

The intensity of pre- and post-copulatory mate guarding in relation to spermatophore transfer in the cricket *Gryllus bimaculatus*

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Abstract While post-copulatory mate guarding has been well documented in field crickets (Orthoptera: Gryllidae), the occurrence of pre-copulatory mate guarding in this family has been largely overlooked. We examined the relationship between the intensity of two components of mate guarding (body judders and antennal whips) and the time before and after copulation. We found that when male *Gryllus bimaculatus* encounter a female but do not have a spermatophore ready to transfer, they engage in pre-copulatory mate guarding that is very similar to post-copulatory mate guarding. The intensity of pre-copulatory mate guarding increased up to the point at which the male was ready to transfer his spermatophore. Following copulation, the intensity of mate guarding initially remained high before declining, after which it began to increase again just before the male resumed courtship stridulation. We interpret this pattern of post-copulatory mate guarding as being consistent with both the ejaculate-protection and spermatophore-renewal hypotheses for the function of mate guarding. We found no significant relationship between mate guarding intensity and male body mass.

Keywords *Gryllus bimaculatus* · Pre-copulatory mate guarding · Mate guarding intensity

Introduction

Post-copulatory mate guarding has been well documented in gryllid crickets (Simmons 1990; Sakaluk 1991; Hockham

and Vahed 1997; Bateman and Macfadyen 1999; Wynn and Vahed 2004; Bussiere et al. 2006; Parker 2009). The occurrence of pre-copulatory mate guarding in gryllids, however, appears to have been largely overlooked. Pre-copulatory mate guarding behaviour has been detailed across a wide range of taxa, including insects (Arakaki et al. 2004), amphibians (Bowcock et al. 2008), spiders (Bel-Venner and Venner 2006), crustaceans (reviewed in Jormalainen 1998; Bauer and Abdalla 2001) and rotifers (Schroder 2003). Pre-copulatory mate guarding behaviour is predicted to occur in species with a high level of first male sperm precedence (Parker 1974; Thornhill and Alcock 1983) and short receptivity periods (Jormalainen 1998).

In many gryllid crickets, such as *Gryllus bimaculatus*, there is neither first nor last male sperm precedence and the receptivity period of females appears to be continual after the final moult (reviewed in Wynn and Vahed 2004). Sperm from males is mixed randomly in the female's spermatheca (Simmons 1987) to produce a 'fair raffle' pattern of paternity, as detailed by Parker (1990). Such random mixing of sperm means a male is able to gain increased paternity by guarding females after copulation by preventing the premature removal of his spermatophore (see Simmons 1986). It may, however, also be advantageous for male gryllids to guard females before mating if a female is encountered by chance and the male does not have a spermatophore ready to transfer. This could occur as a result of the male having ejected his own spermatophore in the absence of copulation—a frequent occurrence in gryllid crickets (Kumashiro et al. 2003; Reinhardt and Siva-Jothy 2005)—or if a male has recently mated with, and then subsequently been displaced from, a female (e.g. via an encounter with a rival male). Following spermatophore ejection or transfer, male *G. bimaculatus* take around an hour to produce another fully formed spermatophore (Nagao and Shimozawa 1987;

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Hall et al. 2000). This ‘sexual refractory period’ has been shown to be a fixed time interval, as the spermatophore needs time to harden in the external genitalia before it can be transferred (see Nagao and Shimozawa 1987; Hall et al. 2000; Ootsubo and Sakai 1992).

It could be argued, however, that pre-copulatory mate guarding is unlikely to occur in the field because male gryllids typically attract females by stridulating when they have a fully formed spermatophore ready to transfer (Loher 1989). Whilst this certainly is the case for a large proportion of males, several studies have shown that at least some males in other species of cricket adopt a strategy of silently intercepting females attracted by the calls of other males (satellite behaviour) (Hissmann 1990; Cade and Cade 1992; Rowell and Cade 1993; Zuk et al. 1993; Hack 1998). It appears, therefore, that it is not essential for a male cricket to call in order to locate and copulate with females.

As guarding is energetically costly (Jormalainen 1998), a male should vary the amount of energy expended on guarding in relation to the benefits that are likely to be gained as a result (Thornhill and Alcock, 1983). Changes in the energy expended on mate guarding have been detailed in various taxa, in terms of either the amount of time spent guarding females (see Parker 1974; Grafen and Ridley 1983) or the intensity of mate guarding (Simmons 1990; Latty 2006; Oku 2009). In the present study, the intensity of pre- and post-copulatory mate guarding was examined in *G. bimaculatus* males over the course of the guard period in order to test the following predictions: (1) that male *G. bimaculatus* without a spermatophore ready to transfer will guard females *prior* to copulation, and (2) that male *G. bimaculatus* will vary their pre- and post-copulatory mate guarding intensity in relation to the value of the benefits likely to be gained from guarding, with males guarding females most intensely when the value of the benefits from guarding are the highest.

