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NEW PERSPECTIVES

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No effect of nuptial gift consumption on female reproductive output in the bushcricket *Leptophyes laticauda* Friv.

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The spermatophores of the majority of bushcrickets (Orthoptera: Ensifera; Tettigoniidae) consist of two parts: a sperm-containing ampulla and an often large, sperm-free spermatophylax. Following the end of copulation, the female eats the spermatophylax before consuming the ampulla (Boldyrev, 1915). The spermatophylax therefore represents a form of nuptial gift (see Thornhill & Alcock, 1983; Simmons & Parker, 1989; Boggs, 1995 for reviews of nuptial feeding in insects).

There are two different, although not mutually exclusive, hypotheses to account for the function of the spermatophylax (for literature, see Vahed & Gilbert, 1996; Gwynne, 1997). The ejaculate protection hypothesis proposes that the spermatophylax functions to prevent the female from removing the ampulla before ejaculate transfer is complete (see, for example, Sakaluk, 1984; Wedell & Arak, 1989; Reinhold & Heller, 1993; Simmons, 1995). The paternal investment hypothesis, on the other hand, proposes that a male benefits from spermatophylax production through the spermatophylax nutrients consumed by his mate being used to increase the fitness and/or number of his own offspring (reviewed by Gwynne, 1990, 1997). In support of this hypothesis, females receiving a spermatophylax at mating have been found subsequently to produce more and/or heavier eggs than females deprived experimentally of the spermatophylax in the tettigoniids *Requena verticalis* (Gwynne, 1984, 1988; but see Gwynne *et al*., 1984, who found no such effect in this species) and *Kawanaphila nartee* (Simmons, 1990; Simmons & Bailey, 1990). However, no effect of spermatophylax consumption on female reproductive output has been found in the tettigoniids *Poecilimon veluchianus* (Reinhold & Heller, 1993) and *Decticus verrucivorus* (Wedell & Arak, 1989) or in the gryllid *Gryllodes sigillatus* (Will & Sakaluk, 1994) even when, in the latter two species, females were maintained on low-quality diets.

In this study, the effect of spermatophylax consumption by females on the number and weight of eggs subsequently produced was examined in the bushcricket *Leptophyes laticauda*. The spermatophylax of this species is large, contributing to a mean loss of 23% (11–33%) of male body weight at mating (Vahed & Gilbert, 1996). Because the effects of male-derived nutrients on female fecundity are expected to be more pronounced when the level of nutrients available to females is below the amount necessary for maximum fecundity (Gwynne, 1984; Gwynne *et al.*, 1984; Boggs, 1990), the effect of spermatophylax consumption on female reproductive output

Table 1. Analysis of the number of eggs produced in the first week after mating (logged) and the weight of eggs (mean weight of twelve eggs per female) with treatments (plus or minus spermatophylax; restricted or normal diets) and the covariate log pronotum length.

Analysis of variance	Log egg number			Egg weight		
Source of variation	*F*	d.f.	*P*	*F*	d.f.	*P*
Log pronotum length (covariate)	2.38	1	0.13	0.08	1	0.78
Spermatophylax	0.23	1	0.64	0.09	1	0.76
Diet	1.28	1	0.27	1.17	1	0.29
Interaction	0.00	1	0.99	0.27	1	0.61
Error		28			28	

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Table 2. Mean number and weight of eggs (mean weight of twelve eggs per female, mg) laid by females in each treatment in the week after mating (means are cited ± SE; numbers in parentheses are the number of females in each treatment).

	Mean number of eggs		Mean egg weight	
Diet	+ Spermatophylax	– Spermatophylax	+ Spermatophylax	– Spermatophylax
Normal	34.9 ± 4.9 (10)	29.9 ± 4.9 (10)	2.19 ± 0.05 (10)	2.25 ± 0.05 (10)
Restricted	23.3 ± 6.3 (6)	23.6 ± 5.8 (7)	2.16 ± 0.07 (6)	2.14 ± 0.07 (7)

Table 3. Analysis of the number of eggs produced over a 4-week period (logged) and the weight of eggs (mean weight of up to forty-eight eggs per female) with treatments (plus or minus spermatophylax; restricted or normal diets) and the covariate log pronotum length.

Analysis of variance	Log egg number			Egg weight		
Source of variation	*F*	d.f.	*P*	*F*	d.f.	*P*
Log pronotum length (covariate)	0.40	1	0.54	0.90	1	0.36
Spermatophylax	0.29	1	0.60	0.27	1	0.61
Diet	42.8	1	< 0.001	0.16	1	0.69
Interaction	0.36	1	0.56	0.35	1	0.57
Error		26			26	

was examined here using females maintained on both normal and impoverished diets.

