Mate Quality and Novelty Influence Post-Copulatory Female Choice in Decorated Crickets, *Gryllodes sigillatus*

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Abstract

Females of many taxa prefer to mate with novel males rather than previous mates, but also favor males that have traits indicative of higher genetic quality or compatibility. However, it may not be possible for females to simultaneously choose males that are both novel and of high quality, and the female response to this dilemma has not previously been examined. In this paper, we ask whether female decorated crickets, *Gryllodes sigillatus*, exert their choice for novel males via post-copulatory choice (sperm ampulla removal) and whether male genetic background (variation in male quality) affects this decision. We found that after matings with inbred males, females removed the ampullae of familiar mates sooner than those of novel males, whereas after matings with outbred males, there was no difference in the ampulla-retention times of familiar mates and novel partners. This suggests that when male do not vary in quality, females prefer novel partners. However, when males vary in quality, female preferences for male traits are more important than preferences for novel partners.

Introduction

Females of numerous animal taxa can secure important indirect genetic benefits by mating multiply, either by increasing the genetic diversity of their offspring, acquiring 'good genes', avoiding genetic incompatibility, or diluting inbreeding effects (Jennions & Petrie 2000; Kokko et al. 2002; Zeh & Zeh 2003). Only by mating with different partners, however, can females secure the diverse ejaculates needed to maximize these kinds of indirect genetic benefits. Selection for indirect benefits can, therefore, favor female preferences for novel males. Indeed, evidence that females prefer novel males to previous mating partners has been documented in a wide array of taxa (Bateman 1998; Zeh et al. 1998; Archer & Elgar 1999; Hughes et al. 1999; Eakley & Houde 2004; Ivy et al. 2005; LaDage & Ferkin 2007; Gershman 2009).

All of these studies, with one exception (Gershman 2009), have focused on females' pre-copulatory mating preferences. However, it well known that females can select the sire(s) of their offspring even after copulation has commenced, by prematurely terminating copulations, failing to store transferred sperm, removing or ejecting stored sperm, or delaying oviposition until after mating with desirable males (Eberhard 1996). In crickets, a male's spermatophore remains secured outside the female's body to her genital opening after mating, and thus females can exert post-copulatory choice by removing the male's spermatophore before complete sperm transfer has occurred (Sakaluk 1984; Simmons 1986; Ivy & Sakaluk 2007). Premature removal of a male's spermatophore reduces his fertilization success relative to those males transferring complete ejaculates to the same female (Sakaluk & Eggert 1996; Calos & Sakaluk 1998; Eggert et al. 2003; Bussière et al. 2006). The timing of spermatophore removal can be influenced by various male traits, such as the size of food gifts offered by males (Fedorka & Mousseau 2002), body size (Sakaluk 1985; Simmons 1986;

Bateman et al. 2001), male courtship song (Hall et al. 2008) and hind-wing morphology (Sakaluk 1997). However, the extent to which female post-copulatory preferences favor novel over previous mates remains largely unexplored (but see Gershman 2009).

The spermatophore transferred by male *Gryllodes sigillatus* at mating includes a large gelatinous mass, the spermatophylax, surrounding a smaller sperm-containing ampulla. Immediately after receiving the spermatophore, the female detaches the spermatophylax from the ampulla and feeds on it for a period of about 40 min, after which the female removes the sperm ampulla, terminating sperm transfer. Thus, the spermatophylax, a form of nuptial food gift, helps mitigate the fitness consequences of the female's ampulla-removal behavior. However, the spermatophylax often is of insufficient mass to ensure complete sperm transfer (Sakaluk 1984, 1985), and females often discard the spermatophylax by simply dropping it prior to its complete consumption, whereupon the female removes the sperm ampulla (Sakaluk 1984, 1987). Thus, while the spermatophylax helps advance the male's fitness interests in the sexual conflict over sperm transfer, it often does not succeed in overriding a female's post-copulatory preference.

In both pre- and post-copulatory phases of a mating encounter, the influence of male novelty on females' preferences may be confounded by male quality. A potentially powerful design for disentangling the effects of partner novelty and quality on female mate choice in this species would be to present females with a choice of novel and previous mates that are otherwise genetically homogenous and presumably, identical with respect to their intrinsic quality. In this study, we examined the post-copulatory mating preferences of female *G. sigillatus*, as measured by the time at which females removed the male's sperm ampulla after mating, under two conditions: (1) when previous mating partners and novel males originated from an outbred colony and were likely unrelated and (2) when previous mates and novel males originated from the same highly inbred line and were thus genetically homogeneous.

