

Females use self-referent cues to avoid mating with previous mates

Tracie M. Ivy*, Carie B. Weddle and Scott K. Sakaluk

Behavior, Ecology, Evolution and Systematics Section, Department of Biological Sciences, Illinois State University, Normal, IL 61790-4120, USA

Females of many species mate repeatedly throughout their lives, often with many different males (polyandry). Females can secure genetic benefits by maximizing their diversity of mating partners, and might be expected, therefore, to forego matings with previous partners in favour of novel males. Indeed, a female preference for novel mating partners has been shown in several taxa, but the mechanism by which females distinguish between novel males and previous mates remains unknown. We show that female crickets (*Grylodes sigillatus*) mark males with their own unique chemical signatures during mating, enabling females to recognize prior mates in subsequent encounters and to avoid remating with them. Because self-referent chemosensory cues provide females with a simple, but reliable mechanism of identifying individuals with whom they have mated without requiring any special cognitive ability, they may be a widespread means by which females across a broad range of animal mating systems maximize the genetic benefits of polyandry.

Keywords: cuticular hydrocarbons; genetic benefits; *Grylodes sigillatus*; mate choice; polyandry; self-referent matching

1. INTRODUCTION

Females frequently mate more often than is necessary to ensure complete fertilization, often at the cost of the increased risk of injury, disease or predation (Daly 1978; Hurst *et al.* 1995; Blanckenhorn *et al.* 2002). Notwithstanding these costs, multiple mating may allow females to maximize the amount of material resources they obtain from males (e.g. food), reduce the level of sexual harassment from males, or replenish their sperm supplies (Arnqvist & Nilsson 2000). In addition to these direct benefits, multiply-mating females may also derive indirect genetic benefits by mating with genetically superior sires (Jennions & Petrie 2000) or by reducing the risk of fertilizing their eggs with the sperm of genetically incompatible mates (Tregenza & Wedell 2000; Zeh & Zeh 2003). Only by mating with different partners, however, can females obtain diverse ejaculates to ensure insemination with viable sperm or to maximize genetic benefits. Hence, females should discriminate against previous mates in favour of seeking matings with novel partners. Indeed, empirical studies have shown that female pseudoscorpions (*Cordylochernes scorpioides*; Zeh *et al.* 1998), field crickets (*Gryllus bimaculatus*; Bateman 1998), hide beetles (*Dermestes maculatus*; Archer & Elgar 1999), and guppies (*Poecilia reticulata*; Eakley & Houde 2004) preferentially mate with novel males rather than with males with whom they have recently mated. However, the mechanism by which females distinguish between novel males and previous mates remains unknown.

Here, we report the results of two studies designed to determine the scope and proximate underpinnings of a female preference for novel partners in the decorated cricket, *Grylodes sigillatus*. Female *G. sigillatus* often mate more than once per evening in the field, but rarely with the

same male twice (Sakaluk *et al.* 2002). Multiple mating in *G. sigillatus* provides significant fitness benefits to females, but these benefits only become evident when females mate polyandrously (Sakaluk *et al.* 2002; Ivy & Sakaluk 2005). Thus, to increase the potential for genetic benefits, females should reject prior mates and seek novel mating partners.

We first confirmed the female preference for novel males that has been documented in other crickets (Bateman 1998) by conducting mating trials involving first-generation offspring of crickets collected in the field. We then tested the possibility that female *G. sigillatus* rely on chemical cues to recognize previous mates. Although females may use a variety of cues to recognize previous mates, chemical cues seem especially likely given their ubiquity as mediators of species and sex recognition in numerous insect species (Bell & Cardé 1984), including crickets (Otte & Cade 1976). Female crickets utilize pheromones to recognize close kin (Simmons 1990), and Tregenza & Wedell (1997) identified a number of sex-specific differences in the relative concentration of cuticular hydrocarbon constituents that mediate sex recognition.

We created a number of inbred lines within which individuals presumably share any chemical cues used in individual recognition to test two non-mutually exclusive hypotheses. In one set of mating trials, we allowed each female to choose between a novel male and one that had mated previously with her inbred sister to determine whether males are imbued with the unique odours of females during mating, and whether females can recognize their own chemical signatures in any subsequent encounters with previous mates (self-referent cues). In a second set of trials, we allowed females to choose between a novel male and the inbred sibling of a male with whom she had previously mated to test the possibility that females learn,

* Author for correspondence (tmivy@ilstu.edu).

and later recognize, the unique chemical signature of previous mates (male-specific cues). It is important to note that in both experiments the female involved in the choice trial was naive to both males. However, if females use self-referent cues to recognize previous mates, then a female should recognize the mate of an inbred sister as her own previous mate and discriminate against him. Similarly, if females use male-specific cues to recognize previous mating partners, then a female should discriminate against the inbred brother of a previous mate as she would a previous mate.