The origin and maintenance in captivity of the *L. laticauda* stocks used in this experiment are outlined in Vahed (1995). Immediately following the final moult, females were housed individually in 2-L polythene containers with nylon mesh set into the lid for ventilation and were assigned at random to one of two experimental groups: in the normal-diet group, females were fed *ad libitum* with *Buddleia* leaves ($n = 20$ females), while in the restricted-diet group, females were provided with only one *Buddleia* leaf (⊤ 8 cm long) per week ($n = 13$ females; the sample size was initially larger but four females in this category died during the first week after mating and a further two died during the second week after mating). In both categories, the *Buddleia* leaves were placed in a vial of water to maintain freshness. A sample of eight females from each dietary group was weighed when newly adult and again when first showing signs of sexual receptivity (i.e. showing response stridulation or attempting to mount a male). Pronotum length was measured (to the nearest 0.1 mm) for all females, using a pair of vernier callipers. On each day following the final moult, each female was placed in a mesh observation cage (measuring ⊤ 10 × 10 × 10 cm) with a stridulating male and observed for 1 h. If females mated, they were assigned to one of two further experimental groups: in the plus-spermatophylax group, females were allowed to consume fully the spermatophylax after mating ($n = 16$ females, including ten normal-diet females and six restricted-diet females); in the minus-spermatophylax group, the spermatophylax was sprinkled with sand to deter the female from eating it ($n = 17$ females, including ten normal-diet females and seven restricted-diet females). This technique was taken from Reinhold & Heller (1993). Females with spermatophylaxes treated in this way invariably left them uneaten. At about 6 h after spermatophore deposition, the entire spermatophore of females in the minus-spermatophylax category was removed with forceps. This time corresponds to the mean time taken for females of this species to consume the spermatophylax and eat the ampulla (Vahed, 1995). Counts of the number of sperm remaining in the spermatophore after this time indicated that the minus-spermatophylax treatment did not interfere with sperm transfer.

Following mating, females were returned to their individual cages and each was provided with a block of polyurethane foam (measuring ⊤ 5 × 5 × 5 cm) in which to lay eggs. Females were maintained on the same diet (normal or restricted) after mating as before mating. Each week following mating, over a period of 4 weeks, the block of foam from each female was removed and replaced. For each female, the number of eggs produced each week following mating was recorded for a period of 4 weeks and a random sample of twelve eggs from each week was weighed to the nearest 0.01 mg on a Cahn 25 electrobalance.

The effects of spermatophylax feeding and diet on the mean weight and number of eggs laid during the 4 week observation period were determined by two-way ANOVA, with spermatophylax feeding (plus or minus spermatophylax) and diet (normal or restricted) as the main factors and log female pronotum length as a covariate. The mean weight and number of eggs laid during the first week of mating were also analysed separately because females might use spermatophylax nutrients in only the first batch of eggs following mating. One week is also the mean sexual refractory period for females of this species (Vahed, 1995). Prior to analysis, egg number was transformed logarithmically to meet the assumptions of parametric ANOVA. The software package nQuery was used to calculate the power of the tests. All means are cited ± standard error.

There was no significant difference in the mean number of days from the final moult to the onset of sexual receptivity between normal and restricted-diet females (mean for normal-diet = 10.3 ± 0.6 days, range 7–15 days, $n = 16$; mean for restricted-diet = 10.3 ± 0.4 days, range 9–12 days, $n = 7$; one-way ANOVA $F_{1,21} = 0.001$, $P > 0.05$). While there was no difference in mean body weight when first adult between normal- and restricted-diet females (mean body weight for normal-diet females = 0.33 ± 0.01 g, $n = 8$; mean body weight for restricted-diet females = 0.33 ± 0.17 g, $n = 7$; one-way ANOVA $F_{1,13} = 0.0$, $P > 0.05$), females in the restricted-diet category were significantly lighter than females in the normal-diet category at the onset of sexual receptivity (mean body weight for restricted-diet females = 0.47 ± 0.03 g, $n = 7$;

Table 4. Mean number and weight of eggs (mean weight of up to forty-eight eggs per female, mg) laid by females in each treatment over a period of 4 weeks after mating (means are cited ± SE; numbers in brackets are the number of females in each treatment).

