SHORT COMMUNICATION

Fitness advantage from nuptial gifts in female fireflies

JENNIFER ROONEY and SARA M. LEWIS Department of Biology, Tufts University, U.S.A.

Abstract. 1. In many insects, males provide nuptial gifts to females in the form of spermatothoraxes, sperm-containing structures produced by male accessory glands.

2. The work reported here examined the influence of both spermatothorax number and spermatothorax size on female reproductive output in two related firefly beetles, Photinus ignitus and Ellychnia corrusca (Coleoptera: Lampyridae). Based on differences in adult diet, male spermatothoraxes were predicted to increase female reproductive output to a greater extent in P. ignitus than in E. corrusca.

3. Female fecundity was significantly higher in triply mated females than in singly mated females in both species, with no difference between mating treatments in female lifespan or egg hatching success. No effects of second male spermatothorax size on fecundity, lifespan, or egg hatching success were detected in either species.

4. These results suggest a direct fitness advantage from multiple mating for females in both species, although enhanced fecundity may be due either to allocation of spermatothorax nutrients to eggs or to other substances transferred within the spermatothorax acting as oviposition stimulants.

Key words. Ellychnia, fecundity effects, multiple mating, nuptial feeding, Photinus, spermatothorax.

Introduction

During courtship and mating in many insects, males provide females with nuptial gifts, including captured prey items, male body parts, or male glandular secretions (Thornhill, 1976; Zeh & Smith, 1985; Vahed, 1998). Male spermatothoraxes, sperm-containing structures of varying complexity produced by male accessory glands, are common nuptial gifts across diverse taxa (Mann, 1984). Previous studies examining how male spermatothoraxes influence female reproductive output have focused primarily on orthopterans and lepidopterans reviewed by Ridley, 1988; Simmons & Parker, 1989; Wedell, 1993; Boggs, 1995; Gwynne, 1997; Vahed, 1998). These studies have revealed considerable variation among species but factors influencing the presence and magnitude of spermatothorax effects on female fecundity have yet to be identified clearly. Adult diet is a potentially key factor that may alter the relative importance of male nutrient donations to female reproductive output (Boggs, 1990).

The work reported here examined whether female reproductive output is influenced by either multiple mating or variation in male spermatothorax size in two lampyrid beetles, Photinus ignitus fall and Ellychnia corrusca LeConte. These closely related species (LeConte, 1881; McDermott, 1964) exhibit marked differences in life history, Photinus ignitus is a nocturnally active, bioluminescent firefly with short-lived, non-feeding adults. Ellychnia corrusca is a diurnally active, non-luminescent beetle with long-lived, feeding adults. During mating, males of both species transfer a single spermatothorax that undergoes digestion over several days in a specialised structure within the female reproductive tract (van der Reijsden et al., 1997; Rooney & Lewis, 1998). Ellychnia corrusca and P. ignitus females showed distinct allocation patterns when male spermatothoraxes were radio-labelled with 3H-amino acid mixtures (Rooney & Lewis, 1999): P. ignitus females allocated the majority of male-derived protein to their maturing oocytes, while E. corrusca allocation was mainly to female somatic tissue. Because P. ignitus do not feed as adults, female vitellogenesis depends on resources acquired either from larval feeding or from male spermatothoraxes, whereas additional dietary input is available to E. corrusca females. Based on both this dietary contrast and differences in the allocation pattern of male-derived
nutrients, it was predicted that male spermatophores would increase female fecundity to a greater extent in *P. ignitus* than in *E. corrugata*. Male spermatophores were predicted to increase female longevity in *E. corrugata*.

**Materials and methods**

**Study organisms**

*Photinus* fireflies are nocturnally active, their courtship consisting of a bioluminescent flash dialogue between flying, signalling males and stationary, responding females (Lloyd, 1966). *Photinus* adults of both sexes live for up to 2 weeks and are capable of mating several times during their ~4-week summer mating season (Lewis & Wang, 1991; Lewis & Monchamp, 1994). *Photinus* do not eat as adults (Williams, 1917; Wing, 1989; Lloyd, 1997), thus both sexes rely on larval reserves to support reproductive activity.