Methods

Gryllodes sigillatus used in this study were the descendents of approximately 500 adult crickets collected in Las Cruces, New Mexico in 2001, and used to initiate a laboratory colony maintained at a population size of approximately 5000 and allowed to breed panmictically. The inbred line used in this study was created by subjecting crickets, randomly selected from the large, panmictic population described above, to 23 generations of full-sib mating followed by 3–4 generations of panmixis (Ivy et al. 2005). Crickets were held in 55-L plastic storage bins in an environmental chamber maintained at 28–30°C on a 14 h:10 h light/dark cycle. Crickets were provisioned with Flukers® cricket chow (Fluker Laboratories, Port Allen, LA, USA), water provided in 40-ml plastic tissue culture flasks plugged with cotton dental rolls, and egg cartons to provide shelter and to increase surface area. Moistened peat moss provided in small plastic containers was made available both as an oviposition substrate and as a source of additional water. Experimental females were collected from the outbred colony within 48 h of adult eclosion to ensure their virginity and housed together for the next 5–8 d until they reached sexual maturity.

Experimental females were first paired with a randomly chosen 'familiar' male with which they were housed for 48 h in containers (7 × 7 × 8.5 cm) provisioned with food, water and oviposition substrate, after which they were housed separately for 24 h prior to experimental matings. At the temperature at which they were held (28–30°C), females mate, on the average, about 6.5 times/d (Kindle et al. 2006), and hence, the 48-h period was more than sufficient to ensure that copulation with familiar males occurred and that females received adequate sperm stores.

Experimental matings were staged in small Plexiglas mating arenas (16 × 30 × 9 cm) illuminated by red light. During mating trials, females were paired either with males with whom they had been housed during the 48-h cohabitation period (familiar mates) or males with whom they had had no previous experience (novel males). To ensure that novel males and familiar males had comparable mating experience, novel males were also housed with females for a 48-h period prior to experimental matings. After an experimental pair had copulated, we immediately removed the male to prevent any effect of mate guarding on the female's propensity to remove the sperm ampulla. Mate guarding is known to deter females from removing the ampulla in some cricket species (Hall et al. 2008), but we have been unable to detect such an influence in *G. sigillatus* (Sakaluk 1991; but see Bateman & MacFadyen 1999). We measured the ampulla-retention time as the time from when the spermatophore was transferred to the female to the time at which she removed the sperm ampulla with her mouthparts.

This protocol was replicated using two groups of sexually mature males, those originating from the outbred colony (previous mates: n = 47; novel males: n = 47) and those originating from the inbred line (previous mates: n = 23; novel males: n = 22). The net result of this experimental design is that the female's familiarity with the male (novel or familiar mate) was varied against both a genetically homogenous (both males of the same quality) and genetically heterogeneous (both males of potentially different quality) male background.

Results

A two-way ANOVA revealed a significant interaction between partner familiarity and genetic background in their effects on the timing of ampulla removal by females (Fig. 1; ANOVA: $F_{1,134}$ = 5.0, p = 0.027). In matings involving inbred males, females removed the ampullae of familiar males sooner than those of novel males (ANOVA: $F_{1,41}$ = 8.11, p = 0.0068), whereas in matings with outbred males, there was no difference in ampulla-retention times of familiar males or novel partners (ANOVA: $F_{1,93}$ = 1.67, p = 0.20). Novel males of both inbred and outbred groups appeared equally attractive to females as there was no significant difference in their ampulla-retention times (Fig. 1; ANOVA: $F_{1,69}$ = 0.174, p = 0.68).

Discussion

Females removed sperm ampullae of familiar males sooner than those of novel males, but only when both males were genetically homogeneous. When males were genetically unrelated, there was no dif-
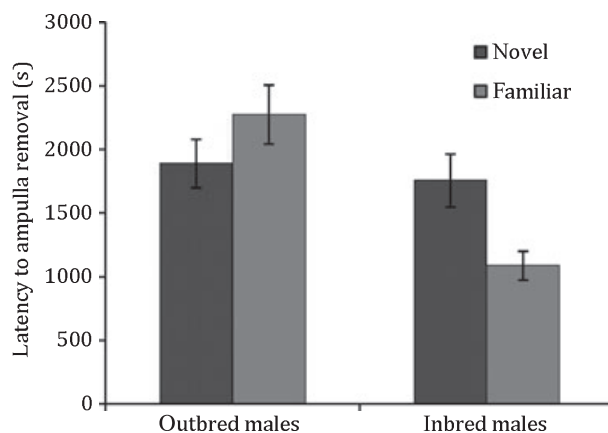
Fig. 1: The effect of male familiarity and genetic background on female ampulla-retention time (mean ± SE).

