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Cryptic Sexual Conflict in Gift-Giving Insects: Chasing the Chase-Away

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ABSTRACT: The chase-away model of sexual selection posits that elaborate male sexual displays arise because they exploit preexisting biases in females' sensory systems and induce females to mate in a suboptimal manner. An essential element of this hypothesis is that such manipulation should quickly lead to female resistance to male displays. Nuptial food gifts may be a frequent conduit by which males attempt to influence the mating behavior of females against females' own reproductive interests. In decorated crickets *Gryllodes sigillatus*, such inducements come in the form of a spermatophylax, a gelatinous mass forming part of the male's spermatophore and consumed by the female after mating. We conducted experiments in which spermatophylaxes obtained from male *G. sigillatus* were offered as novel food gifts to females of a non-gift-giving species (*Acheta domesticus*) having no evolutionary history of spermatophylax consumption. Female *A. domesticus* that were allowed to consume the spermatophylax took significantly longer to remate than when given no such opportunity. In contrast, when female *G. sigillatus* were prevented from consuming their partners' nuptial gifts, there was no difference in their propensity to remate relative to females permitted to consume a food gift after mating. These results suggest that the spermatophylax synthesized by male *G. sigillatus* contains substances designed to inhibit the sexual receptivity of their mates but that female *G. sigillatus* have evolved reduced responsiveness to these substances.

Keywords: antagonistic coevolution, chase-away, nuptial food gifts, sensory exploitation, sexual conflict, sexual selection.

An evolutionary conflict between the sexes ensues whenever the reproductive strategy pursued by one sex reduces the fitness of the other (Parker 1979; Rice and Holland 1997; Zeh and Zeh 2003). The reproductive interests of males and females often diverge with respect to the occurrence and timing of mating, number of mating partners, parental investment, and paternity of offspring. These evolutionary disagreements may lead to a variety of sex-specific adaptations designed to thwart the interests of the opposite sex. In males, such adaptations include structures to impose forced copulations on females (Thornhill 1980; Arnqvist 1989; Sakaluk et al. 1995), behaviors such as mate guarding that function to prevent remating by females (Alcock 1994; Johnsen et al. 1998; Jormalainen 1998), and substances in males' ejaculates that reduce female receptivity to subsequent courtship attempts (Eberhard 1996; Wolfner 1997; Miyatake et al. 1999). Counteradaptations in females include devices that permit females to reject copulations (Arnqvist and Rowe 1995), postcopulatory mating preferences that afford females greater control of the paternity of their offspring (Eberhard 1996), and behavioral tactics that function to discourage male polygyny and thereby maximize male parental investment (Davies 1992; Eggert and Sakaluk 1995).

The rate at which males and females mate is an aspect of reproduction that is especially rife with the potential for sexual conflict (Holland and Rice 1998). In the majority of animal species, the greater investment by females in offspring means that females, in terms of their reproductive success, are limited primarily by the number of eggs they are able to produce, whereas males are limited mainly by the number of mates they are able to acquire (Trivers 1972). This means that selection typically favors males that mate at a higher rate than is optimal for females. As a consequence, sexual selection should favor traits in males that induce females to mate at a suboptimal rate and traits in females that allow them to resist such inducements (Holland and Rice 1998).

Holland and Rice (1998) proposed a new model to account for the evolution of elaborate male sexual displays that incorporates this fundamental conflict over mating rate. According to their model, display traits initially arise in males because they exploit preexisting sensory biases in

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females and consequently induce females to mate in a suboptimal manner. This in turn selects for female resistance or decreased attraction to the trait, which in turn leads to greater selection on males to exaggerate the display trait to overcome this resistance. The resultant cycle of antagonistic coevolution forms the basis of what Holland and Rice (1998) term the "chase-away" model of sexual selection (see also Gavrilets et al. 2001).

Although the chase-away model of sexual selection is not mutually exclusive of other, more well-established models for the evolution of exaggerated mating displays (e.g., direct benefits, indirect benefits, and sensory drive; review in Kokko et al. 2003), it does make two unique predictions (Holland and Rice 1998). First, the chase-away sexual selection model predicts that females will evolve decreased attraction to male display traits, whereas established models predict increased attraction (direct and indirect benefits) or static attraction (sensory drive) to male display traits. Second, only the chase-away model of sexual selection predicts that female attraction to male display traits will result in a decrease in female fitness. All of the other models predict either that the fitness of females is enhanced via their preferences for the most conspicuous males (direct and indirect benefits) or, at worst, that it is unaffected (sensory drive).

An essential element of this hypothesis is that such manipulation should quickly lead to female resistance or decreased attraction to male display traits. Although anecdotal evidence in certain taxa appears to provide support for the evolution of female resistance (Holland and Rice 1998; Forstmeier 2004), the hypothesis has not been directly tested. Nuptial food gifts, an integral feature of the mating systems of a wide variety of insects, may be a frequent conduit by which males attempt to influence the mating behavior of females against females' own reproductive interests. Such 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*b*; Vahed 1998). A pervasive feature of these gifts is that they give the male direct access to the female's physiology. Indeed, a recent meta-analysis of female multiple mating in gift-giving insects revealed that although female fitness increases markedly with increased mating rate, females of most species mate at a lower than optimal rate (Arnqvist and Nilsson 2000); this finding prompted the authors to suggest that food gifts represent male refractory-inducing substances disguised as nutritional offerings ("Medea gifts").

