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Sensory exploitation as an evolutionary origin to nuptial food gifts in insects

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Nuptial food gifts given by males to females at mating are widespread in insects, but their evolutionary origin remains obscure. Such gifts may arise as a form of sensory trap that exploits the normal gustatory responses of females, favouring the selective retention of sperm of gift-giving males. I tested this hypothesis by offering foreign food gifts, synthesized by males of one cricket species, to females of three non-gift-giving species. Females provisioned with novel food gifts were 'fooled' into accepting more sperm than they otherwise would in the absence of a gift. These results support the hypothesis that nuptial food gifts and post-copulatory female mating preferences coevolve through a unique form of sensory exploitation.

Keywords: crickets; nuptial food gifts; mate choice; sexual selection; sensory exploitation

1. INTRODUCTION

Nuptial food gifts come in various forms, including prey items acquired by males, adjuncts to males' spermatophores, secretions produced by specialized glands, regurgitated crop contents and even portions of the male's body (Thornhill 1976*a*; Vahed 1998). A sexual conflict over paternity of offspring is the likely selective context within which courtship feeding has evolved (Sakaluk & Eggert 1996). In many species of insects, females are well positioned to determine the fate of their mates' gametes through various means, including control of copulation duration (Thornhill 1976*b*), premature removal of spermatophores (Sakaluk 1984; Simmons 1986) and internal manipulation of ejaculates (Ward 1993; Eberhard 1996). Because it is in the interests of males to have their sperm used in fertilizations, female control of sperm transfer and usage is often at odds with the reproductive interests of their sexual partners. Courtship food gifts function to entice females into relinquishing at least some of their control of the insemination process, and help further males' reproductive interests either by improving their prospects of mating (Steele 1986; Eggert & Sakaluk 1994) or increasing their chances of paternity once copulation has occurred (Thornhill 1976*b*; Sakaluk 1984).

Any hypothesis concerning the evolution of nuptial food gifts must account not only for their astonishing diversity, but also for the pervasiveness of female post-copulatory mate choice that occurs in gift-giving species. Why do females across species as diverse as crickets (Sakaluk 1984, 1985), katydids (Wedell & Arak 1989; Reinhold & Heller 1993), hangingflies (Thornhill 1976*b*), scorpionflies (Thornhill & Sauer 1991) and dance flies (Svensson *et al.* 1990), preferentially use the sperm of males providing the largest gifts? The conventional explanation for species in which males control resources sought by females is that females derive direct benefits through their mating preferences (reviewed in Andersson 1994). There is, however, a simple reason why the direct benefits hypothesis cannot account for female choice of nuptial food gifts: females typically exert their preferences only after they have accepted males' offerings. In a number of gift-giving species, females accept copulations without regard to the size of the gift being offered, and choice only occurs during or after copulation (Thornhill 1976*b*; Sakaluk 1984, 1985; Wedell & Arak 1989). In these species, it is not at all clear what females gain from discriminating against males after they have already accepted the males' nuptial offerings; with or without a post-copulatory preference, females derive precisely the same benefits through the use of any nutrients contained in food gifts.

The sensory exploitation hypothesis, which posits that male sexual ornaments can arise because they exploit pre-existing biases in females' sensory systems (reviewed in Ryan 1998), offers an alternative explanation. Nuptial food gifts may evolve as a form of sensory trap (Christy 1995) that exploits a female's underlying motivation to feed. If the proximate mechanisms regulating females' gustatory responses take precedence over other impulses, including those that might otherwise disrupt sperm transfer, then gift-giving males may succeed in fertilizing more eggs than they otherwise would in the absence of a food gift. This hypothesis might also explain why the food gifts of certain species apparently provide no significant nutritional benefits to females (Wedell & Arak 1989; Vahed & Gilbert 1997; Will & Sakaluk 1994; reviewed in Vahed 1998).

Crickets offer an ideal model with which to examine the evolution of post-copulatory female choice: the ejaculate of a male typically remains attached outside the female's genital opening after mating in the form of an externally attached spermatophore, and females are thus well positioned to determine the fate of their mates' gametes by removing their spermatophores (Sakaluk 1984; Simmons 1986). In decorated crickets, *Gryllodes sigillatus*, the spermatophore includes a gelatinous mass, the spermatophylax, which the female detaches and feeds on after mating. While the female consumes this nuptial food gift, sperm are evacuated into her reproductive tract from the remaining portion of the spermatophore, the sperm ampulla. Smaller spermatophylaxes require less time to consume, and males providing such gifts transfer fewer sperm because their ampullae are removed sooner (Sakaluk 1984, 1985). Females mated to more than one male can greatly influence the paternity of their offspring

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through their ampulla-removal behaviour (Sakaluk 1986; Sakaluk & Eggert 1996; Calos & Sakaluk 1998).

