Report

Guarding Males Protect Females from Predation in a Wild Insect

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Summary

Males frequently remain in close proximity to their mate immediately postcopulation. This behavior has generally been interpreted as a guarding tactic designed to reduce the likelihood that a rival male can rapidly displace the ejaculate of the guarding male [1, 2]. Such attempts by males to control their mates represent a potential source of conflict [3–5], but guarding behaviors in species where it is difficult for males to control their mates suggest that conflict is not inevitable [6, 7]. We employed a network of infrared video cameras to study a wild population of individually marked and genotyped field crickets (*Gryllus campestris*). Lone females or males suffer similar rates of predation, but when a pair is attacked, the male allows the female priority access to their burrow, and in doing so dramatically increases his probability of being killed. In compensation for this increased predation risk, paired males mate more frequently and father more of the female's offspring. By staying with a male, females increase the sperm contribution of preferred males as well as reducing their predation risk. In contrast to conclusions based on previous lab studies, our field study suggests that mate guarding can evolve in a context of cooperation rather than conflict between the sexes.

Results

We used a natural population of crickets to investigate whether associations between males and females after they mate are characterized by conflict or cooperation. Continuous video surveillance of an entire population of individually marked adults allowed us to observe behaviors and predation. Reproductive output was estimated using molecular markers. *Gryllus campestris* are polygamous and live in and around burrows that they use to escape from predators. Although each individual frequently moves between burrows, both sexes are territorial and, when using a burrow, defend it against conspecifics. Burrow sharing only occurs between individuals of opposite sex (see Supplemental Experimental Procedures available online).

Female presence had a strong effect on how males used the space in and around their burrows. Moreover, males stayed for longer with females that mated with them and were more successful in fighting against rival males when sharing a burrow than when on their own. When occupying a burrow on their own, individuals of both sexes used the space around the burrow entrance in a similar manner (median distance to the burrow entrance over a 2 hr period for females on their own versus males on their own, 1.07 screen units [SU] versus 2.05 SU) (distributions are right skewed; Mann-Whitney U test, U = 74, n = 27, p = 0.435). However, when sharing a burrow, males tended to be further from the burrow entrance than when they were on their own (median distance for solitary males versus paired males, 2.05 SU versus 18.90 SU; sign test of differences between lone and paired males, Z = 2.58, n = 15, p = 0.01). This greater distance from the burrow allowed their partners to keep using the area in the immediate vicinity of the entrance in the same manner as when they were on their own (median distance for females on their own versus paired females, 1.07 SU versus 3.66 SU; sign test, Z = 0.60, p = 0.55, n = 11; see Figure S1).

Whether the female mated to the male or not had a significant effect on the time that the pair stayed together: when sharing a burrow with a female that did not mate with them, males stayed long enough to allow a mating to occur, i.e., as long as it took them to get their first mating with a female that did mate (median stay of males when nonmated versus time it took them to mate; 0.52 hr versus 0.07 hr; sign test of differences when the same males were in each of these situations, Z = 0.38, p = 0.70, n = 27). However, they left much earlier than when they were successful in getting at least one mating (median stay of mated males, 6.29 hr; sign test of differences, Z = 3.59, p < 0.001, n = 27; Figure 1). Furthermore, males left the burrow before the female more often when they got no matings (median proportion of times male left the burrow before female when not mated versus mated, 0.37 versus 0.25; Wilcoxon matched-pairs test, Z = 3.94, n = 75, p < 0.001).

There was no difference in how often resident males fought with intruders or retreated from their burrow relative to whether a female was present or not (contingency chi-square test, $\chi^2_1 = 0.20$, n = 364, p = 0.65; paired males fought 60 of 71 times [84%]; unpaired males fought 241 of 293 times [82%]). However, for resident males, individual success in winning fights was higher (albeit marginally nonsignificantly, so with a very weak sign test of differences) when they were paired (64%) than when they were on their own (43%) (sign test, Z = 1.92, n = 22, p = 0.055). Overall, there was a significant association between being paired and winning fights (contingency chi-square test, $\chi^2_1 = 6.69$, n = 301, p = 0.010; paired males won 35 of 60 fights [58%]; unpaired males won 96 of 241 fights [40%]). After a fight, 56% of females (n = 69) mated to the winner, and in the vast majority of cases this mating took place very soon afterwards (median time between fighting and mating 6 min; third quartile 49 min; range 11 hr).

