

The role of the male's cerci in copulation and mate guarding in decorated crickets (*Gryllodes sigillatus*)

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Abstract

Cerci are paired, sensory appendages extending from the terminal abdominal segment of crickets. While the cerci are acutely sensitive to air currents and thereby function in the detection of potential predators, they are also known to play a role in co-ordinating movements of males and females during copulation. The role of the male's cerci at four stages of the mating sequence (courtship, copulation, mate guarding and spermatophore removal) was examined by experimentally removing the cerci of male decorated crickets *Gryllodes sigillatus* and comparing their mating success with that of control males. The mating success of cercectomized males was significantly reduced relative to control males, primarily because of their greater inability to induce females to mount. Even when they succeeded in securing an initial mount, cercectomized males took significantly longer to transfer a spermatophore than did control males. Cercectomy had no influence on the efficacy of post-copulatory mate guarding by males, nor did females discriminate against cercectomized males by removing their spermatophores sooner than those of control males. We conclude that the primary function of the male's cerci in sexual interactions in *G. sigillatus* is to provide tactile stimuli to females that either elicit or guide their mounting response.

Key words: cerci, crickets, *Gryllodes sigillatus*, mating, mate guarding, spermatophore

INTRODUCTION

The cerci of crickets consist of a pair of slender, sensory appendages extending from the terminal abdominal segment. They are covered by a dense array of filiform sensory hairs that mediate the detection of air currents (Edwards & Palka, 1975; Dambach & Rausche, 1985; Landolfi & Jacobs, 1995), and that are also sensitive to sound and substrate-transmitted vibrations (Petrovskaya *et al.*, 1972; Dambach & Rausche, 1985). While the cerci facilitate detection and evasion of potential predators (Gnatzy & Heußlein, 1986; Gras & Hörner, 1992; Tauber & Camhi, 1995), they also play a critical role in copulation. Sensory feedback from the male's cerci facilitates the transfer of the spermatophore to the female, and ablation of the male's cerci results in a significant decrease in male mating success in several species (Loher & Rence, 1978; Sakai & Ootsubo, 1988; Snell & Killian, 2000); in contrast, ablation of the female's cerci has no effect on

mating success (Pollack, Givois & Balakrishnan, 1998; Snell & Killian, 2000).

Courtship in crickets is initiated when a male comes into antennal contact with a female. During courtship, the male exhibits rhythmic lateral movements of his body and produces a characteristic courtship song (Alexander & Otte, 1967; Loher & Rence, 1978). While courting, the male flattens his body against the substrate and makes repeated attempts to back under the female. If the female is sexually responsive, she mounts the male (Fig. 1a) at which point the male attempts to secure her subgenital plate with his epiphallus, a necessary prelude to spermatophore transfer (Sakai *et al.*, 1991). During courtship and copulation, the male's cerci make large-amplitude vibratory movements that may induce the female to mount, assist the male in securing the female's genitalia, or both (Sakai & Ootsubo, 1988; Snell & Killian, 2000). Copulation is completed with the successful transfer of the spermatophore, which normally consists of a small, sperm-containing ampulla that remains attached to the female's genital opening at the base of her ovipositor (Fig. 1b).

In addition to their role in copulation, the male's cerci may function in sexual interactions that occur after

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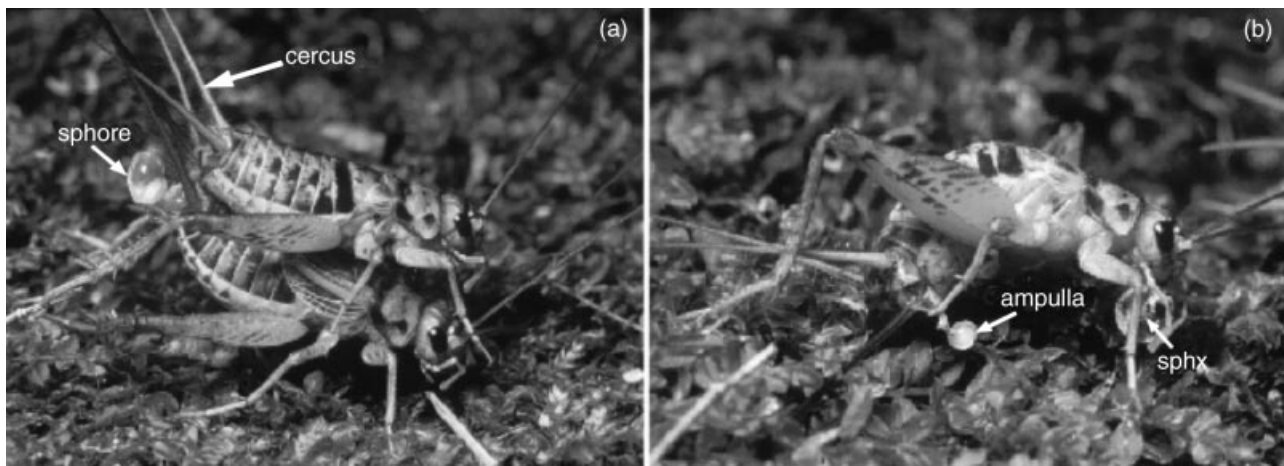