Although females in a number of species often derive no significant nutritional benefits from the consumption of nuptial food gifts (Wedell and Arak 1989; Reinhold and Heller 1993; Will and Sakaluk 1994; Vahed 1998), they nevertheless eagerly consume them and differentially accept the sperm of their mates based on the size of the gifts they receive (Thornhill 1976*a*; Sakaluk 1984, 1985; Wedell and Arak 1989). These observations are not readily explicable within the direct- or indirect-benefits models of sexual selection, but they are consistent with the sensory drive and chase-away models. Although the conventional approach has been to seek evidence of the benefits to females of consuming food gifts (e.g., Gwynne 1984; Ritchie et al. 1998; Ivy et al. 1999), the possibility that the consumption of such gifts could be detrimental to female fitness has rarely, if ever, been considered. Such evidence would provide critical support to the chase-away sexual selection model.

We believe that we have identified a male display trait that presents a unique opportunity to seek unequivocal evidence of female resistance and to demonstrate that female attraction to a male display trait can lead to decreased female fitness. The male display trait in question is a courtship food gift, the spermatophylax, a component of the spermatophore that is transferred by male decorated crickets *Gryllodes sigillatus* (Orthoptera: Gryllidae) to females at mating. In a recent study, Sakaluk (2000) tested the hypothesis that the spermatophylax of male decorated crickets evolved as a form of sensory trap, exploiting the normal gustatory responses of females and favoring the selective retention of sperm from gift-giving males. He tested this hypothesis by offering spermatophylaxes that had been synthesized by male *G. sigillatus* to mated females of 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, suggesting that food gifts evolve through a unique form of sensory exploitation.

These results provide support to the incipient stage of Holland and Rice's (1998) chase-away process, namely, that display traits such as the spermatophylax first arise in males because they exploit preexisting sensory biases in females. However, what is the evidence that the ancestral spermatophylax induced females to mate in a suboptimal manner, thus giving rise to the evolutionary conflict fueling the chase-away process? The sensory exploitation study described above provides a possible clue. An interesting but entirely unexpected result emerged with respect to the remating interval of female *Acheta domesticus*, a non-gift-giving species: females that were given a spermatophylax to consume in their first mating trial took significantly longer to remate than females that did not receive a spermatophylax in their first trial. What makes this difference of particular interest is that over many years of study, we have observed no obvious effect of spermatophylax consumption on the propensity to remate in female *G. sigillatus*.

How do we account for the reduction in the sexual receptivity of females of a non-gift-giving species that follows the consumption of the spermatophylax and the apparent absence of such an effect in female *G. sigillatus*? The results may best be explained by the chase-away sexual selection model. We hypothesize that the spermatophylax transferred by male *G. sigillatus* contains substances that at one time inhibited the subsequent sexual receptivity of their mates but that female *G. sigillatus* have evolved reduced responsiveness (i.e., resistance) to these substances to retain control of their mating rate. We suggest that the reason why females of the non-gift-giving species show a reduction in sexual receptivity is that, having had no evolutionary experience with spermatophylax consumption, they have been under no selection to evolve any kind of immunity to the receptivity-inhibiting substances contained in the spermatophylax.

Here we test the chase-away sexual selection model as it applies to the coevolution of the spermatophylax and female resistance in decorated crickets. We first report the unpublished data from Sakaluk's (2000) initial study showing a reduction in the receptivity of female *A. domesticus* fed food gifts synthesized by male *G. sigillatus*, and then we report the results of two new experiments that, first, replicate this result but rule out the possibility that differential receipt of ejaculates could account for the observed decrease in female receptivity; second, demonstrate that female *G. sigillatus* are resistant to the receptivity-inhibiting substances contained in males' food gifts; and third, show that this result is repeatable under conditions in which females are given ample opportunity to evade males.

Material and Methods

Cricket Mating Behavior

Copulation in crickets is completed with the successful transfer of the spermatophore, which normally consists of a small, sperm-containing ampulla that remains attached to the female's genital opening at the base of her ovipositor (Zuk and Simmons 1997). In *Gryllodes sigillatus*, the spermatophore includes a large gelatinous mass, the spermatophylax, that envelopes the sperm-containing ampulla. Almost immediately after the spermatophore has been transferred, the female detaches the spermatophylax from the ampulla with her mandibles and begins to consume it. The sperm ampulla remains secured to the female, and its contents are emptied into the female reproductive tract while the female feeds on the spermatophylax. It normally takes the female about 40 min to fully consume the spermatophylax, and typically within a few minutes of doing so, she removes and eats the sperm ampulla. Smaller spermatophylaxes require less time to consume, and consequently, males providing such gifts experience premature ampulla removal and reduced sperm transfer (Sakaluk 1984, 1985, 1987). The amount of sperm transferred is vital to male fitness because it is the principal determinant of a male's fertilization success, particularly when his sperm must compete with those of a female's other mating partners (Sakaluk 1986; Sakaluk and Eggert 1996; Calos and Sakaluk 1998; Eggert et al. 2003).