Materials and methods

Gryllus bimaculatus used in this study were taken from a laboratory population at the University of Derby. The colony was maintained in semi-natural conditions (28°C, 12 h light:12 h dark cycle) in plastic aquaria (30 cm × 20 cm × 15 cm) and fed on dried rat and rabbit pellets and fresh vegetables (see Wynn and Vahed 2004).

Both the male and female *Gryllus bimaculatus* used in this study were sexually mature virgins of similar age (2–4 days after eclosion), as a number of studies have shown that female crickets prefer older males (Zuk 1988; Simmons 1995), which could have affected mate guarding behaviour. To minimise variation in female body mass, only females with a body mass of 0.68 ± 0.05 g were

used. Male body mass was measured using an electronic balance accurate to 0.01 g.

In order to determine if males were ready to transfer a spermatophore, each male was placed into a small plastic container (15 cm × 8 cm × 8 cm) with a mesh barrier which physically separated the male from a female (the female was subsequently excluded from mate trials) but still allowed visual and chemical communication. A male was considered not to have a spermatophore ready if courtship stridulation was not produced after 1 min. The male ($n = 43$) was then placed with a female into a small transparent plastic container (15 cm × 8 cm × 6 cm) and the mate guarding intensity (the number of body judders and antennal whips/rotations; see Simmons 1990) was recorded every minute until the male entered into courtship (determined by production of long courtship song whilst backing towards the female; Alexander 1961). Body judders appear to reduce the female’s movement, while antennal whips and rotations may allow the male to locate the female and monitor her movements, both of which enable the male to keep in close contact with the female (Simmons 1990; Wynn 2001). These behaviours were used as a measure of mate guarding intensity for two reasons. Firstly, out of the variety of mate guarding behaviours a male may produce (see Simmons 1990) body judders and antennal whips occur with the highest frequency. Secondly, because body judders appear to be produced when the female is active and antennal whips when she is stationary (Simmons 1990; Wynn 2001), using both these behaviours as a measure of mate guarding intensity allows a male’s mate guarding intensity to be recorded regardless of female movement (see Simmons 1990). Between each trial all containers were washed to ensure that pheromones from previous experiments did not affect results.

The intensity of post-copulatory mate guarding was recorded for 30 of the males used in the pre-copulatory mate guarding trials (above), by recording the number of judders and antennal whips every minute until the male re-entered into courtship. Again any instances of stridulation were also recorded.

All statistical analyses were carried out using SPSS version 16. All data analysed was found to be normally distributed using the Kolmogorov–Smirnov test ($P > 0.1$ in all instances).

Results

Pre-copulatory mate guarding intensity

Males were found to guard females more intensely closer to copulation (Fig. 1) [mate guarding intensity (judders and whips combined) = $1.661 \exp(0.066 \times \text{time})$, $r^2 = 0.826$,

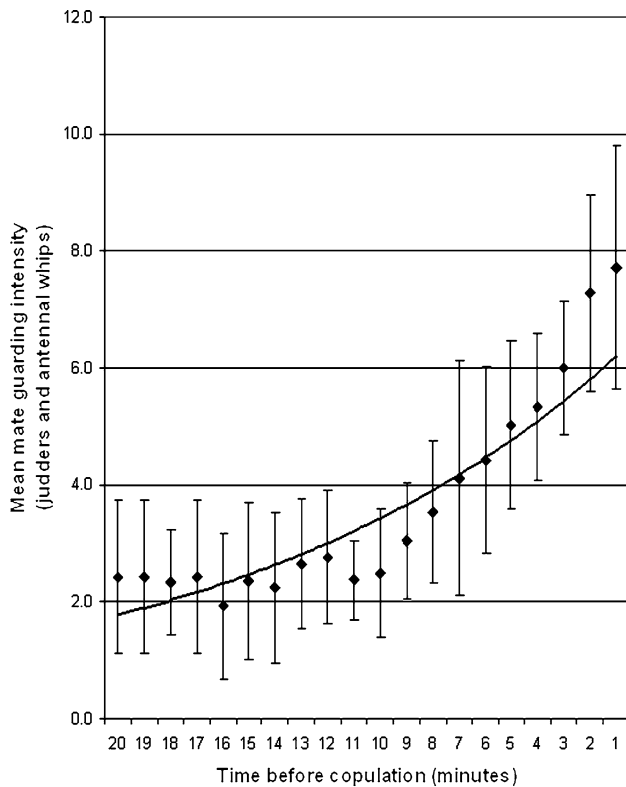


Fig. 1 Relationship between pre-copulatory mate guarding intensity (number of judders and whips combined per minute) and time before copulation (mean \pm SD)