Sexual Conflict or Mutual Benefit?

In the thousands of hours of video that we watched, there was no sign of females being coerced into remaining with a male. Males did not show any signs of hindering female movements to or from the burrow (see typical behavior in Movie S1) and were never aggressive toward their mates (in contrast to their aggressive interactions with other males). We found evidence for mutual benefits derived from mate guarding, with males experiencing higher predation when paired but gaining

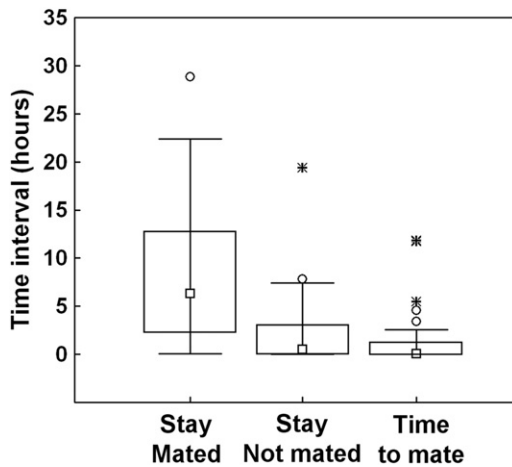*Correspondence: r.rodriguez-munoz@exeter.ac.uk (R.R.-M.), t.tregenza@exeter.ac.uk (T.T.)

Figure 1. Time Spent by Males with a Female in Relation to Mating Status

Time interval between male arrival at a burrow occupied by a female and departure when mated, time interval to departure when not mated, or time to first mating (indicating that unmated males had time to mate). Squares represent median; boxes, interquartile range; whiskers, nonoutlier range (values within one interquartile range outside the closest quartile); circles, outliers; stars, extremes (n = 27).
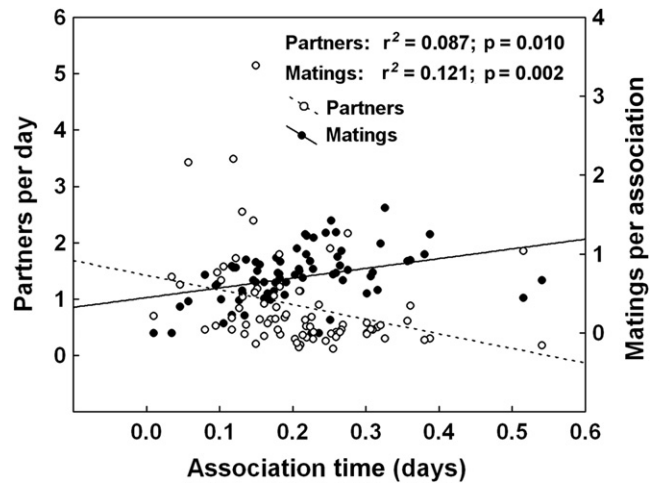

Figure 2. Effects of Male-Female Association Time on Mating Frequency and Number of Mates

Relationship between the duration of male-female associations, the number of matings per association, and the number of different partners encountered per day (n = 75).

increasing paternity over the progeny of guarded females, who in turn experienced lower predation rates.

The mean time a male spent guarding his mates had a positive relationship with the number of matings he engaged in during that time (b = 1.24, r^2 = 0.121, p = 0.002, n = 75; Figure 2) and a negative relationship with the number of different mates he encountered per day (b = −2.60, r^2 = 0.087, p = 0.010, n = 75; Figure 2). However, there was no significant correlation between time spent guarding and the number of females that the male mated with per day (r = 0.17, p = 0.143, n = 75). We found a positive correlation between the share of matings that a male achieved with his mates (relative to his competitors) and his share of their offspring (Spearman correlation, r = 0.526, p = 0.004, n = 28).

Paired males were always vigilant, responding rapidly to approaching conspecifics and apparently monitoring female movements, but we never observed any behavior that could be interpreted as males trying to force females to remain at their burrow. Within pairs, males stayed further from the burrow than females (median distance for males versus females, 18.33 SU versus 2.75 SU; Sign test of differences, Z = 3.39, p < 0.001, n = 17; see Figure S2) and were nearly always outside when at least one of the crickets was inside the burrow, or closer to the surface when both of them were inside the burrow (19 of 20 times; chi-square test, χ^2_1 = 17.19, p < 0.001). However, we found no indication that males attempted to limit female movements; instead, they always granted unimpeded passage to the female moving in and out of or around the burrow (see Movie S1 showing typical male behavior). For females, there was no significant correlation between the duration of each association and the number of different males encountered per day, regardless of whether they mated to them (paired time as a function of total mates encountered per day, r^2 = 0.028, p = 0.095, n = 100; paired time as a function of mates encountered per day when at least one mating occurred, r^2 = 0.025, p = 0.114, n = 100).