Diet	Mean number of eggs + Spermatophylax	– Spermatophylax	Mean egg weight +Spermatophylax	– Spermatophylax
Normal	99.9 ± 6.3 (10)	97.8 ± 6.3 (10)	2.17 ± 0.05 (10)	2.25 ± 0.05 (10)
Restricted	48.4 ± 8.9 (5)	50.5 ± 8.1 (6)	2.19 ± 0.07 (5)	2.17 ± 0.06 (6)

mean body weight for normal-diet females = 0.60 ± 0.03 g, $n = 8$; one-way ANOVA $F_{1,13} = 8.9$, $P = 0.01$). This indicates that, as expected, the restricted diet was suboptimal in terms of energy and/or nutrient intake for the females. The fact that six females in the restricted-diet category died during the 4-week observation period supports this assertion.

For eggs laid in the first week after mating (Tables 1 and 2), diet, spermatophylax feeding and the covariate female pronotum length were not found to have a significant effect on egg number or egg weight. For eggs laid in the 4 weeks after mating (Tables 3 and 4), the restricted diet was found to have a significant negative effect on egg number, while spermatophylax feeding and female pronotum length had no significant effect. Spermatophylax feeding and female pronotum length did not have a significant effect on egg weight (Tables 3 and 4). There were no significant interactions (Tables 1–4). It should, however, be noted that the power of the above tests was low (10–16%), so it is possible that the failure to find significant differences between groups was an artefact of the small sample sizes. Nevertheless, the alpha levels were far from significant and the differences between plus- and minus-spermatophylax groups in mean egg number and weight were small (Tables 2 and 4), and in half of the comparisons were actually in the opposite direction to that predicted by the paternal investment hypothesis.

A negative effect of a restricted diet on the number of eggs laid over a period of 4 weeks following mating was found for female *L. laticauda* in this experiment. A similar decrease in the number of eggs produced with a decrease in diet quality has been found in other ensiferans (Gwynne, 1988; Wedell & Arak, 1989; Simmons & Gwynne, 1993; Will & Sakaluk, 1994) and is well documented in insects in general (for literature, see Wheeler, 1996). In *Kawanaphila nartee*, the difference in fecundity between females maintained on high- and low-quality diets found by Simmons & Gwynne (1993) appeared to be due to the fact that females on the low-quality diet allocated a greater proportion of the nutrients obtained from both the general diet and spermatophylax consumption to somatic maintenance rather than to reproduction (Simmons & Gwynne, 1993). When eggs laid by female *L. laticauda* in the first week after mating in the present study were analysed, however, no significant effect of diet on the number of eggs laid was found. This suggests that females might have used energy reserves to compensate for poor diet quality in the first week after mating.

No significant difference in either the number of eggs produced or the weight of these eggs was found between female *L. laticauda* that were allowed to consume the spermatophylax and females prevented from doing so in this study, even when females were maintained on a restricted diet (although it should be noted that the power of the statistical tests in this study was low). The spermatophylaxes produced by male *L. laticauda* are large, representing up to a third of male body weight (Vahed & Gilbert, 1996). The paternal investment hypothesis would therefore predict a positive effect of spermatophylax consumption on female reproductive output in this species (see Gwynne, 1990). Taking into consideration other recent studies that have failed to detect any effect of spermatophylax consumption on female reproductive output in ensiferans (see Wedell & Arak, 1989; Reinhold & Heller, 1993; Will & Sakaluk, 1994), the generality of the phenomenon of an increase in egg weight and/or number as a result of spermatophylax-feeding would seem to be in doubt.

There could, of course, be more subtle effects of spermatophylax-feeding on offspring fitness than an increase in egg weight. While therefore the failure to find a positive effect of spermatophylax-feeding on egg weight or number does not provide support for the paternal investment hypothesis, it does not refute it. Furthermore, in highly fecund species such as *L. laticauda*, any effect of spermatophylax nutrients on egg weight will be diluted over a large number of eggs and will therefore be more difficult to detect than in less fecund species such as *Kawanaphila nartee* (studied by Simmons, 1990).

It should be noted that this experiment was primarily concerned with the possible benefits, in terms of an increase in egg weight or number, resulting from spermatophylax feeding from the perspective of the male that produced the spermatophylax. Experiments examining the female's perspective (i.e. the benefits to females of multiple mating in terms of receiving male-donated nutrients) might offer females a wider range of spermatophylaxes than in the present study (as in Gwynne, 1984, 1988) and might concentrate more on the possible benefits to a female from spermatophylax consumption in terms of reduced foraging activity (see Boggs, 1990; Heller, 1996) and increased lifespan (see Burpee & Sakaluk, 1993; Will & Sakaluk, 1994).

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