When females were played songs manipulated to sound like those produced by males with high or low encapsulation abilities (a measure of immunity), they responded more rapidly to the songs corresponding to a high immune response.

Acoustically signalling insects represent one of the most conspicuous examples of a mating system in which females choose between potential mates. The calling songs of Orthopterans, particularly crickets, have been studied extensively in relation to female choice (reviewed in Zuk & Simmons 1997; Brown 1999; Robinson & Hall 2002). However, in many species, notably grasshoppers and crickets, males produce a second type of song when in close proximity to a female (Alexander 1961). This courtship song is somewhat enigmatic: why produce a second type of signal that is energetically costly (Hack 1998), and has the potential to attract predators and parasites, at a point where the female and male have already found one another?

Courtship song in the cricket *Acheta domesticus* is twice as energetically expensive as calling song (Hack 1998) and in the one species pair that has been studied does not appear to have evolved as a species recognition signal (Fitzpatrick & Gray 2001; Gray 2005) suggesting that it is under selection driven by female choice. Courtship song might provide information to females about male quality that is not provided by calling song. Calling song may be constrained to be energetically less expensive because males need to call for long periods during the night to attract females, and females need to distinguish between conspecific males and those of other species. Courtship song is known to be important in inducing females to mate, with evidence from a number of species of crickets where females are reluctant to mount silenced males (Crankshaw 1979; Libersat et al. 1994; Balakrishnan & Pollack 1996; Nelson & Nolen 1997). However, evidence that females actually discriminate between males on the basis of courtship song remains equivocal. Boake (1984) found that males differing in courtship song did not differ predictably in their mating success in the gregarious cricket *Amphiacusta maya*. Similarly, Nelson & Nolen (1997) failed to find any aspect of courtship song that

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0003–3472/06/$30.00/0

could be used to predict male mating success in *A. domesticus*. The only published direct evidence for mate choice in relation to the structure of courtship song comes from Wagner & Reiser's (2000) finding that *Gryllus lineaticeps* females were more likely to move towards courtship song with a higher chirp rate.

The condition dependence hypothesis (Williams 1966) predicts that male secondary sexual traits should be particularly sensitive to the overall condition of the individual, and hence provide honest signals of male genetic quality (reviewed in Tomkins et al. 2004). Despite limited evidence for female preferences for particular courtship song elements, previous studies have examined the possibility that courtship song is an honest indicator of male genetic quality. One approach to this question is to manipulate factors affecting male condition such as diet during development. Wagner & Reiser (2000) found that in *G. lineaticeps* there was no difference in courtship song chirp rate between males on good and poor diets. Similarly, Gray & Eckhardt (2001) found that in *G. texensis* manipulations of larval and adult diet did not affect courtship song, and although parameters of the song itself were weakly correlated with male age, they were not related to the male's fat content. An alternative approach is to measure immune function since this represents a character that is likely to be under directional selection in all environments and hence should be closely correlated with overall condition. If courtship song is an accurate indicator of male condition we would expect correlations between song properties and immunity. In *Gryllus bimaculatus*, Rantala & Kortet (2003) found that males with higher cellular immune responses to a foreign object (encapsulation rate) differed in temporal properties of their courtship songs from males with lower encapsulation rates, and that the courtship songs of males with high encapsulation rates were preferred by females to those with low encapsulation rates. Similarly, the calling songs of *A. domesticus* and *Teleogryllus commodus* correlate weakly with immune response measured, respectively, as haemocyte load and encapsulation rate (Ryder & Siva-Jothy 2000; Simmons et al. 2005).

Related to the role of courtship song as an honest indicator of immune function is the question of whether courtship song reflects the overall phenotypic condition of an individual. This leads to the insight that if males are placed under stress this should be reflected in their condition-dependent traits, a prediction that has typically been examined by stressing animals during development and then measuring effects on sexually selected traits relative to nonsexual traits (reviewed in Cotton et al. 2004). Acoustic signals provide the opportunity to examine whether these sexually selected 'ornaments' can respond dynamically as well as developmentally to reduced phenotypic condition by acutely stressing males and looking for changes in song properties that might be detected by females.

Our aims in this study were to examine courtship song in the Australian cricket *Teleogryllus oceanicus* to discover whether females prefer certain elements of courtship song, as would be expected if song is an indicator of male quality, and to determine whether these (or any other elements) are linked to immune function.

METHODS

Study Animals

Crickets used in the study were offspring of females collected in the field in Carnarvon, Western Australia and brought into the laboratory. Hatchlings were reared to maturity and maintained at 29°C with a 12:12 h light:dark cycle in 5-litre plastic containers with food (cat chow) and water provided ad libitum. The sexes were separated before the final moult. All crickets were at least 5 days old when they were used in experiments.

Song Recordings

All recordings were made under dim red light at 25°C in an anechoic room. Recordings were made during the first 3 h after the dark portion of the light cycle on a Sony Pro-Walkman with a Sennheiser directional microphone. Crickets were housed in transparent plastic tubs (7×7 cm and 5 cm high) with a mesh lid during the recording process. A female was added to a male's container to induce him to sing; if the female mounted before a complete recording was obtained, we interrupted the pair by gently tapping the container. We attempted to obtain at least 10 complete courtship songs for each male.

Song Analysis

Songs were digitized at 22 kHz and analysed with the Raven software package (Cornell Laboratory of Ornithology, New York, U.S.A.). They were filtered to remove noise at <3.5 kHz and >6 kHz. We did not measure song amplitude because recorded volume is extremely sensitive to the orientation of the singing cricket and the distance to the microphone. We examined a set of temporal features of the song, which can be divided up into a chirp and a trill, each of which contains a number of pulses (Fig. 1). The parameters measured were: chirp length (CL), the duration of the final pulse of the chirp (chirp pulse length; CPL), the interval between the final and penultimate pulses of the chirp (chirp pulse interval; CPI), the interval between the end of the chirp and the start of the trill (song interval; SI), the duration of the first and fifth trill pulses (trill pulse length; TPL1 and TPL5) and the intervals following these pulses (trill pulse interval; TPI1 and TPI5), the duration of the first five pulses and intervals of the trill (trill pulse; TP), the total duration of the trill up to the first break at least as long as a missing pulse (trill (to) break; TB) and the duration of the trill until the next chirp (trill length; TL). All acoustic analyses were conducted by T.T., and recordings from different treatments were analysed during the same session. Because multiple measurements were made on each song, we used multivariate analyses in SPSS 11.0 (SPSS Inc., Chicago, IL, U.S.A.) to compare overall patterns of variation between songs and between treatments.