The total amount of time that we observed single crickets occupying burrows was six times greater than that of cricket pairs. However, predators attacked burrows occupied by pairs over four times more often than they attacked lone crickets (attacks on burrows with single crickets, 38 of 2138; attacks on burrows with pairs, 20 of 420; contingency chi-square test, χ^2_1 = 13.97, n = 2548, p < 0.001; Figure 3A). Attacks on pairs did not improve predator efficiency, though; the proportion of successful attacks was similar for pairs and single crickets (successful attacks on pairs, 8 of 22; successful attacks on single crickets, 20 of 48; contingency chi-square test, χ^2_1 = 0.18, n = 70, p = 0.674). Males and females on their own did not differ in how often they were attacked by predators (attacks on burrows with isolated males, 16 of 935; attacks on burrows with isolated females, 22 of 1,192; χ^2_1 = 0.05, n = 2,138, p < 0.816) or in the success of the attacks (contingency chi-square test, χ^2_1 = 1.28, n = 48, p = 0.28; Figure 3B). However, there was a highly significant difference between male and female survival in successful attacks on shared burrows. Whereas only one female was predated (both partners were caught at once by a magpie during mating), males were always killed in successful attacks when paired (contingency chi-square test, χ^2_1 = 17.09, n = 22, p < 0.001). Overall, paired males had a 3.9 times greater chance of being predated when paired than when on their own, whereas for females that probability was 5.6 times smaller. This was not because paired males sing more and are hence more likely to be detected by predators (mainly birds; see Figure S3); only 4 of 22 paired males sang within the 10 min prior to a predator attack, and 5 of 22 within the previous hour.

Discussion

We found that *G. campestris* males form postinsemination associations with their mates in the wild, and that this guarding behavior allows them to increase their contribution to their mate's total matings and so increases paternity at a cost of increased predation risk. Females benefit from associating with males through experiencing reduced predation and potentially by increasing the sperm contribution of preferred males.
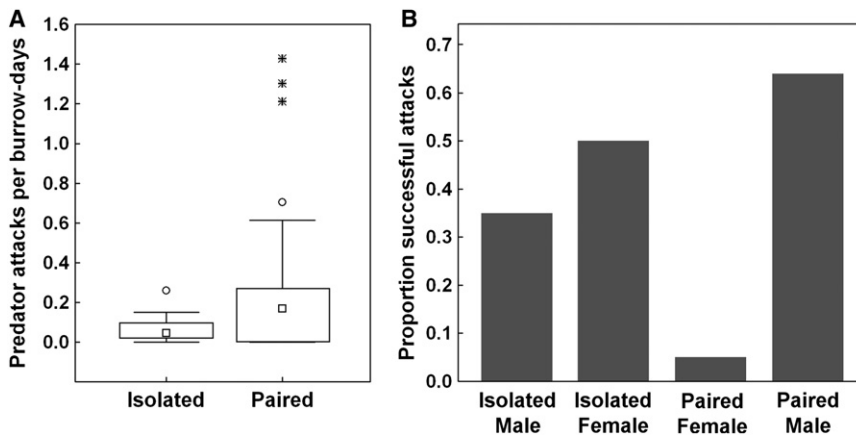
Figure 3. Predator Attacks in Relation to Burrow Occupancy

(A) Predator attacks on single and paired crickets (n = 50). Squares represent median; boxes, interquartile range; whiskers, nonoutlier range (values within one interquartile range outside the closest quartile); circles, outliers; stars, extremes.
(B) Proportion of successful attacks in relation to burrow sharing status and sex (n = 50).

[6, 13]. The differences between our observations of wild crickets and these previous lab studies raises the question of whether lab situations may produce anomalous behaviors and at the least suggests that observations of wild insects may lead to differing conclusions.