2. MATERIAL AND METHODS

(a) *Novel male mate choice*

Experimental *G. sigillatus* were F₁ offspring of approximately 500 individuals collected in Las Cruces, New Mexico in May 2001. All crickets were housed in an environmental chamber at 32 °C on a 16 : 8 light : dark cycle and provisioned with Flukers cricket chow, water supplied in 40 ml plastic tissue culture flasks plugged with cotton dental rolls, and egg cartons for shelter. Moistened peat moss was provided as an oviposition substrate.

Newly-eclosed male crickets were housed individually in 0.47 l containers and given a female with which to mate for a period of 7 days. Twenty-four hours prior to mating trials, females were removed from the containers to afford males the opportunity to replenish spermatophores. Males were then randomly designated as either 'familiar' or 'novel' males.

Newly-eclosed females of the same age were housed together in plastic cages. Previous work on this species has indicated that virgin females are more eager to mate than non-virgin females (unpublished data), so the experiment was replicated with both virgin and multiply-mated females. Multiply-mated females were provided with sexually experienced males in a 2 : 1 (male : female) ratio for a period of 6 days to ensure ample mating experience. On day 7, males were removed from the boxes to allow females time to regain their sexual receptivity.

Experimental females were first paired with a randomly chosen familiar male in Plexiglas viewing chambers (10.5 × 7.5 × 3 cm) lined with paper towel and viewed under red-light illumination. To ensure that novel males and familiar males experienced comparable resource depletion as a consequence of mating, the novel male was presented with a non-experimental female twenty-four hours before the choice trial (i.e. the same time at which the familiar male initially mated) and observed until mating occurred. After both males had completed their initial mating, we marked one of them with a spot of correction fluid to facilitate their identification the following day. In half of the cases, we marked the familiar male, and in the other half, we marked the novel male.

On the day after her initial mating, each experimental female was simultaneously presented with the familiar male and a novel male and given an hour in which to mate. Matings were staged as described above, except we used larger arenas to offer females additional room to elude males (30.5 × 16.5 × 8.5 cm plastic cages lined with paper towel). Because females will not mate with males that do not produce courtship song (Adamo & Hoy 1994), we included for analysis only those trials in which both males actively courted the female during the trial. At the time of a successful spermatophore transfer, we recorded the identity of the male with whom the female mated.

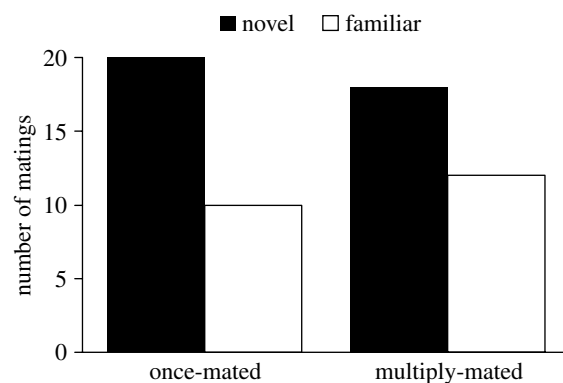


Figure 1. The number of once-mated and multiply-mated outbred females choosing familiar mating partners and novel males.

(b) *Chemical cues in mate recognition*

Six genetic lines were developed by subjecting individuals, randomly selected from the large, panmictic population described above, to three generations of full-sib mating (coefficient of inbreeding, $F=0.5$). All crickets were maintained under the conditions described above. In each of two experiments, we used seven females from each line as focal individuals. In the first set of trials, inbred sisters of females who had mated the previous day were offered a choice between the same male with which her sister had mated and a novel male. In the second set of trials, we allowed females to choose between a novel male and an inbred male sibling of the male with which she had mated the previous day. Matings were staged in the same manner as above.

We evenly assigned the lines from which the novel and familiar male originated, so that females from the same line never had the same combination of males from which to choose. We also ensured that males from each line were assigned as both novel and familiar males in all possible combinations. To avoid any confounding effects of inbreeding avoidance, novel and familiar males used in mating trials never came from the same line as the female. We standardized mating experience between all individuals, including females in the first set of trials, in a manner similar to the above experiment. When mates were provided to standardize mating experience, the allocated mates were never from the same lines as the males involved in the choice trials.

3. RESULTS

(a) *Novel male mate choice*

Outbred female *G. sigillatus* showed a significant preference for novel males over previous mates (38/60, χ^2 test for equal proportions: $\chi^2=4.27$, $p=0.04$; figure 1). The preference for novel males was a bit weaker in multiply-mated females than in once-mated females, but not significantly so (once-mated females 20/30, multiply-mated females 18/30; likelihood ratio χ^2 : $\chi^2=0.29$, $p=0.59$; figure 1). The decreased mating success of the familiar male cannot be attributed to any difference in the sexual motivation of the males because both the familiar and novel male had mated 24 h earlier.