ference in ampulla-retention times of previous mates and novel males. A parallel result has been reported with respect to the pre-copulatory choices of female *G. sigillatus*: when females were simultaneously presented with a novel male and a previous mate, females mated more often with the novel male, but this effect was more pronounced when the pair of rival males came from the same inbred line as opposed to the outbred population (Ivy et al. 2005). Because females recognize previous mates based on females' own cuticular hydrocarbon cues left on their partners during mating (Ivy et al. 2005), the degree of genetic similarity between males should have no effect on the ability of females to distinguish novel males from previous mates. Instead, it seems likely that when males vary in quality, females take into account both male quality and familiarity when choosing partners. In trials using outbred males, the female preference for novel partners likely collides on occasion with the lower quality of a prospective mate, and thus, the preference for novel males may become obscured. However, in inbred males, the variation in quality has been standardized and thus, a novel male is never of lower quality than a female's previous mate; hence, in this situation, the preference for novel partners is expected to predominate.

In this study, we assumed that males of the outbred line varied more in their intrinsic quality than males of the inbred line. However, if inbreeding leads to a decrease in male quality, then the two groups of males might also differ in their average quality. Indeed, measurements made in 2007 revealed evidence of significant inbreeding depression in our inbred lines, as evidenced by lower hatching success, decreased offspring production and longer developmental times compared with the outbred population (J. M. St. John & S. K. Sakaluk, unpubl. data). While there is no obvious reason why females would be less inclined to exhibit a novel-male mating preference when presented with males of higher quality, we cannot rule out the possibility that differences in average quality between the two groups of males influenced the interaction between partner familiarity and genetic background in their effects on post-copulatory female choice.

One final caveat with respect to the interpretation of our results is that we employed only a single inbred line, and so we cannot rule out the possibility that the pattern of female choice we observed was an artifact of the particular inbred strain of males used in experimental trials. However, there was no obvious difference in the ampulla-retention times of

outbred and inbred males when they were introduced as novel mates; a difference only emerged when males of the two groups were used in the familiar-male position. Although we can provide no obvious explanation as to why an interaction between male novelty and male genetic background on the post-copulatory preferences of outbred females might arise when one inbred line is used but not another, the generality of our findings must await studies employing multiple inbred lines.

Although female *G. sigillatus* are able to recognize and reject previous mates prior to copulation (Ivy et al. 2005), this study demonstrates that females readily mate with such males when there is no other option. It is possible that by choosing to mate with previous mates, females are able to replenish depleted sperm stores or receive oviposition-stimulating substances from these males (reviewed in Arnqvist & Nilsson 2000). Even after mating with previous mates, however, females retain the option of prematurely removing the sperm ampulla, reducing the fertilization success of these males relative to novel (or more desirable) partners that they might encounter in the future. Although the provision of a spermatophylax by male *G. sigillatus* functions to promote increased sperm transfer (Sakaluk 1984), an interesting result emerging from this study is that females are able to at least partially offset this form of male manipulation when it is in their interests to do so. The fact that a female's ampulla-removal behavior is influenced by both male quality and novelty underscores the complex sexual conflict over the fate of males' ejaculates that has been documented in previous studies (Sakaluk 2000; Sakaluk et al. 2006).

In conclusion, the results of our study add to a growing body of evidence suggesting that female mating preferences are not fixed, but rather, context dependent, influenced by a variety of social and environmental factors including previous mating experience (Moore & Moore 2001; Martin & Hosken 2002; Mautz & Sakaluk 2008), the mating preferences of other females (Dugatkin 1992), the operational sex ratio (Jirotkul 1999), population density (Welch 2003) and the risk of predation (Forsgren 1992; Hedrick & Dill1993). Of particular importance is the role of social context (Westneat et al. 2000; Royle et al. 2008), given that plastic mating preferences of females can select for signal modulation in males (Bateson & Healy 2005) or even lead to divergence in recognition systems between populations (Ryan et al. 2007). Female decorated crickets clearly have at their disposal an array of pre- and post-copulatory mechanisms that permit them to carefully

vet mate quality at different stages of the mating sequence and to respond opportunistically to variation in mate quality both in time and space (Sakaluk 1984, 1997; Ivy et al. 2005; Ivy & Sakaluk 2007).

Acknowledgements

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