Experiment 1: Does the Spermatophylax Contain Receptivity-Inhibiting Substances?

As part of an earlier study designed to assess the intrinsic gustatory appeal of the spermatophylax, Sakaluk (2000) offered the spermatophylax as a novel food gift to sexually experienced females of a non-gift-giving species, *Acheta domesticus*. In that study, he employed a paired design in which each female was mated twice to the same conspecific 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 *G. sigillatus* immediately before trials. The spermatophylax was offered to females by holding it directly in front of them at the end of fine forceps, or by placing it on the substrate directly in front of them but without direct physical contact with the female. Treatment order was reversed for every other female, and matings were staged at least 24 h apart. If a female failed to mate within a 60-min observation period, the trial was terminated and she was given the opportunity to mate the next day and each subsequent day until she completed both treatments. Although the study was designed specifically to examine the effect of spermatophylax consumption on female retention of the ampulla, we also recorded the number of days that elapsed before the female remated, both for those females that received a food gift at their first mating and those that did not. Although the data on the time to remating were not reported in the original study, we include them here because they offer the first test of the effect of spermatophylax consumption on female receptivity. A complete description of the protocol employed in staging the matings is described by Sakaluk (2000).

Experiment 2: Testing for Resistance

In Sakaluk's (2000) earlier study, mated female *A. domesticus* that were permitted to consume the spermatophylax of a male *G. sigillatus* retained their mate's sperm ampulla significantly longer than when not offered one;

hence, in addition to receiving a spermatophylax, these females received both greater amounts of sperm and other ejaculatory products. If males' ejaculates contain receptivity-inhibiting substances of the kind that have been documented in other insects (Cordero 1995; Wolfner 1997; Miyatake et al. 1999; Andersson et al. 2000; Xue and Noll 2000), then any reduction in sexual receptivity observed in females of the non-gift-giving species could be attributed as much to the differential receipt of ejaculates as it could to the effects of spermatophylax consumption. Indeed, it is known that certain constituents of cricket ejaculates, including prostaglandins, sperm, and other male-derived factors, have important effects on the rate of vitellogenesis and oviposition in females (e.g., Destephano and Brady 1977; Loher 1979; Murtaugh and Denlinger 1987) and conceivably could alter female receptivity (but see Orshan and Pener 1991; Fleischman and Sakaluk 2004).

A second concern is that although female *G. sigillatus* do not appear to exhibit the same reduction in sexual receptivity following consumption of the spermatophylax as has been observed in *A. domesticus*, this lack of responsiveness is based solely on anecdotal evidence. Until this study, we have never compared the remating latency of female *G. sigillatus* that were experimentally prevented from feeding on the spermatophylax with that of control females, a test that is critical to any comparison with a non-gift-giving species. A robust test of the evolution of resistance in female *G. sigillatus* would entail the following elements: first, a control for any difference in the amount of ejaculate transferred to females permitted to consume the spermatophylax and those prevented from doing so and, second, a comparison of the remating propensity of female *G. sigillatus* experimentally precluded from feeding on the spermatophylax and those permitted to do so. Here we describe such a test.

Experimental *G. sigillatus* were the descendants of approximately 200 crickets collected at Tucson, Arizona, in October 1995. Experimental *A. domesticus* were obtained from a commercial supplier (Fluker Farms, Baton Rouge, LA). All crickets were maintained according to standard procedures (Sakaluk 1991; Burpee and Sakaluk 1993). Late-instar females were held in a separate terrarium to ensure their virginity upon the adult molt.

To assess the effects of spermatophylax consumption on the latency to remating, two treatments were established: in one treatment, females were given a spermatophylax to consume after mating, whereas in the other, females were given no spermatophylax to consume. For each species, 60 females were randomly assigned to each of the two treatments, 30 of whom were initially virgin and 30 of whom were sexually experienced (total $N = 120$). We reasoned that once-mated females might have a greater incentive to remate because they have reduced sperm stores in comparison with females that have mated multiple times. Sexually experienced females were held continuously with adult males of similar age for the first 2 weeks of their adult life before their initial experimental mating, whereas virgin females were held with other virgin females before their initial mating.

Initial matings were staged in specially constructed Plexiglas viewing chambers (4.4 [w] × 10.5 [l] × 7.9[h] cm) observed under red light illumination. Females given a spermatophylax to consume after mating were presented with the spermatophylax on the tip of a dissecting probe held directly in front of their mandibles. In mating trials involving *G. sigillatus*, we first detached and discarded the original spermatophylax that was transferred by the male at mating before assigning females to their prescribed treatments. This ensured that any differences in the latency to remating between female *G. sigillatus* and female *A. domesticus* did not arise because of a difference in the way that food gifts were presented.