Fig. 1. (a) Copulation in *Grylloides sigillatus*; (b) recently mated female *G. sigillatus* that has just detached the spermatophylax from the sperm ampulla. Original photos by D. Funk.

copulation has been completed. In most cricket species, males guard females after mating by remaining in close proximity to them and aggressively repelling rival males (Loher & Dambach, 1989; Zuk & Simmons, 1997). In decorated crickets *Grylloides sigillatus*, males typically stand motionless next to their mates with their cerci directed towards the female during mate guarding. Sakaluk (1991) proposed that males adopt this posture to facilitate detection of any air currents generated by movements of the female, thereby ensuring continued contact with the female should she opt to move away. In support of this possibility, Landolfa & Jacobs (1995) demonstrated that cercal filiform hairs show their greatest sensitivity to air currents originating from behind a cricket. This hypothesis predicts that males lacking cerci should be less efficient at guarding females than males with intact cerci.

A final context in which a male's cerci may play a role is in post-copulatory female mating preferences. In crickets, females are well positioned to determine the fate of their mates' gametes because they can remove and consume the male's externally attached spermatophore any time after mating, and often do so before it has been completely evacuated of sperm (Sakaluk, 1984; Simmons, 1986). In *G. sigillatus*, the spermatophore includes a large gelatinous mass, the spermatophylax, which surrounds the sperm-containing ampulla and is detached and eaten by the female after mating (Fig. 1b). This nuptial gift functions to keep the female preoccupied while sperm are evacuated into her reproductive tract from the sperm ampulla (Sakaluk, 1984). However, females often discard the spermatophylax before its complete consumption, whereupon they remove the sperm ampulla and terminate sperm transfer (Sakaluk, 1984, 1987). Females can greatly influence the paternity of their offspring through their ampulla-removal behaviour, and hence this behaviour provides females with a powerful mechanism of post-copulatory female choice (Sakaluk & Eggert, 1996). If the vibratory movements

made by the male's cerci during copulation constitute one of the signals by which females assess their mates, then ampulla removal remains an obvious mechanism by which females could act on this assessment. This hypothesis predicts that not only would females be less inclined to mate with a male lacking cerci, but they might also be inclined to remove the sperm ampulla of such a male sooner than they would that of a male with intact cerci.

In this study, the role of the cerci in male *G. sigillatus* was examined at all four stages of the mating sequence: courtship, copulation, mate guarding and spermatophore removal. The cerci of males were removed and their success at each of these four stages was compared with intact males.

MATERIALS AND METHODS

Experimental individuals were descendants (F_{14} and F_{15}) of *c.* 200 adult *G. sigillatus* collected in October 1995 at Tucson, Arizona (U.S.A.). Crickets were housed communally in 75-l glass terraria or 55-l plastic storage containers, and provisioned with Fluker's[®] cricket chow, water in vials plugged with cotton wicks, and egg cartons for shelter. Eggs were collected in 0.1-l weigh boats filled with a mixture of sand and vermiculite, and moistened every other day. Nymphs were reared in plastic shoe boxes (30 × 16 × 9 cm) in an environmental chamber maintained at 28 °C on a 12:12 h light:dark cycle.