There are three diagnostic criteria by which sensory exploitation can legitimately be viewed as a basis for the coevolution of male traits and female mating preferences: (i) an absence of the trait as the ancestral condition within the clade of interest, (ii) a female preference for the trait in species in which the trait is present, and (iii) a female preference for the trait in species in which the trait is absent (Basolo 1995). With respect to post-copulatory female choice of the spermatophylax in *Gryllodes sigillatus*, the first two criteria have been satisfied. First, a recent phylogenetic analysis of the ensiferan Orthoptera confirms that a simple, externally attached sperm ampulla and female consumption of the ampulla were the likely ancestral character states within this suborder, and that spermatophylax feeding and other forms of courtship feeding arose only after the origin of female ampulla consumption (Gwynne 1995). The analysis reveals that the absence of a spermatophylax constitutes the ancestral condition within the Gryllidae (true crickets). Second, females control sperm transfer through their ampulla-removal behaviour, and the timing of ampulla removal is determined principally by the mass of the spermatophylax provided by the male at mating (Sakaluk 1984, 1985). The number of sperm transferred by males has important fitness consequences for males in the context of sperm competition, and hence the ampulla-removal behaviour of females can be viewed as a mechanism of post-copulatory female choice (Sakaluk & Eggert 1996; Calos & Sakaluk 1998). It is with respect to the third criterion that we lack compelling evidence: to what extent does the preference for a larger spermatophylax exist in cricket species that lack a spermatophylax? I attempted to determine the existence of any such preference by giving mated females of non-spermatophylax-donating cricket species an opportunity to feed on the spermatophylaxes of *Gryllodes sigillatus*. I predicted that (i) recently mated females of non-spermatophylax-bearing species would readily consume *Gryllodes* spermatophylaxes, and (ii) spermatophylax consumption by mated females of these species would delay their removal of the sperm ampulla, resulting in greater sperm transfer by their mates.

2. METHODS

Tests for pre-existing sensory biases were conducted on recently mated females of three non-spermatophylax-bearing cricket species, *Gryllus veletis* ($n=32$), *Gryllus integer* ($n=22$), and *Acheta domesticus* ($n=33$), two to three weeks beyond the imaginal moult. Crickets used in mating trials were reared from individuals collected in the field (*Gryllodes sigillatus*: Tucson, AZ; *Gryllus integer*: Austin, TX; *Gryllus veletis*: Normal, IL) or in the case of *A. domesticus*, obtained from a commercial supplier (Fluker Farms, Port Allen, LA). All three species exhibit ancestral mating characteristics, namely, a simple sperm ampulla and female consumption of the ampulla after mating (Gwynne 1995). Experiments followed a paired design in which each female was mated twice to the same male: immediately after one mating, the female was given the opportunity to feed on a *Gryllodes* spermatophylax, whereas no spermatophylax was provided after the other mating. Spermatophylaxes presented to experimental females were obtained by removing them from the spermatophoric pouches of male *Gryllodes* immediately before trials. The spermatophylax was either placed on the substrate directly in front of the female or held on a probe in front of her. Treatment order was reversed for every other female, and immediately after each mating, the male was removed so as to eliminate any possible effect of post-copulatory mate guarding on female ampulla-removal behaviour. Mated females were observed for 60 min after copulation or until they removed the sperm ampulla.

The effect of ampulla removal on the number of sperm transferred was assessed through direct counts of sperm retained in ampullae removed from mated females at various times after copulation. The ampulla was placed in 4 ml distilled water and cut into several pieces using microscissors, after which the mixture was forced repeatedly through a syringe until the ampulla had been sheared into smaller pieces. To prevent sperm agglutination, the solution was stirred vigorously for 1 min using a Fisher Vortex Genie 2^{TM} (Bohemia, New York). Five 10-µl samples were pipetted onto a microscope slide equipped with a grid, which subsequently was set aside to dry. The total number of sperm contained in the smear was determined at $\times 100$ magnification. Sperm counts were made by a research assistant blind to the duration of ampulla attachment.