The potential for a female to have previously mated or to subsequently mate with another male creates a conflict of interests between partners [4]. In many species, it is possible for males to guard females, limiting their opportunity to mate multiply and reducing paternity losses to rival males [1, 8]. This has driven the evolution of a potentially dynamic equilibrium between benefits and costs to males of guarding and benefits and costs to females of resisting coercion, which will lead to loss of guarding altogether when females can resist efficiently. In species where coercion is not possible or the costs exceed the benefits, guarding behavior should not be expected. An alternative scenario to this intersexual tension is one where females obtain some benefit from associating with their partners, in which case they may promote male guarding behavior in a context of sexual cooperation rather than conflict. It has been suggested that females of some species can retain a male by soliciting repeated copulations and so increase the male's contribution to parental care [9]. A decrease in predation risk has also been reported as a consequence of mate guarding in the amphipod *Hyalella azteca*, but with no indication of female cooperation [10, 11], and in the white-tailed ptarmigan, *Lagopus leucurus*, male vigilance behavior seems to increase foraging efficiency of females before the onset of incubation [12].

We suggest that mate guarding can evolve in two different ways, depending on a species' life history. It can evolve through sexual conflict, driven solely by male benefits, if males can enforce female monandry through a predictable and/or short duration of the fertile period and the possibility of preventing female remating during that period. When enforcement is not possible or is too costly, for instance when there is a long fertile period or one that is under female control, guarding can evolve providing it also carries benefits for the female. In these situations, females can drive the evolution of cooperative mate guarding in a context of mutual benefit.

We have demonstrated that male mate guarding provides mutual benefits for both sexes in a natural insect population, with no overt sexual conflict. Field crickets have frequently been used as a model for the study of mate guarding [13–16], with numerous reports of males guarding their mates after copulation, preventing them from removing the male's spermatophore and/or mating with rival males [15, 17, 18]. However, with the exception of Simmons' study [6], which was carried out indoors but used enclosures of up to 3 m², previous studies have been conducted in small boxes, which greatly limits movement and may prevent females from escaping postmating

We show that when a female is present, *G. campestris* males change the way that they use the territory around a burrow. Moreover, after mating, they stay for longer with their mate and are more successful in fighting potential takeovers. Males that remain for longer get more matings, and this increases their contribution to female sperm stores and increases paternity over the female's progeny, a fundamental prediction for the evolution of mate guarding that is rarely demonstrated in the wild. Additionally, it is possible that by protecting females from predation, males gain indirect benefits if the female subsequently lays eggs fertilized by his sperm, so the male behavior may function as a mate protection strategy as well as a guarding strategy. However, although living in burrows would allow males to block the entrance and so force females to stay with them, males do not seem to limit the movements of their mates at all. Instead, they simply remain in the vicinity and fight any approaching male. Females have the choice of remaining with a male or moving away in search of a new partner. Females gain two potential benefits from remaining with a male: first, they can use it to bias paternity in favor of that male by mating repeatedly with him, and second, they reduce their chances of being predated. Both of these have been shown to increase fitness in female crickets [19–22].

The economics of mate guarding depend not only on the benefits of the association but also on the balance between these and the associated costs [1]. Time spent with a mate may limit opportunities to acquire additional mates [8, 23], and increased predation risk can reduce life span [10, 24]. Although we found a significant negative relationship between the time a male and female spent together and the rate at which males encountered other potential mates, this did not translate into a negative relationship between guarding time and number of mates in a given day, for either sex. The opportunity to find a new mate varies with density and sex ratio [25], so in years where either factor changes, the relationship between paired time and the number of mates encountered per day might change, and this might shift the duration of male-female associations.

We found a substantial increase in predation risk for males when they were paired as compared to when they were alone, showing that there is a compromise between benefits and costs of guarding. In contrast, females showed no evidence of paying any cost from being guarded. They were never forced to stay with a male, and we found no relationship between how long a female was with their partner and how many partners that female mated to per day.

Our study shows that mate guarding can involve cooperation between the sexes and that field-based studies are an important counterpoint to laboratory experiments. The understanding of mate guarding in general may benefit from an approach that accounts for the benefits and costs to both sexes and gives full consideration to both conflict and cooperation scenarios.