After their initial mating, females were prevented from removing the sperm ampulla for 1 h, a period that is sufficient to ensure complete evacuation of the ampulla (Sakaluk 2000). Removal of the ampulla was prevented by placing a probe between the female's mandibles and the ampulla whenever she tried to reach around and grasp it. Hence, females were inseminated equally across treatments, which thereby controls for any effects the ejaculate might have on the female's willingness to mate again. Females and males that completed their initial mating were housed in individual containers with ample food (Fluker's Cricket Chow) and water for 24 h. At the end of the 24-h period, each female was given the opportunity to mate with the male with whom she had mated the day before. Females were given a maximum of 4 h within which to complete their second matings. No food or water was provided during mating trials.

Experiment 3: Resistance in a Complex Environment

This experiment was designed to test whether putative receptivity-inhibiting substances in the spermatophylax have a detectable influence on female receptivity when females have a wider latitude to elude males. The experimental protocol was similar to that of the second experiment, with several key alterations. First, experimental *G. sigillatus* were the descendants of approximately 500 adults collected in Las Cruces, New Mexico, in May 2001 (the previous experiments were conducted using *G. sigillatus* from Arizona). To prevent inbreeding and the loss of genetic variation, crickets were maintained in a large, panmictic population of approximately 5,000 individuals. Second, although initial matings were staged in the small mating arenas described earlier, remating trials were staged in large glass terrariums (31 [w] × 76 [l] × 32 [h] cm) in which four shelters had been placed to provide locations where females could avoid detection by males should they choose to do so. Shelters were constructed of cylindrical PVC couplers (diameter = 12.7 cm; height = 9.5 cm) with four symmetrically located entrances at the bottom of each shelter to provide a means of access for the crickets. These shelters were rapidly adopted as refuges by free-living *G. sigillatus* in a previous field study (Sakaluk et al. 2002). Third, females were given 1 h to remate from the time when the male first courted them in the large arena. If the female failed to remate during the 1-h observation period, she was re-paired the following day (and each subsequent day to a maximum of 3 days) until she remated. For each female, we recorded the total time taken to complete the second mating in relation to the time when the male first initiated courtship, pooled over successive observation periods if she failed to remate at her first opportunity to do so. Fourth, all females were sexually experienced prior to experimental trials.

As in the previous experiment, the duration of ampulla attachment was standardized across treatments, except that in *G. sigillatus*, the ampulla was removed after 1 h, whereas in *A. domesticus*, the ampulla was removed after 25 min; these times correspond to the time when females normally remove the ampulla in the absence of experimental intervention in the two species, respectively (Sakaluk 1987, 2000). In addition, remating trials for *G. sigillatus* were conducted in the entire terrarium, whereas remating trials for *A. domesticus* were conducted in one side or the other of the terrarium, which was partitioned by a plastic wall. Remating trials were conducted for 40 females in *G. sigillatus* (20 in each treatment), whereas in *A. domesticus*, remating trials were conducted for 23 females that were allowed to consumed a *Gryllodes* spermatophylax after their initial mating compared with 20 females that were not offered a food gift.

Results

Experiment 1: Does the Spermatophylax Contain Receptivity-Inhibiting Substances?

Female *Acheta domesticus* that consumed a spermatophylax after their initial mating took significantly longer to remate than did females that experienced the no-spermatophylax treatment first (fig. 1). Eighty-two percent (14/17) of females that did not consume a spermatophylax in their first mating remated the next day, whereas only 43% (7/16) of females that consumed a spermatophylax in their first mating did so (likelihood ratio $\chi^2 = 5.48$, $P = .019$).

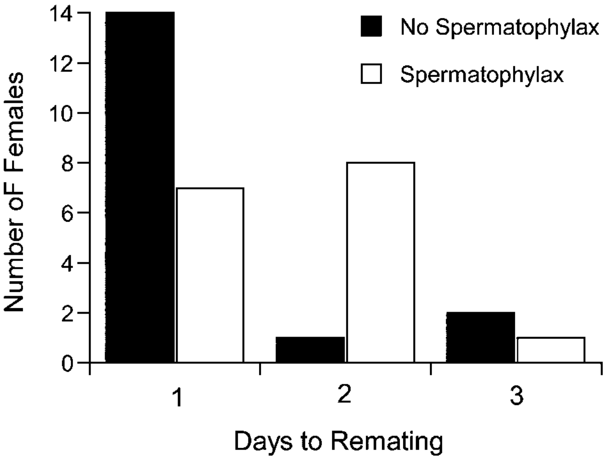

Figure 1: Frequency distribution of the number of days to remating of female *Acheta domesticus* permitted to consume the spermatophylax of a male *Gryllodes sigillatus* (i.e., a novel food gift) after an initial mating or having been given no spermatophylax to consume. Females permitted to consume a spermatophylax took significantly longer to remate ($P = .019$).