3. RESULTS

The majority of females of all three species voluntarily grasped the *Gryllodes* spermatophylax when it was first presented to them (*Gryllus veletis*: 91% (29 out of 32 females); *Gryllus integer*: 95% (21 out of 22 females); *A. domesticus*: 100% (33 out of 33 females)). Females began feeding on the spermatophylax shortly after copulation (median = 1.5–4.3 min), and continued feeding on it for an extended period thereafter (median duration of spermatophylax consumption, first and third quartiles in parentheses; *Gryllus veletis*: 30.23 min ($Q_1=18.56$, $Q_3=50.71$), *Gryllus integer*: 51.20 min ($Q_1=46.81$, $Q_3=57.87$), *A. domesticus*: 42.03 min ($Q_1=20.71$, $Q_3=58.38$)).

Females of all three species retained the sperm ampulla of their mates significantly longer when provided with a *Gryllodes* spermatophylax than when not given a nuptial food gift (figure 1, mean difference in ampulla attachment duration ± s.e., *Gryllus veletis*: 18.2 ± 4.6 min, paired samples *t*-test, $t=3.98$, $p<0.001$; *Gryllus integer*: 9.5 ± 2.9 min, $t=3.28$, $p<0.005$; *A. domesticus*: 19.3 ± 3.8 min, $t=5.05$, $p<0.001$). Direct counts of sperm retained in ampullae removed at various times after copulation revealed that such differences lead to greater insemination of females presented with novel food gifts. There was a significant linear decrease in the number of sperm retained in the ampulla with increasing ampulla attachment duration for all three species (figure 1; *Gryllus veletis*: $n=31$, $b=-10.16\pm3.12$ (± s.e.), intercept = 751 ± 151, $r^2=0.27$, $F_{1,29}=10.6$, $p<0.003$; *A. domesticus*: $n=34$, $b=-76.51\pm3.6$, intercept = 5138 ± 587, $r^2=0.50$, $F_{1,32}=31.4$, $p<0.001$; *Gryllus integer*: $n=51$, $b=-6.52\pm1.95$, intercept = 492 ± 92, $r^2=0.19$, $F_{1,49}=11.2$, $p<0.002$). Based on these trajectories, male *Gryllus veletis* transferred about 2.4 times as many sperm when their mates were provided with a spermatophylax as when they were not, and male *Gryllus integer* and *A. domesticus* transferred 1.3 times and 5.6 times as many sperm, respectively, under these same conditions.