$F = 85.259, P < 0.01$]. This relationship was also found to be statistically significant when the number of judders and antennal whips were analysed separately (number of antennal whips = $0.048 \times \text{time} + 1.448, r^2 = 0.550, F = 22.008, P < 0.01$; number of judders = $1.498 \exp(0.058 \times \text{time}), r^2 = 0.720, F = 46.375, P < 0.01$). The average pre-copulatory guard period was found to be 13.3 min, with few males guarding for over 25 min (variation in guard time was probably due to the difference in spermatophore maturation stage in the males), therefore only results for the last 20 min of guarding intensity were used for the analysis.

Post-copulatory mate guarding intensity

The refractory period (time from spermatophore transfer to initiation of courtship stridulation) for each male varied from 42 to 56 min with an average refractory period of 49 min. This variation in refractory period resulted in larger SD values for the intensity of guarding closer to the second mating (Fig. 2), as the first copulation was used as the reference point for each male. Mate guarding intensity (judders and whips combined) was found to be

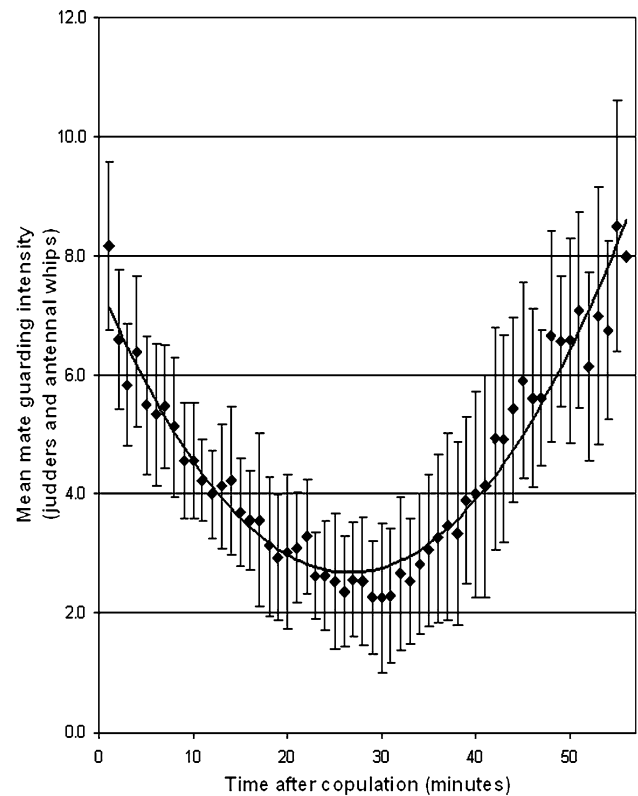


Fig. 2 Relationship between post-copulatory mate guarding intensity (number of judders and whips combined per minute) and time after copulation (mean \pm SD)

initially high after mating, declining gradually, before increasing again up to the next copulation (Fig. 2) (mate guarding intensity = $-0.36 \times \text{time} + 0.007 \times \text{time}^2 + 7.505, r^2 = 0.942, F = 427.510, P < 0.01$). When analysed separately, the number of antennal whips (number of antennal whips = $-0.063 \times \text{time} + 0.001 \times \text{time}^2 + 2.761, r^2 = 0.292, F = 10.945, P < 0.01$) and the number of judders (number of judders = $-0.244 \times \text{time} + 0.005 \times \text{time}^2 + 5.040, r^2 = 0.886, F = 205.449, P < 0.01$) followed a similar pattern.

No significant relationship was found between male body mass and pre-copulatory mate guarding intensity (judders and whips combined) in the last 2 min prior to copulation ($r = -0.275, df = 41, P = 0.074$) nor between male body mass and post-copulatory mate guarding intensity (judders and whips combined) in either the first 5 min following copulation ($r = -0.025, df = 28, P = 0.89$) or the last 5 min of the guarding period ($r = 0.096, df = 28, P = 0.629$) (the points analysed were selected because they were the points at which mate guarding intensity was at its highest and thus the points most likely to show any effects that mass may have had on mate guarding intensity).