In contrast, *Ellychnia corrugata* adults are diurnally active and lack light organs. Adults overwinter following autumn emergence, and mate in early spring ~7 months after adult eclosion. Adults feed on tree sap and flowers (Dillon & Dillon, 1972; Rooney & Lewis, 2000), and both sexes mate multiply (Rooney & Lewis, 2000).

**Effects of multiple mating**

The influence of multiple spermatophores on female reproductive output was examined by mating females of each species either once to a single, conspecific male or sequentially to three different, conspecific males. These mating treatments were based on field estimates of female mating frequency ranging from zero to four matings over 2 weeks in a related firefly, *Photinus marginellus* (Lewis & Wang, 1991). The natural mating frequency for *E. corrugata* females is unknown but females in captivity had a median of four matings over 10 days (Rooney & Lewis, 2000).

*Ellychnia corrugata* adults (n = 40 females) were collected from Belmont, Massachusetts (42°23′N, 71°10′W) during their mating season in April – May 1997. The experiment was repeated in 1998 using virgin females (n = 30) collected prior to their mating season; adults in both years were ~7 months old when used in experiments. Results were similar between years so data were pooled for analysis. *Photinus ignitus* adults (n = 40 females) were collected early in their mating season during July 1998 from Lincoln, Massachusetts (42°25′N, 71°18′W) to increase the likelihood that beetles had not mated previously.

Females were weighed to the nearest 0.1 mg within 24 h of collection, and were assigned randomly to either a single mating or three matings with different, field-collected males. Females were kept individually in 100-ml plastic containers with moist filter paper, and beetles were not fed for the duration of the experiment. Females assigned to the singly mated group were paired with a male and were observed until mating occurred. Females assigned to the triply mated group were allowed to mate sequentially with three field-collected males, with no more than 48 h between each mating. Following their final mating, females were given *Plagiomniun* sp. moss for oviposition, and eggs were collected daily until the female died. Eggs laid between matings by triply mated females were included in total egg counts. Eggs were incubated at 29 °C until they hatched into first-instar larvae (~15 days). For both *E. corrugata* and *P. ignitus*, female lifespan (days from collection until death), female fecundity (number of eggs laid), and hatching success (percentage of eggs surviving to first larval instar) were measured.

**Effects of variation in spermatophore size**

Previous studies have demonstrated a significant decrease in male spermatophore mass and protein content across sequential matings for *P. ignitus* males, with a 35% decrease in spermatophore mass observed between the first and second laboratory matings of field-collected males (Rooney, 2000). In this study, second male spermatophore size was altered by manipulating male mating history. For each species, 30 females were weighed to the nearest 0.1 mg within 24 h of collection, and allowed to mate in the laboratory with a randomly chosen, field-collected male to standardise female mating history. Forty-eight hours after this initial mating, half the females were assigned randomly to mate with a second male that had mated recently (within 48 h) in the laboratory (*n* = 15 small spermatophore females for each species). The remaining females were mated next to a control male that had not mated for at least 48 h (*n* = 15 large spermatophore females for each species). Following the second mating, eggs were collected from females, and female lifespan, fecundity, and egg hatching success were measured as described above.

**Statistical analysis**

Because female fecundity increases with body weight, female weight was included as a covariate in ANCOVAs to examine treatment effects on fecundity in both experiments. Before testing for treatment effects, homogeneity of regression slopes between treatments was confirmed (for singly mated vs. triply mated females: *P. ignitus*: *F*<sub>1,36</sub> = 1.1, *P* = NS; *E. corrugata*: *F*<sub>1,66</sub> = 2.3, *P* = NS; for large spermatophore vs. small spermatophore females: *P. ignitus*: *F*<sub>1,26</sub> = 0.65, *P* = NS; *E. corrugata*: *F*<sub>1,25</sub> = 0.07, *P* = NS). T-tests were used to compare female lifespan and hatching success (hatching data ln-transformed to normalise distributions) between treatments.

**Results**

**Effects of multiple mating**

Females of both species laid more eggs when they mated multiply (Fig.1). Triply mated *P. ignitus* females

had significantly increased fecundity compared with singly mated females (ANOVA for mating frequency, F_{1,26} = 8.8, P = 0.005). *Ellychnia corrusca* females also showed increased fecundity following three matings compared with a single mating (ANOVA for mating frequency, F_{1,26} = 6.8, P = 0.01). Comparison of adjusted least-squares means (Table 1) indicated that in *P. ignitus*, the fecundity of triply mated females increased 73% relative to singly mated females, with an increase of 41% in *E. corrusca* females.