(b) *Chemical cues in recognition*

Inbred females mated significantly more often with novel males than with males that had previously mated with an inbred female sibling (34/42; χ^2 test for equal proportions: $\chi^2=16.1$, $p<0.001$; figure 2). However, females did not



Figure 2. Frequency at which females mated with: (i) a novel male and a male with whom her inbred sister had previously mated (self-referent cues) and (ii) a novel male and the inbred sibling of a male with whom she had already mated (male-specific cues).

show a significant preference for novel males over the inbred siblings of males with whom they had previously mated (23/42; χ^2 test for equal proportions: $\chi^2=0.38$, $p=0.5371$).

4. DISCUSSION

The results of this study demonstrate that female *G. sigillatus* prefer to mate with novel males rather than males with whom they have already mated and that they rely on self-referent cues to identify previous mates. Females did not differentiate between inbred siblings of males with whom they had mated and novel mates, suggesting that females do not rely on the individual recognition of males with whom they have mated to avoid mating with these males in future encounters (male-specific cues). Females did, however, distinguish between novel mates and males with whom their inbred sisters mated. Because the test female had no prior experience with either the 'familiar' or the novel male, this preference cannot be attributed to any learning on the part of the female. Instead, this result can only be explained by the presence of a distinguishing cue left on the familiar male by the test female's inbred sibling that had mated with this male 24 h earlier (self-referent cues). Other than by chemical means it is difficult to imagine a plausible mechanism by which a female could mark males in this manner. However, the results of this study do not rule out the possibility that females in nature use additional means to recognize previous mating partners, such as acoustic and/or visual cues. Further study will be needed to evaluate these mechanisms.

It seems likely that during the physical contact that ensues when a female mounts a male to initiate mating, she imbues the male with her own 'scent', enabling her to recognize him as a previous mate in later interactions. The most probable chemosensory cues in this regard are cuticular hydrocarbons, especially given their role in species and sex recognition in crickets (Otte & Cade 1976; Simmons 1990; Tregenza & Wedell 1997).

Despite demonstrated differences in their composition between species, populations and the sexes, it is uncertain to what extent cuticular hydrocarbons actually vary among individuals within a population. Blows (2002) demonstrated a complex interaction between the effects of

natural and sexual selection in shaping cuticular-hydrocarbon profiles in *Drosophila serrata*. Strong selection against hybrid mating might result in cuticular-hydrocarbon profiles becoming more uniform among individuals to reduce the likelihood of mate recognition errors. However, strong sexual selection may generate completely different patterns of cuticular-hydrocarbon composition. Nonetheless, uniformity in cuticular-hydrocarbon profiles does not rule out a role in individual recognition. Polerstock *et al.* (2002) found that relative proportions of components of the cuticular-hydrocarbon profile changed in female mosquitoes before and after mating. It is conceivable that differences between female chemical signatures are a result of the variation in the relative proportion of cuticular hydrocarbon constituents rather than differences in the components themselves.

By choosing novel mating partners, females may benefit in several ways. Diverse ejaculates may result in higher offspring diversity, which can increase offspring survival in stochastic environments (e.g. Tary 2003). Females may also forgo previous partners as a bet-hedging strategy, whereby females mating with many different males enjoy higher geometric mean fitness over time due to a reduction in the variance of fitness (Philippi & Seger 1989; Fox & Rauter 2003). Moreover, polyandry may enable females to avoid fertilizing all of their eggs with sperm from genetically incompatible males (Tregenza & Wedell 2000; Zeh & Zeh 2003), or ameliorate the negative effects of mating with relatives (Stockley 1999; Tregenza & Wedell 2002). Regardless of the precise nature of the genetic benefits involved, the simple form of self-referencing documented here could easily be a widespread mechanism by which females enhance their opportunities for polyandrous matings and, thereby, maximize the genetic benefits obtained through their preference for novel males.

The apparent chemosensory self-referencing that female *G. sigillatus* employ to recognize previous mating partners represents a variant of the 'armpit effect', a form of self-referent phenotype matching that was first described as a possible mechanism used in kin recognition (Dawkins 1982; Mateo & Johnston 2000). While it has generally been assumed that individuals relying on self-referencing must learn their own features as a basis for comparison in later interactions, it seems more likely that female crickets use a form of 'on-line processing' (Hauber & Sherman 2001), during which they directly compare their own 'scent' with that of the individual with whom they interact. Such a mechanism does not require a female to form a learned template because her phenotype is always available for direct comparison. Indeed, the type of self-referencing demonstrated in our study may be employed by animals in a variety of social situations, as it would allow animals to 'remember' individuals with whom they have interacted without requiring any special cognitive ability.

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