Sexually experienced males and females, 1–7 weeks past the adult moult, were removed from communal colonies and held separately for 24 h before experimental trials to ensure their sexual receptivity. Males were assigned to 1 of 2 treatments: (1) males whose cerci were experimentally removed ($n = 53$); (2) unmanipulated control males ($n = 32$). The cerci of cercectomized males were severed using fine dissecting scissors, resulting in little or no loss of haemolymph. Cercectomized

males were given 24 h to recover from their operations before mating trials.

Matings were staged in clear plastic shoeboxes and observed under a 25-W incandescent red light. Trials were initiated at ambient laboratory temperature (25–29 °C) within 4–10 h of the onset of the scotophase, the time of greatest sexual activity in *G. sigillatus* (Sakaluk, 1987; Burpee & Sakaluk, 1993). The floor of each shoebox was covered with a thin layer of sand to afford experimental individuals a degree of purchase during mating. Two inverted Petri dishes (8.5 cm diameter) were placed in the centre of each shoebox to add spatial complexity and to afford females the opportunity to elude guarding males should they choose to do so; 2 openings, each *c.* 3 cm wide, were made in the walls of each Petri dish to accommodate the entry of crickets. Mating chambers and Petri dishes were washed with detergent and rinsed between trials to eliminate any potential pheromonal cues.

At the beginning of each mating trial, a single female was placed into the mating chamber and allowed to acclimatize to her surroundings for *c.* 5 min. Following the acclimation period, a male (the focal male) from either of the 2 treatments was introduced into the chamber. If copulation occurred, a second male (the rival male) was introduced into the chamber immediately thereafter. The following were recorded: (1) the time at which the female first mounted the focal male; (2) the total number of mounts; (3) the time at which successful transfer of the spermatophore occurred. For each successful mating, we recorded: (1) the time spent feeding by the female on the spermatophylax; (2) the time after mating at which the female removed the sperm ampulla; (3) the time spent guarding by the focal male. The end of guarding was recorded when the focal male voluntarily walked away from the female or made no attempt to stay near her when she walked away, or exhibited none of the stereotypical responses that guarding males show upon contact with a rival (Sakaluk, 1991). If a mated female subsequently mounted the rival male, we also recorded the time at which she did so.

Experimental pairs were observed for a minimum of 60 min or, in successful matings, until the female had removed the sperm ampulla and the focal male had ceased guarding. Data were analysed using SAS software for personal computers (SAS Institute, 1988).

RESULTS

Of the 85 experimental pairings established, males actively courted the female in all but two trials; these two pairings, both involving cercectomized males, were excluded from further analysis. Of those males exhibiting courtship, a significantly greater proportion of control males (31/32) mated than did cercectomized males (26/51; Fisher exact test, $P < 0.0001$). To further identify the source of this difference, we employed failure time analysis to compare treatments with respect