Experiment 2: Testing for Resistance

All of the experimental females remated within the specified 4-h time limit, so there were no censored data. The two species were analyzed separately because, owing to logistical constraints, experiments on the two species had to be conducted at different times. The effect of spermatophylax consumption on female latency to remating was assessed using ANCOVA, with date of experimental pairing entered as the covariate and treatment (spermatophylax or no spermatophylax consumed) and mating status (virgin or nonvirgin) entered as categorical variables. The analysis was conducted using the PROC GLM module of the Statistical Analysis System for personal computers (SAS Institute 2000). Data were $\log_{10}$-transformed to meet the assumptions of the ANCOVA. Preplanned pairwise comparisons were adjusted using the Bonferroni method (Sokal and Rohlf 1995).

There was no effect of treatment or mating status on female remating latency in *Gryllodes sigillatus*, nor were any of the interactions significant (table 1; fig. 2). There was, however, a significant treatment by mating status interaction on female remating latency in *A. domesticus* (table 1; fig. 2). Pairwise comparisons revealed that nonvirgin *A. domesticus* females that consumed a spermatophylax after their initial mating took significantly longer to remate than did nonvirgin females that were not offered a spermatophylax ($P = .0042$). However, spermatophylax consumption had no significant effect on the time to remating

Table 1: ANCOVA of time to remating (log-transformed) of female *Acheta domesticus* and *Gryllodes sigillatus*

	Sum of squares	df	*F*	*P*
Acheta domesticus:				
Treatment	.839	1	3.54	.0623
Mating status	.184	1	.78	.3798
Treatment × status	1.204	1	5.08	.0261
Date (covariate)	1.030	1	4.35	.0392
Error	27.249	115	…	…
Gryllodes sigillatus:				
Treatment	.0242	1	.06	.7997
Mating status	.235	1	.63	.4294
Treatment × status	.227	1	.61	.4380
Date	1.429	1	3.82	.0531
Error	43.049	115	…	…

Note: Females were assigned randomly to mating status groups (virgin or sexually experienced) and were given either a *G. sigillatus* spermatophylax to consume after their initial mating or nothing at all. Females were paired with the same male 24 h later. Date of experimental pairing did not interact significantly with any combination of mating or treatment, so all interactions involving date were excluded from the model.

in virgin female *A. domesticus* ($P = .79$). The date of experimental pairing had a significant effect on female remating latency in *A. domesticus* but was not significant in *G. sigillatus* (table 1; fig. 2).

Experiment 3: Resistance in a Complex Environment

Because experiments on the two species were conducted at different times and using slightly different methodologies, results from the two species were analyzed separately. To determine the effect of spermatophylax consumption on remating propensity, we used PROC LIFETEST in SAS (ver. 8.02), which permits the inclusion of right-censored data (i.e., observations in which females had not remated by the end of the 3-day period; Allison 1995). In comparisons between females permitted to consume the spermatophylax and those prohibited from doing so, differences in remating propensity were assessed using the log-rank test.

Consumption of the spermatophylax had no significant effect on the remating propensity of female *G. sigillatus* (median time to remating, first and third quartiles in parentheses; when the spermatophylax was consumed: 3.77 min [$Q_1 = 3.54$, $Q_3 = 7.72$]; when no spermatophylax was consumed: 4.41 min [$Q_1 = 2.86$, $Q_3 = 10.94$]; log-rank $\chi^2 = 0.05$, $P = .83$; fig. 3). However, female *A. domesticus* given a *Gryllodes* spermatophylax to consume took significantly longer to remate (median = 70.4 min [$Q_1 = 26.93$, $Q_3 = 156.22$]) than did females not given a spermatophylax (median = 17.03 min [$Q_1 = 4.78$, $Q_3 = 64.57$]; log-rank $\chi^2 = 4.73$, $P = .029$; fig. 3).

Discussion

Our results support the hypothesis that the spermatophylax, a nuptial food gift synthesized by male *Gryllodes sigillatus* and transferred to the female at mating, has been the target of chase-away sexual selection in the context of a sexual conflict over female remating. When food gifts of male *G. sigillatus* were fed to females of a non-gift-giving species, *Acheta domesticus*, females took significantly longer to remate than did females that were not given food gifts to consume after their initial mating. In contrast, when female *G. sigillatus* were prevented from consuming

