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- \* To whom requests for reprints should be addressed.

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## Male Crickets Feed Females to Ensure Complete Sperm Transfer

**Abstract.** The spermatophore transferred by the male decorated cricket *Gryllobates supplicans* to the female during copulation includes a large gelatinous portion (spermatophylax), which the female removes and feeds on immediately after mating. Females usually removed and ate the smaller sperm-containing portion (ampulla) within 1 to 7 minutes after fully consuming or losing the spermatophylax. Complete sperm transfer requires that the ampulla remain attached for a minimum of 50 minutes; this corresponds to the average time at which females actually removed ampullae,  $52.0 \pm 2.2$  minutes after mating. These results indicate that nuptial feeding of the female cricket functions to deter females from removing the sperm ampulla before sperm transfer is complete.

Nuptial feeding of females by males occurs in various insects although its adaptive significance is often unclear. Food gifts presented to females before, during, or after mating include prey captured by the male, glandular products and, in some instances, the male's own body (1). The bipartite spermatophore

which the male decorated cricket, *Gryllobates supplicans*, transfers to a female during copulation consists of a large gelatinous mass or spermatophylax attached to a smaller sperm-containing ampulla (2). Immediately after mating, the female detaches the spermatophylax and feeds on it before removing and eating

the sperm ampulla. Alexander and Otte (2) proposed that the spermatophylax of *G. supplicans* might prevent the female from removing the sperm ampulla before it was emptied of sperm. Although this "sperm protection" function has been attributed to glandular feeding in other gryllids as well (3-5), it never has been empirically demonstrated. I now report that nuptial feeding of female crickets deters females from removing the sperm ampulla until complete sperm transfer has occurred.

After mating, the spermatophylax was normally removed by a female *G. supplicans* within 1 to 5 seconds of dismounting the male (202 of 228 matings) (6). Alexander and Otte's hypothesis (2) predicts that the female should remove the sperm ampulla shortly after the spermatophylax is fully consumed. Females finished eating the spermatophylax  $39.8 \pm 0.7$  minutes (mean  $\pm$  standard error) after mating and subsequently removed and ate the ampulla  $12.2 \pm 1.5$  minutes later ( $N = 143$ ) (Fig. 1) (7). In 64 percent of the matings (91 of 143), females removed ampullae less than 7 minutes after fully consuming the spermatophylax. These results reveal that a female removes the sperm ampulla shortly after feeding regardless of the feeding-phase duration.

Loss of the spermatophylax to the female should result in her removing the sperm ampulla within a similar time period as occurs after normal feeding. In many cases, the female only ate part of the spermatophylax before dropping what remained. This normally occurred  $10.5 \pm 2.0$  minutes after mating ( $N = 85$ ). In these cases, the female removed the ampulla  $10.1 \pm 1.6$  minutes later, regardless of the amount of time that had

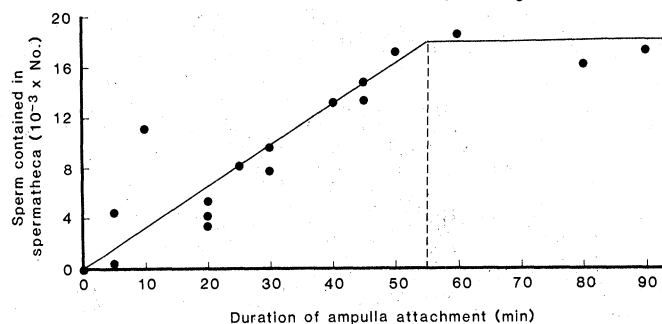
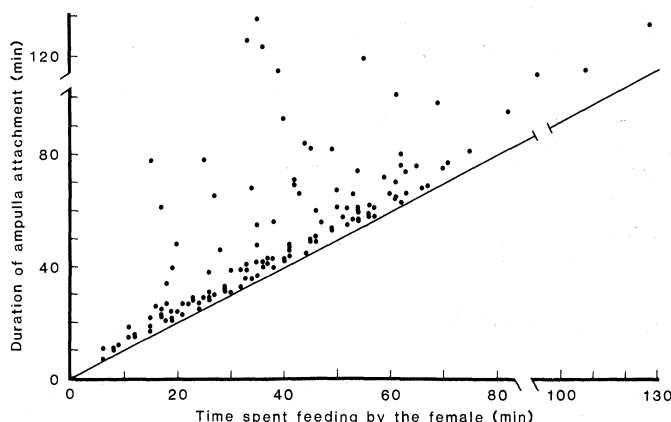


Fig. 1 (left). The time after mating at which female *G. supplicans* removed the sperm ampulla as a function of the time at which they fully consumed the spermatophylax. The line describes those points where the duration of ampulla attachment equals the time spent

feeding by the female. Females removed the ampulla  $12.2 \pm 1.5$  minutes (mean  $\pm$  standard error) after fully consuming the spermatophylax (median time, 5 minutes; range, 1 to 105 minutes). Fig. 2 (right). The number of sperm transferred to the female's spermatheca as a function of the duration of ampulla attachment ( $N = 18$ ). The point at which the dotted line intersects the x-axis (abscissa) represents the time at which sperm transfer is probably complete. The lines drawn are based on the assumption that the 55-minute cutoff after sperm ejaculation ceases would lead to a constant value after that time, thus:  $y = 0.322x$  for  $y < 55$  minutes.

been spent feeding on the spermatophylax. This positive correlation between the time at which females dropped the remnants of the spermatophylax and the time at which they removed the ampulla was statistically significant (Spearman rank correlation;  $r = 0.605$ ;  $P < 0.001$ ).