Supplemental Information

Acknowledgments

References

1. Alcock, J. (1994). Postinsemination associations between males and females in insects: the mate-guarding hypothesis. Annu. Rev. Entomol. *39*, 1–21.
2. Birkhead, T. (1987). Sperm competition in birds. Trends Ecol. Evol. (Amst.) *2*, 268–272.
3. Tregenza, T., Wedell, N., and Chapman, T. (2006). Introduction. Sexual conflict: a new paradigm? Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 229–234.
4. Arnqvist, G., and Rowe, L. (2005). Sexual Conflict (Princeton, NJ: Princeton University Press).
5. Parker, G.A. (1979). Sexual selection and sexual conflict. In Sexual Selection and Reproductive Competition in Insects, M.S. Blum and A.N. Blum, eds. (London: Academic Press), pp. 123–166.
6. Simmons, L.W. (1986). Female choice in the field cricket *Gryllus bimaculatus* (De Geer). Anim. Behav. *34*, 1463–1470.
7. Simmons, L.W. (1991). On the post-copulatory guarding behaviour of male field crickets. Anim. Behav. *42*, 504–505.
8. Parker, G.A. (1974). Courtship persistence and female-guarding as male time investment strategies. Behaviour *48*, 157–184.
9. Hunter, F.M., Petrie, M., Otronen, M., Birkhead, T., and Pape Møller, A. (1993). Why do females copulate repeatedly with one male? Trends Ecol. Evol. (Amst.) *8*, 21–26.
10. Cothran, R.D. (2004). Precopulatory mate guarding affects predation risk in two freshwater amphipod species. Anim. Behav. *68*, 1133–1138.
11. Cothran, R.D. (2008). Direct and indirect fitness consequences of female choice in a crustacean. Evolution *62*, 1666–1675.
12. Artiss, T., and Martin, K. (1995). Male vigilance in white-tailed ptarmigan, *Lagopus leucurus*: mate guarding or predator detection? Anim. Behav. *49*, 1249–1258.
13. Simmons, L.W. (1990). Postcopulatory guarding, female choice and the levels of gregarine infections in the field cricket, *Gryllus bimaculatus*. Behav. Ecol. Sociobiol. *26*, 403–407.
14. Frankino, W.A., and Sakaluk, S.K. (1994). Post-copulatory mate guarding delays promiscuous mating by female decorated crickets. Anim. Behav. *48*, 1479–1481.
15. Bateman, P.W., and MacFadyen, D.N. (1999). Mate guarding in the cricket *Gryllodes sigillatus*: Influence of multiple potential partners. Ethology *105*, 949–957.
16. Bussière, L.F., Hunt, J., Jennions, M.D., and Brooks, R. (2006). Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. Evolution *60*, 792–800.
17. Hockham, L., and Vahed, K. (1997). The function of mate guarding in a field cricket (Orthoptera: Gryllidae; *Teleogryllus natalensis* Otte and cade). J. Insect Behav. *10*, 247–256.
18. Parker, D.J., and Vahed, K. (2010). The intensity of pre- and post-copulatory mate guarding in relation to spermatophore transfer in the cricket *Gryllus bimaculatus*. J. Ethol. *28*, 245–249.
19. Rodríguez-Muñoz, R., Bretman, A., Slate, J., Walling, C.A., and Tregenza, T. (2010). Natural and sexual selection in a wild insect population. Science *328*, 1269–1272.
20. Wagner, W.E., Jr., Kelley, R.J., Tucker, K.R., and Harper, C.J. (2001). Females receive a life-span benefit from male ejaculates in a field cricket. Evolution *55*, 994–1001.
21. Wagner, W.E.J., Jr., and Harper, C.J. (2003). Female life span and fertility are increased by the ejaculates of preferred males. Evolution *57*, 2054–2066.
22. Simmons, L.W. (1987). Female choice contributes to offspring fitness in the field cricket, *Gryllus bimaculatus* (De Geer). Behav. Ecol. Sociobiol. *21*, 313–321.
23. Hasselquist, D., and Bensch, S. (1991). Trade-off between mate guarding and mate attraction in the polygynous great reed warbler. Behav. Ecol. Sociobiol. *28*, 187–193.
24. Cooper, W., and Vitt, L. (2002). Increased predation risk while mate guarding as a cost of reproduction for male broad-headed skinks (*Eumeces laticeps*). Acta Ethol. *5*, 19–23.
25. Carroll, S.P. (1993). Divergence in male mating tactics between two populations of the soapberry bug: I. Guarding versus nonguarding. Behav. Ecol. *4*, 156–164.