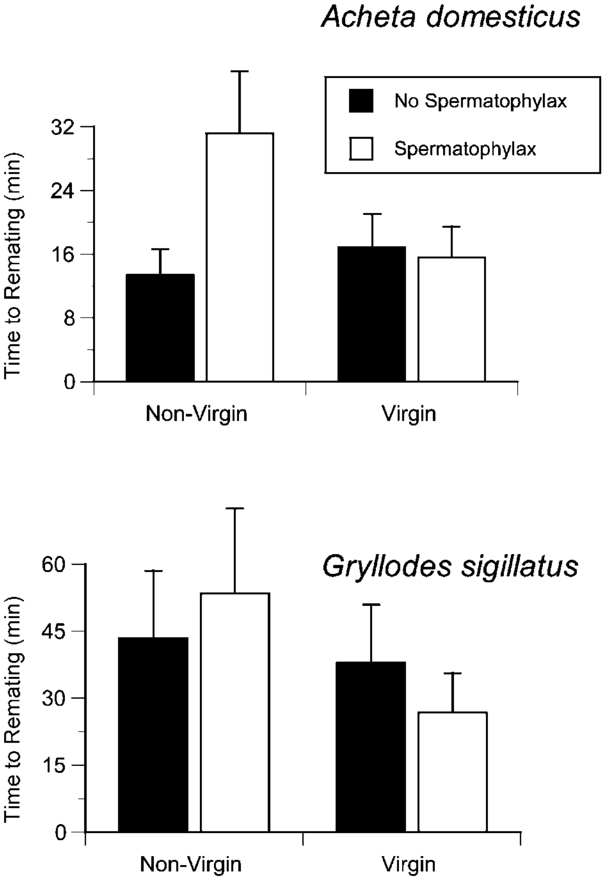

Figure 2: Mean time of remating (±SE; $\log_{10}$-back-transformed) of virgin and nonvirgin female *Acheta domesticus* and *Gryllodes sigillatus* permitted to consume the spermatophylax of a male *G. sigillatus* after an initial mating or being prevented from doing so. Females were equally inseminated across treatments and given 4 h within which to remate 1 day after the initial mating had occurred. Nonvirgin *A. domesticus* females that consumed a spermatophylax after their initial mating took significantly longer to remate than did nonvirgin females that were not offered a spermatophylax ($P = .0042$). However, spermatophylax consumption had no significant effect on the time to remating in virgin female *A. domesticus* ($P = .79$). There was no effect of treatment or mating status on female remating latency in *G. sigillatus* (all $P > .05$).

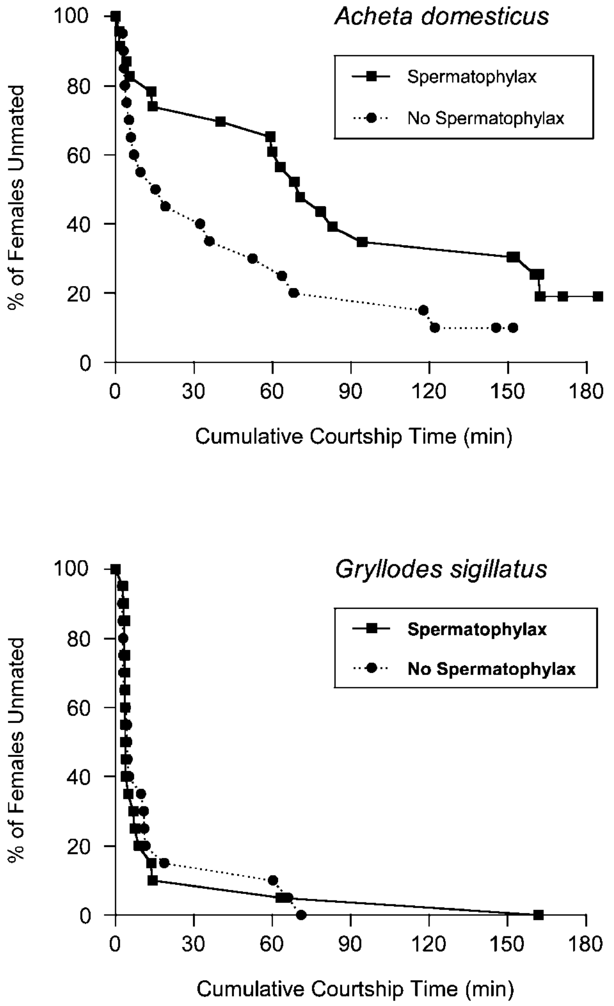

Figure 3: Percent of females remaining unmated as a function of the cumulative courtship time of their mates. Remating trials were staged in large glass aquariums in which females were free to elude their mates. Females *Acheta domesticus* that were permitted to consume the spermatophylax of a male *Gryllodes sigillatus* after an initial mating took significantly longer to remate the next day, compared with females that were not offered a spermatophylax ($P = .029$). In contrast, there was no difference in the latency to remating of female *G. sigillatus* permitted to consume a spermatophylax after an initial mating and those prevented from doing so ($P = .83$).

their partners' nuptial gifts, there was no difference in their propensity to remate relative to females permitted to consume a food gift after mating. These results suggest that the spermatophylax synthesized by male *G. sigillatus* contains substances designed to inhibit the sexual receptivity of their mates but that female *G. sigillatus* have evolved reduced responsiveness (i.e., resistance) to these substances. Female *A. domesticus*, having had no evolutionary experience with spermatophylax consumption, have been under no selection to evolve any resistance to these substances and hence are more susceptible to this form of manipulation.

The decreased mating propensity of female *A. domesticus* permitted to consume a *Gryllodes* spermatophylax cannot be attributed to the differential receipt of sperm or other ejaculatory products because ampulla attachment durations were equalized across treatments in two of the experiments. Moreover, a recent study in which ampulla attachment durations of female *A. domesticus* were experimentally manipulated showed no effect of premature ampulla removal on the time to female remating, either when females were tested immediately or 24 h after a previous mating (Fleischman and Sakaluk 2004). However, we cannot rule out the possibility that females internally manipulate stored sperm and ejaculatory products even after they have been inseminated (see Eberhard 1996).