Discussion

Male *G. bimaculatus* without a spermatophore ready to transfer were found to guard females prior to copulation. This appears to be the first time such pre-copulatory mate guarding behaviour has been detailed in this species, or indeed in a gryllid cricket. Pre-copulatory mate guarding intensity was found to increase as the guarding period progressed, being most intense just before the male entered into courtship. The increase in mate guarding intensity closer to copulation could be a result of the change in the value of the benefits a male gains from guarding as the guard period shortens. The value of guarding is likely to increase nearer to copulation as the guard time required is shorter and thereby less costly (Parker 1974) and there is also less chance of takeover from a rival male (Grafen and Ridley 1983). Mate guarding intensity has been shown to change in relation to the benefits of guarding in other arthropods (Alcock 1992; Oku 2009).

Post-copulatory mate guarding intensity was found to be initially high following copulation, before declining and then increasing to be high just before the male re-entered courtship. We propose that this pattern may be explained by the interaction between two of the three hypotheses for the function of post-copulatory mate guarding in gryllid crickets: ejaculate protection and spermatophore renewal (Sakaluk 1991; Bateman and MacFadyen 1999). The relative importance of each of these strategies is likely to alter over the guarding period as the benefits of each strategy change in value. Benefits gained from protection of the ejaculate decrease the longer the spermatophore is attached, with the male gaining the greatest rate of increased fertilisation in the first 10 min of spermatophore attachment (Simmons 1987). Furthermore, attachment times of less than 10 min result in the transfer of sperm loads that suffer considerable long-term dilution consequences (Simmons 1987). In contrast, the benefits gained from guarding the female in order to transfer a new spermatophore increase as the guarding period advances due to the reduced guarding costs and reduced chance of takeover from rival male as with pre-copulatory mate guarding (above). Thus, as efforts of the latter half of the post-copulatory mate guarding period appear to be directed towards the next mating (spermatophore renewal), the latter part of the post-copulatory mate guarding period could be interpreted as pre-copulatory mate guarding, analogous to that already detailed above. This is in contrast to the first half of the mate guarding period following mating, which appears to increase the male's paternity in the current mating (ejaculate protection). In reality, however, the guarding period following mating is unlikely to be able to be split into two halves representing post- and pre-copulatory mate guarding, respectively. This is because

benefits from each of the strategies (ejaculate protection and spermatophore renewal) are likely to be gained across the *whole* guard period. For example, although the benefits of protecting the ejaculate are much reduced in the latter half of the guarding period, benefits are still gained, as sperm transfer continues for approximately the whole guard period (around an hour) (Simmons 1987; Nagao and Shimozawa 1987; Hall et al. 2000).

The 'interaction' model described above helps to explain some conflicting results within the literature. For example, the ejaculate protection hypothesis was rejected in *G. bimaculatus* by Wynn and Vahed (2004) as the spermatophore attachment time was actually shorter when the male was allowed to guard than when removed. This reduction in attachment time, however, resulted from either the male dislodging his own spermatophore in subsequent mating attempts or from the female removing it to prepare for the next mating (also observed in this study). This may seem counter-productive, however the present study suggests that the value of the benefits from spermatophore renewal and ejaculate protection are likely to shift as the guard period advances (see above). Thus, the benefits to the male of attaching a new spermatophore (spermatophore renewal) are likely to be higher than the benefits of protecting the previous spermatophore (ejaculate protection) after a certain period, meaning a male may be selected to remove his previous spermatophore when he is able to remate.

The third non-mutually exclusive hypothesis for the function of mate guarding, rival exclusion, whereby males attempt to prevent or delay rival males from copulating with the female (Sakaluk 1991), is also expected to be affected as the benefits of guarding the female change. This is because the intensity with which males fight has been shown to change in response to the perceived value of a resource (Elwood et al. 1998). It has also been shown that male aggression is higher in male crickets following chemo-tactile interaction with a female, as this causes the perceived value of winning the fight to increase (Wynn 2001; Killian and Allen 2008). As the present study has suggested that the value of guarding a female changes over time, it is predicted that the degree of aggression that a guarding male exhibits towards a rival will also change over the guarding period in parallel with the changes in mate guarding intensity documented here.

Female movement was not measured in this study, but could have either affected mate guarding intensity, or have been influenced by mate guarding intensity. For example, it is conceivable that females could have used mate guarding intensity as a measure of an aspect of male quality such as male size or vigour (see Simmons 1990, 1995; Eberhard 1996; Bateman et al. 2001) and could have attempted to escape from unfavoured males. In the present study, however, we found no evidence to suggest that mate guarding intensity

might be used by females to assess male fitness in terms of body mass as no significant relationship between male body mass and mate guarding intensity was found. This result might be due to the use of virgin females in this study as female *G. bimaculatus* have been shown to exhibit reduced mate choice on their first copulation as insurance against a lack of further mating opportunities (Wynn and Vahed, 2004).

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