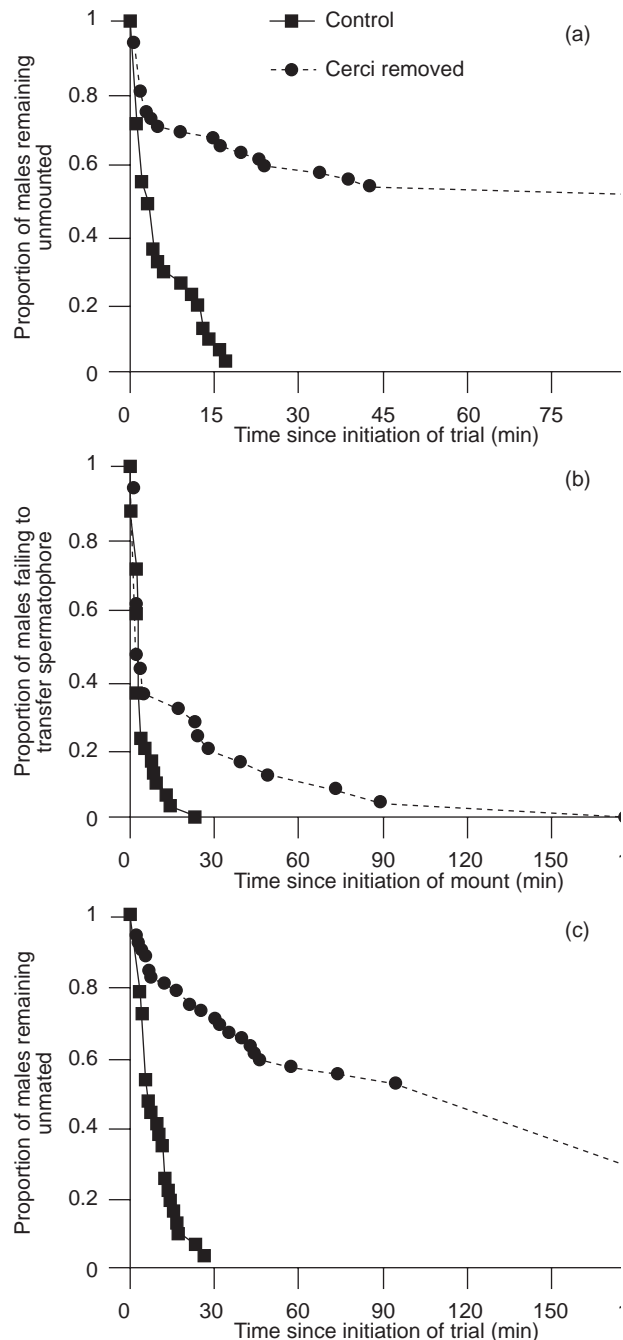


Fig. 2. Effect of male cercectomy of *Grullodes sigillatus* on: (a) ability of males to induce females to mount; (b) ability of males to transfer the spermatophore once mounted; (c) overall copulation success.

to the time taken by females to mount males (Fig. 2a), the success of males at transferring the spermatophore once mounted (Fig. 2b), and the total time taken by males to secure copulations (Fig. 2c). Failure-time analysis accommodates censored data, observations in which an event such as mating may not have occurred by the end of the study, as was the case here. Omission of such data, as is frequently done in behavioural studies, may lead to a serious bias in comparisons across treatments (Fox, 1993).

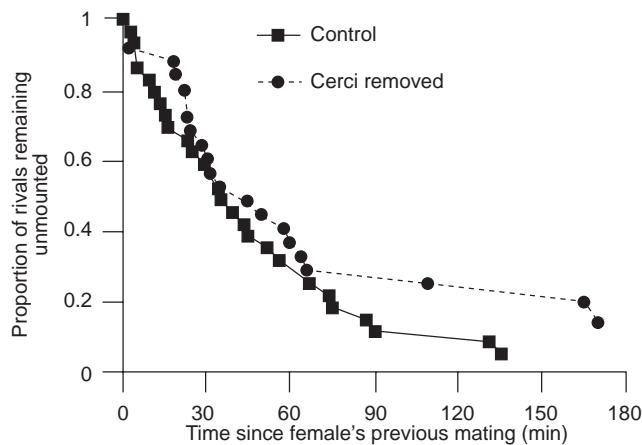


Fig. 3. Effect of cercectomy of *Gryllobates sigillatus* on the efficacy of male post-copulatory mate guarding, measured as the time since the female's initial copulation with the guarding male and her subsequent copulation with the rival male.

Females took significantly longer to mount cercectomized males than they did control males (Fig. 2a, log-rank test, $\chi^2 = 32.0$, $P < 0.0001$), and a significantly greater proportion of cercectomized males were never mounted (21/51 cercectomized males versus 1/32 control males; Fisher exact test, $P < 0.0001$). Cercectomized males, even when they succeeded in securing an initial mount, took significantly longer to mate than control males (Fig. 2b, $\chi^2 = 6.65$, $P < 0.01$), partly because the mean time between consecutive mountings per successful mating was longer for cercectomized males ($n = 12$, 13.6 ± 4.1 min) than for control males ($n = 10$, 3.6 ± 1.1 min; Mann-Whitney test $Z = -2.085$, $P = 0.037$), and also because mountings of cercectomized males were less likely to lead to spermatophore transfer. Of the 26 cercectomized males that succeeded in mating, 13 required more than one mounting by the female (50%), whereas only nine of the 31 successful control males required more than one mounting (29%). However, this difference was not statistically significant (Fisher exact test, $P = 0.19$). Overall, measured with respect to the initiation of the trial, cercectomized males took significantly longer to mate than did control males (Fig. 2c, $\chi^2 = 44.3$, $P < 0.0001$).