If male production of the spermatophylax has evolved to ensure maximum sperm transfer, males should be selected to provide no more than an adequate amount of spermatophylax material to deter a female from removing the ampulla until it is essentially depleted of its contents. Thus, the time required for complete transfer of sperm from the ampulla should correspond to the average time taken after mating for removal of the sperm ampullae. To test this prediction, virgin female *G. supplicans* were mated, and the durations of ampulla attachment were varied by experimentally removing ampullae at set intervals. Females were then killed and their sperm-storage organs (spermathecae) were removed. The number of sperm contained in the spermatheca was determined by staining the sperm nuclei with Hoechst 33258 and counting the number under epifluorescence microscopy (8). The number of sperm transferred to the spermatheca showed a linear increase with increasing durations of ampulla attachment until approximately 55 minutes; at this time sperm transfer leveled off (Fig. 2). An ampulla attachment duration of 1.5 hours resulted in essentially the same sperm transfer as one of 50 minutes. In certain cases, the experimentally removed ampullae were placed in phosphate-buffered saline. When such ampullae had only been attached for 0 to 45 minutes, the ejaculation of the sperm could be observed under a dissecting microscope ( $N = 12$ ). However, such evident ejaculation did not occur with ampullae that had been removed more than 45 minutes after mating ( $N = 5$ ) (9). The complete transfer of sperm from the ampulla requires, therefore, a minimum ampullar attachment time of about 50 minutes; females remove ampullae at about the same time, an average of  $52.0 \pm 2.2$  minutes after mating. Thus, it

appears that male *G. supplicans* provide females with a nuptial meal no larger than that required to prevent premature removal of the sperm ampulla.

The results show that male *G. supplicans* feed females and thereby ensure transfer of their sperm. The costs to a male *G. supplicans* of producing the spermatophore are not trivial. The spermatophore can assume up to about 6 percent of a male's body weight, and males remate  $3.3 \pm 0.1$  hours after a previous mating (10). In other crickets, males transfer a smaller spermatophore consisting solely of the ampulla without a spermatophylax and remate within as little as 15 minutes (2). Nonetheless, even in these species females routinely remove and eat the spermatophore after mating (11). Males do not provide additional feeding material (for example, a spermatophylax) in these species probably because they exert direct control over the duration of spermatophore attachment; male field crickets, *Teleogryllus commodus*, guard females after copulation and this behavior results in longer durations of spermatophore attachment (12).

In katydids (Tettigoniidae), spermatophylaxes are a common component of spermatophores, often accounting for up to 20 percent of a male's body weight (13). Recent evidence suggests that the generally larger spermatophores of katydids contain feeding material far in excess of that required to guarantee insemination (14) and represent significant male nutrient investment (14–16). The data for *G. supplicans* illustrate an important intermediate step in the evolution of this form of male investment. Nuptial feeding by males may have initially functioned to ensure maximal insemination of females; selection has subsequently acted on this evolutionary substrate to increase the size of the nuptial meal when circumstances favored increased nutrient investment by males.

SCOTT K. SAKALUK

Department of Zoology,  
Erindale College,  
University of Toronto, Ontario,  
Mississauga, Canada L5L 1C6

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6. Each of 15 virgin female *G. supplicans* was placed in individually marked, plastic containers (2 liters) covered with screened lids. Two randomly selected adult males were introduced into each container on each of the 20 consecutive nights of the study. The mating behavior of the crickets was subsequently monitored continuously for 4 hours. Introduced males were removed immediately after the 4-hour observation period. Crickets were kept under a photoperiod of 12 hours light and 12 hours of darkness (L:D, 12:12) at  $28^\circ \pm 2^\circ\text{C}$ .
7. Ampulla removal did not occur before the spermatophylax was fully consumed or lost to the female [as predicted by Alexander and Otte's (2) hypothesis]. Values for the time at which ampullae were removed could not, therefore, be normally distributed for any given value of  $X$  (time spent feeding), precluding the use of a linear regression analysis.
8. The spermatheca of a female was placed in a test tube containing 1 ml of phosphate-buffered saline and subsequently sheared by repeated forcing through a plastic syringe (Lure tip, 7236A-Stylex). Random dispersal of the sperm was further accomplished mechanically (Vortex). Each of five drops (10  $\mu\text{l}$ ) were placed on glass microscope slides. Sperm were subsequently stained with a DNA-specific stain (Hoechst 33258); the heads of the sperm were a fluorescent bright blue when observed with a Zeiss photomicroscope equipped for epifluorescence microscopy. The total number of sperm circumscribed on the slides was used to estimate the number of sperm contained within the spermatheca (S. K. Sakaluk and D. H. O'Day, in preparation).
9. This result is corroborative only if the rate at which the ejaculate is expelled from the ampulla in vitro parallels the in situ condition.
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16. My study does not preclude the possibility that spermatophylax feeding by female *G. supplicans* may result incidentally in an increase in female reproductive success. D. T. Gwynne [*Nature (London)*, in press] recently has shown that consumption of spermatophores by female katydids (*Requena verticalis*) leads to an increase in the size and number of eggs produced.
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