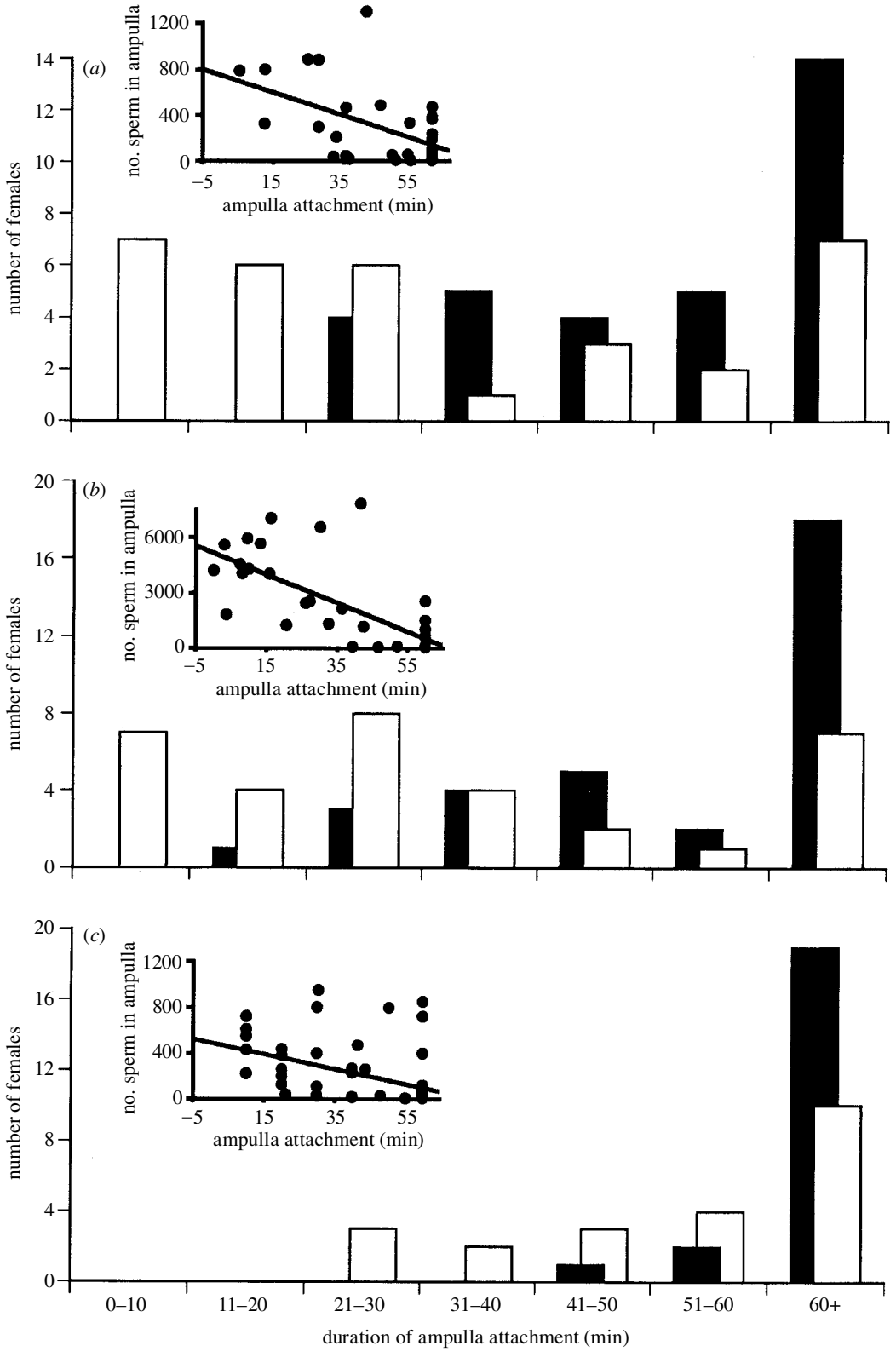

Figure 1. Frequency distribution of the duration of ampulla attachment of females mated twice to the same male and either given the opportunity to feed on a novel food gift, a *Gryllodes* spermatophylax (solid bars), or given no such opportunity (control: open bars). Females of all three non-gift-giving species ((*a*) *Gryllus veletis*, (*b*) *A. domesticus*, and (*c*) *Gryllus integer*) retained their mate's ampulla longer when provided with a *Gryllodes* spermatophylax than when not given one, and consequently were more fully inseminated (all $p<0.005$). The inset in each frequency distribution shows the number of sperm remaining in the sperm ampulla as a function of the time the ampulla remained attached, based on 50-µl samples. There was a significant decrease in the number of sperm retained in the ampulla over the time period during which female ampulla removal occurred; the regression was highly significant in each case (all $p<0.003$). The apparent number of data points shown in each inset is less than the actual number owing to some overlap in the data.

4. DISCUSSION

Sensory traps refer to male signals that mimic stimuli to which females respond in other contexts, and elicit female behaviours that enhance male fertilization success (Christy 1995). What distinguishes sensory traps from other forms of sensory exploitation, is that the biases they engage are currently advantageous to females outside the context of mate choice. In water mites, for example, male courtship signals apparently mimic the vibrations of copepod prey, resulting in the attraction of hunting females (Proctor 1991). Sensory trap signals are intrinsically deceitful, so that males are selected to exaggerate their attractiveness while minimizing their costs (Christy 1995).

Whether the spermatophylax of *Gryllodes sigillatus* can be regarded as a sensory trap hinges, in part, on the extent to which the spermatophylax is perceived by females as a valuable food item without being especially nutritious. Previous work suggests that with respect to its nutritional value to females, the spermatophylax is, in fact, a sham. Will & Sakaluk (1994) systematically varied food levels under which female *Gryllodes sigillatus* were maintained (three levels: starved, 30% *ad libitum*, and *ad libitum*) and the number of spermatophylaxes females were permitted to consume per day (three levels: 0, 1, and 3). There was no effect of spermatophylax consumption on female survival, egg size or lifetime reproduction, a result that has since been replicated in another study (Kasuya & Sato 1998). The absence of detectable nutritional benefits to courtship feeding is not unique to *Gryllodes sigillatus*, but appears to be widespread across a variety of gift-giving taxa (reviewed in Vahed 1998). However, since all of these studies rely on the absence of treatment effects to infer a lack of nutritional benefits, they must be viewed with some caution; it may well be that the conditions under which females might benefit through substances contained in food gifts are difficult to duplicate in the laboratory.