There was no significant difference in the mean time (\pm SE) spent guarding by control males ($n = 30$, 34.1 ± 2.8 min) and cercectomized males ($n = 26$, 39.6 ± 4.9 min; Student's t -test, $t = 1.03$, $P = 0.31$). The effectiveness of male guarding, measured as the time since the female's initial copulation with the guarding male and her subsequent copulation with the rival male, also did not differ between the two treatments (Fig. 3, $\chi^2 = 1.87$, $P = 0.17$). There was no difference in the mean time spent feeding on the spermatophylax by females mated to control males ($n = 26$, 19.2 ± 3.8 min) and those mated to cercectomized males ($n = 22$, 22.5 ± 5.7 min; $t = 0.41$, $P = 0.68$). There was also no difference between the two treatments in the mean time after mating at which the female removed the sperm ampulla

(control: $n = 30$, 31.5 ± 3.3 min; cercectomized: $n = 26$, 28.0 ± 4.6 min; $t = 0.64$, $P = 0.53$). For all mated females pooled across treatments, there was a positive correlation between the time spent feeding on the spermatophylax and the time after mating at which the ampulla was removed ($n = 48$, $r = 0.90$, $P < 0.0001$). There was also a positive correlation between the guarding duration of the male and the retention of the sperm ampulla by the female ($n = 56$, $r = 0.42$, $P = 0.0012$).

DISCUSSION

The mating success of male *G. sigillatus* was significantly reduced by the ablation of their cerci. This reduction in mating success did not seem to be the result of any apparent difference in the sexual motivation of males of the two treatments, as only two of the 53 cercectomized males failed to actively court the females and these were omitted from further analysis. Males unsuccessful at mating typically courted females throughout the trial, although we cannot rule out the possibility that there were qualitative differences in males' courtship across treatments. For example, cercectomized males may have been unable to court females as vigorously as control males owing to the stress of their operations, but if this were true, it would not explain why cercectomized males were as capable as control males of guarding their mates against intruders.

The reduced mating success of cercectomized males is attributed primarily to their greater inability to induce females to mount, as has also been reported in *Teleogryllus commodus* (Loher & Rence, 1978). The tactile stimulus that a female receives from the male's cerci during courtship seems to be critical in either eliciting or guiding her mounting response. While there was some suggestion that cercectomized males experienced greater difficulty in transferring the spermatophore once they were mounted, this trend was not statistically significant. This result is in contrast to the effect of cercal ablation on male mating success in two other cricket species, *Acheta domesticus* and *Gryllus bimaculatus*. Snell & Killian (2000) and Sakai & Ootsubo (1988) reported that even when mounted, cercectomized males experience greater difficulty in hooking their epiphallus on to the female's subgenital plate, a prerequisite to successful spermatophore transfer. However, Snell & Killian's (2000) and Sakai & Ootsubo's (1988) observations fail to take into account the active involvement of the female in these failed interactions. Our results, and those of Loher & Rence (1978), suggest that the inability of cercectomized males to complete matings has as much to do with the decision of the female to mount as it does to any physical impairment of males *per se*.

Cercal ablation had no influence on the efficacy of mate guarding. Females guarded by cercectomized males did not mount rivals any sooner than when they were guarded by control males. We conclude, therefore, that the posture assumed by the male during guarding is

not adopted to facilitate cercal detection of any air currents produced by the movement of the female, alerting him to her imminent departure. Instead, we suggest that the male faces away from his mate because by so doing, he is more likely to detect an intruding rival with his antennae. In support of this possibility, previous studies have shown that when a guarding male is removed after mating, the female is more likely to mount a courting rival (Sakaluk, 1991; Frankino & Sakaluk, 1994).

There was no evidence that cercal ablation of males influenced post-copulatory preferences of females, as females mated to cercectomized males retained their sperm ampullae after mating for as long as those females that were mated to control males. Similarly, females of the two treatments did not differ in the time they spent feeding on the male's spermatophylax. Across both treatments, there was a positive correlation in the duration of mate guarding and the female's retention of the sperm ampulla. While such a correlation has, in the past, been taken as evidence that guarding functions to deter premature removal of the ampulla (e.g., Loher & Rence, 1978; Hockham & Vahed, 1997; Bateman & MacFadyen, 1999), it is also consistent with the possibility that it is an incidental consequence of the female's decision to leave the male once she has consumed the remainder of the spermatophore (Sakaluk, 1991).

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