Although a reduction in female receptivity following consumption of the spermatophylax was observed in nonvirgin *A. domesticus* (experiments 1–3), no such effect was observed in virgin females (experiment 2). This result suggests that the efficacy of antiaphrodisiacs contained in males' nuptial food gifts is contingent on the hormonal state of the females. A number of hormonal changes in female crickets, triggered by mating or the accumulation of sperm or ejaculatory products in the female's spermatheca, have been documented. It is known, for example, that juvenile hormone titers increase dramatically in mated female crickets (review in Loher and Zaretsky 1989), and this appears to influence both female phonotaxis and sexual receptivity (review in Strambi et al. 1997). The ejaculates of male *A. domesticus* contain prostaglandin synthetase (Destephano and Brady 1977), which activates prostaglandin-synthesizing enzymes (PGE and $PGF_{2\alpha}$) in the female and promotes oviposition behavior (Murtaugh and Denlinger 1982, 1987). If the receptivity-inhibiting substances contained in the spermatophylax influence the receptors of these hormones or if the hormones serve as the substrate on which these substances act, it might explain why the sexual receptivity of the nonvirgin females was affected by the ingestion of the spermatophylax, whereas the receptivity of virgin females was not. Regardless of the underlying proximate mechanism, virgin females, who may remain sperm limited even after a single mating, would clearly benefit more than sexually experienced females from any delay in mating-induced hormonal changes that incidentally affect their response to the consumption of receptivity-inhibiting substances.

Notwithstanding its effect on female receptivity, with respect to its nutritional value, the spermatophylax appears to be a sham. Will and Sakaluk (1994) systematically varied food levels under which female *G. sigillatus* were maintained and the number of spermatophylaxes females were permitted to consume per day. There was no effect of spermatophylax consumption on female survival, egg size, or lifetime reproduction, a result that has been replicated in another study (Kasuya and Sato 1998). The absence of detectable nutritional benefits to courtship feeding is not unique to *G. sigillatus* but appears to be widespread across a variety of gift-giving taxa (review in Vahed 1998). Thus, it seems unlikely that the composition of the spermatophylax, as well as that of the nuptial gifts of at least some other insect species, can be explained in the context of a direct-benefits model of female choice. However, it remains possible that conventional female choice could favor increased transfer of particular spermatophylax substances if females receiving below-average quantities of such substances showed a greater propensity to remate than females receiving larger quantities. Indeed, just such a mechanism has been invoked as an example of cryptic mate choice in female scorpionflies that remate sooner if provided with smaller-than-average nuptial prey at their previous mating (Thornhill 1983). While such a mechanism could generate a pattern of remating that superficially resembled a male-induced sexual inhibition, it cannot easily account for the absence of a treatment effect in female *G. sigillatus* prevented from consuming the spermatophylax, nor can it explain the species × treatment interaction documented in this study.

If the evolution of the spermatophylax is explicable within the context of the chase-away model of sexual selection, it requires that males benefit by inducing a delay in remating by their mates and that females suffer a reduction in fitness from any such delay. There is clear evidence to support both of these underlying assumptions. Multiple mating by females is widespread in crickets (review in Zuk and Simmons 1997), and females are capable of storing sperm of multiple mates for extended periods (Sakaluk 1986); these are attributes conducive to a high degree of sperm competition (Parker 1970). The success of a male in sperm competition depends in part on the number of sperm that he transfers to the female because the sperm of a female's various mating partners are recruited for fertilizations in direct proportion to their relative abundance in the female's spermatheca (Sakaluk 1986; Simmons 1987; Sakaluk and Eggert 1996; Eggert et al. 2003; García-González and Simmons 2005). These results suggest that even a modest delay in female remating would reduce the level of sperm competition confronting a female's most recent mate, enhancing his overall reproductive success (see also Calos and Sakaluk 1998). Delayed remating would not, however, necessarily be in the best interests of the female. Studies of a number of cricket species have shown that when females are permitted to mate repeatedly with the same male or with multiple partners, they produce a greater number of offspring or offspring of higher fitness than females whose mating opportunities have been curtailed (Sakaluk and Cade 1980, 1983; Simmons 1988, 2001; Burpee and Sakaluk 1993; Tregenza and Wedell 1998, 2002; Wagner et al. 2001; Fedorka and Mousseau 2002; Sakaluk et al. 2002; Ivy and Sakaluk 2005).

If, as our data suggest, the food gifts of male *G. sigillatus* contain antiaphrodisiacs to which females have evolved resistance, then, assuming there is some cost to their manufacture, why has selection not favored the elimination of such substances? The most likely answer is that there is variation in the level of the resistance in females and that males occasionally encounter females that succumb to the receptivity-inhibiting substances contained in their food gifts. This seems particularly likely when males of one population encounter females from another (i.e., where there has been no recent history of coevolution between the sexes). Alternatively, it may be that these substances serve another important function such as enhancing the phagostimulatory properties of the spermatophylax, promoting its consumption by the female and thereby discouraging premature removal of the sperm ampulla (Sakaluk 1984, 2000).