The absence of a nutritional benefit of spermatophylax consumption does not, of course, preclude the existence of other kinds of benefits; indeed Ivy *et al.* (1999) recently showed that female *Gryllodes sigillatus* subject to water stress secure significant hydration benefits through nuptial feeding. Notwithstanding the existence of these or other undetected nutritional benefits to nuptial food gifts, they cannot alone account for the evolution of post-copulatory female choice in gift-giving species. The direct benefits hypothesis of female choice was originally conceived to explain female choice in species in which females select among males before mating; thus, if males differed in the level of material resources they had to offer, females would receive direct benefits by selectively mating with the males offering the greatest level of material resources. The direct benefits hypothesis cannot, however, account for female choice in gift-giving insect species; in these species, females normally mate with males irrespective of the resources males have to offer and exercise their preferences only after they have received males' gifts. Consider, for example, a typical female *Gryllodes* who prematurely removes her mate's ampulla if the spermatophylax he provides her is small, versus a hypothetical non-choosy female who retains the same male's ampulla until its complete evacuation, despite receiving a small spermatophylax. The female receives precisely the same direct benefit from the gift whether she is choosy or not. If the benefit is the same irrespective of a female's post-copulatory choice, then the benefit cannot be invoked to account for that choice; indeed, such an explanation would commit what Simmons & Parker (1989) termed a 'sequence fallacy'.

Females of the non-gift-giving species clearly found the spermatophylax of *Gryllodes sigillatus* appealing, and in accordance with the sensory trap hypothesis, accepted more sperm from males when offered a spermatophylax than when not. That females found the spermatophylax enticing is underscored by the fact that experimental females were maintained on a normal rearing diet and should not, therefore, have been particularly hungry. Recent biochemical analyses of the *Gryllodes* spermatophylax by Stuart Warwick (unpublished data) suggest that the phagostimulatory effect of the spermatophylax may stem from high concentrations of free amino acids, which in some species, serve as a reliable indicator of food quality. The amino-acid composition of the spermatophylax is, however, highly unbalanced, with unusually low concentrations of essential amino acids, leading Warwick to conclude that males are exaggerating the nutritional quality of their nuptial gifts. His analyses provide independent support of the sensory trap hypothesis as it applies to the spermatophylax.

The provision of a spermatophylax was probably an adaptive male response to the early removal of the sperm-containing ampulla by females (Alexander & Otte 1967; Sakaluk 1984), as occurs in numerous cricket species (Sakaluk 1984; Simmons 1986; Vahed 1998). Consumption of the ampulla, even before sperm transfer was complete, would have benefited the female because the male frequently would have provided a replacement spermatophore; in a number of cricket species, females often mate repeatedly with the same male (Hissmann 1990; Zuk 1987; Burpee & Sakaluk 1993). The spermatophylax would have served to delay female consumption of the actual sperm-containing vessel. At first, the ancestral spermatophylax might also have provided some nutritional benefit to the female, but over time, males would have been selected to minimize its value while maintaining its appeal. Indeed, courtship feeding bears all the hallmarks of an unending coevolutionary spiral between the sexes (Parker 1979; Alexander *et al.* 1997), where once gift giving was established, there was continuous selection on males to deceive females with respect to the nutritional quality of their offerings, and selection on females to see through these deceptions. Such unending sexual conflicts may account for the variation in courtship feeding observed in dance flies (Diptera: Empidinae), whose nuptial gifts reveal an evolutionary transition from the provision of insect prey, to insect prey wrapped in silk, inedible objects wrapped in silk, and finally, to hollow, inedible silken balloons (Downes 1970; Cumming 1994).

The sensory exploitation process also offers a possible explanation for the bewildering variety of nuptial gifts manifest in insects (Thornhill 1976*a*; Vahed 1998) and other arthropods (Andrade 1996). Because the process requires only that males elicit a gustatory response from females, the exact nature of the phagostimulatory

material may be largely a matter of chance. Any device, structural or secretory, that first succeeds in eliciting a feeding response, will be subsequently elaborated through sexual selection, and there is no reason to assume that males of different taxa will necessarily converge on the same mimetic signal.

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