Notwithstanding the burgeoning interest in sexually antagonistic sexual selection (see Hosken and Snook 2005 and associated articles in the same supplemental volume), the chase-away model has been challenged on both theoretical grounds (e.g., Pizzari and Snook 2003) and on the basis of comparative morphological evidence (Eberhard 2004*a*, 2004*b*). Rosenthal and Servedio (1999) argued that the chase-away model fails to adequately distinguish between female preference and female resistance, and Getty (1999) argued that chase-away sexual selection represents a "noisy" signaling system that is otherwise inherently honest. At least part of the disagreement appears to stem from the difficulty in establishing female resistance, particularly because the chase-away sexual selection model postulates an initial preference for the trait via sensory exploitation of a preexisting sensory bias followed by selection on females to exhibit decreased attraction. In response to these criticisms, Rice and Holland (1999) outlined an explicit model by which female resistance could evolve. While conceding that a test of female preference at a single point in time might make it difficult to empirically distinguish between the evolution of female preference and female resistance, Rice and Holland (1999) argued that within the context of a phylogenetic comparison, such a distinction should be possible. In this regard, it is worth noting that a phylogenetic analysis of the ensiferan Orthoptera (crickets and katydids) suggests that a simple, externally attached sperm ampulla and female consumption of the ampulla were the probable 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). Although some of the details of this phylogeny have been called into question (Flook et al. 1999; Desutter-Grandcolas 2003; Gäde et al. 2003), it seems certain that the absence of a spermatophylax constitutes the ancestral condition within the Gryllidae (true crickets).

Because the inherent conflict between male and female *G. sigillatus* over the timing of female remating is not readily apparent in normal interactions between males and females but becomes manifest only when the spermatophylax is fed to females of other species, it can be regarded as a form of cryptic sexual conflict. Many such kinds of intrinsic sexual conflicts may go overlooked when antagonistic traits in one sex remain concealed as a consequence of counteradaptations in the other (Arnqvist 2004). To the extent that our data provide support to this interpretation, they have established a critical facet of the chase-away model, namely, that females frequently prevail in sexual conflicts, encumbering males with sexual display traits that often have little or no effect on female mating decisions (Holland and Rice 1998). While a single empirical study will not resolve ongoing controversies concerning the role of sexual conflict in shaping courtship traits in males, it does reinforce Arnqvist's (2004) suggestion that phenotypic manipulation of male persistence represents a powerful tool in the array of methods used to uncover sexual antagonism (cf. Pizzari and Snook 2003).

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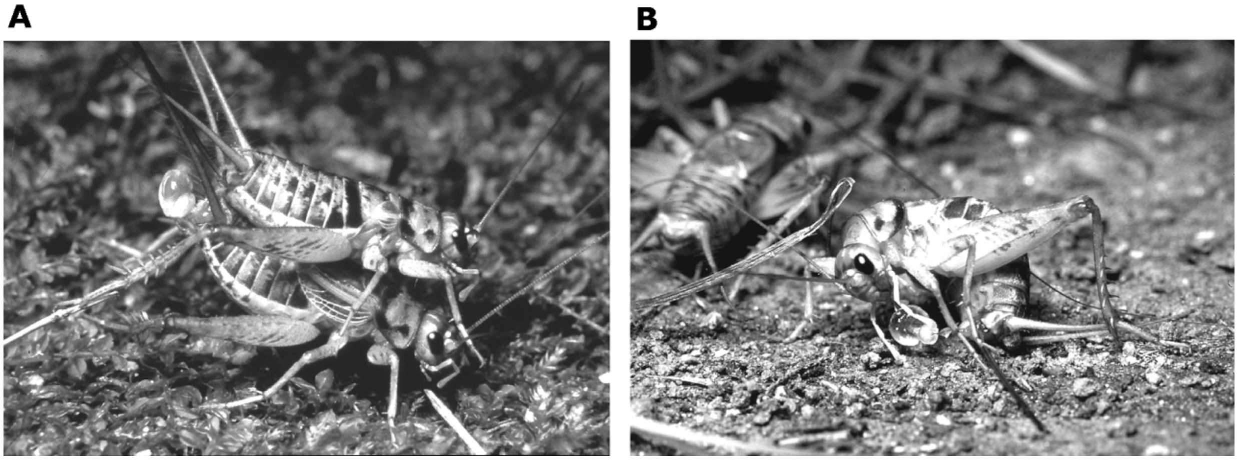

A, Mating in decorated crickets. The male's nuptial gift (spermatophylax) is visible as a translucent ball emerging from the tip of the male's abdomen (cricket on the bottom). *B*, Mated female decorated cricket in the act of detaching the nuptial food gift (translucent ball) from the male's ejaculate (small white capsule). Photographs by David Funk.