Egg hatching success was high for both singly mated and triply mated females in both *P. ignitus* (mean ± SE = 87.8 ± 5.5% and 93.0 ± 1.6% respectively) and *E. corrusca* (92.7 ± 1.6% and 90.9 ± 1.5%), and was unaffected by number of matings (t-tests on In-transformed data, *P. ignitus*: t = 0.2, d.f. = 36, P = NS; *E. corrusca*: t = 0.8, d.f. = 66, P = NS).

In *P. ignitus*, triply mated females lived 5.8 ± 0.3 days while singly mated females lived 4.9 ± 0.3 days, but this difference was not significant at the 5% level (t = 1.9, d.f. = 38, P = 0.068). No difference in lifespan was found for triply mated *E. corrusca* females (8.2 ± 0.3 days) compared with singly mated females (7.8 ± 0.3 days; t = 0.8, d.f. = 68, P = NS).

**Effects of spermatophore size**

Spermatophore size did not affect female reproductive output in either *P. ignitus* or *E. corrusca* (Table 2). Fecundity did not differ between females whose second mate was a control male (large spermatophore) and females whose second mate was a recently mated male (small spermatophore) (ANOVA for mating frequency: *P. ignitus*, F_{1,26} = 0.4, P = NS; *E. corrusca*, F_{1,25} = 0.2, P = NS; both tests had power ≈70% to detect a difference between treatment means as large as 1 SD). The percentage of eggs hatching also did not differ between spermatophore size treatments in either species (In-transformed data: *P. ignitus*, t = 0.1, d.f. = 28, P = NS; *E. corrusca*, t = 1.3, d.f. = 27, P = NS), and no difference was found in female lifespan (*P. ignitus*, t = 1.3, d.f. = 28, P = NS; *E. corrusca*, t = 1.1, d.f. = 27, P = NS).

**Discussion**

Previous studies have revealed major differences among species in the extent to which nuptial gifts affect female reproductive output, and adult diet has been suggested as one explanation for such variability (Boggs, 1990). The present study confirmed the prediction that multiple spermatophores would increase female fecundity in *Photinus* fireflies, which lack adult feeding; female fecundity increased 73% in triply mated compared with singly mated females. Radiolabelling studies have shown that *P. ignitus* females allocate 62% of total radiolabelled protein from male spermatophores to their mature oocytes within 2 days after mating, with only 27% found in female somatic tissue (Rooney & Lewis, 1999). In contrast, in *E. corrusca* 64% of male-derived protein is found in female somatic tissue (primarily fat body) by 4 days after mating, with only 21% in oocytes. In spite of this much lower allocation of male-derived protein to oocytes, *E. corrusca* showed a 41% increase in fecundity in triply mated females compared with singly mated females. No effect of spermatophore size was
Table 2. Female fecundity (adjusted least-square means), egg hatching success, and female lifespan for *Photinus ignitus* and *Elychnia corrusca* females assigned second mates that were either recently mated males (small spermatophore) or control males (large spermatophore). Means ± 1 SE based on 15 females per treatment, except 14 in the *E. corrusca* small spermatophore treatment.

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<tr>
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<th>Small spermatophore females</th>
<th>Large spermatophore females</th>
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<tr>
<td><em>P. ignitus</em></td>
<td></td>
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<tr>
<td>Fecundity</td>
<td>27.1 ± 2.3</td>
<td>29.2 ± 2.3</td>
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<tr>
<td>Hatching success (%)</td>
<td>97.3 ± 2.4</td>
<td>99.2 ± 4.3</td>
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<tr>
<td>Lifespan (days)</td>
<td>5.8 ± 0.4</td>
<td>6.5 ± 0.4</td>
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<tr>
<td><em>E. corrusca</em></td>
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<tr>
<td>Fecundity</td>
<td>32.1 ± 2.4</td>
<td>35.2 ± 2.4</td>
</tr>
<tr>
<td>Hatching success (%)</td>
<td>92.7 ± 4.2</td>
<td>97.9 ± 0.9</td>
</tr>
<tr>
<td>Lifespan (days)</td>
<td>8.1 ± 0.6</td>
<td>8.9 ± 0.6</td>
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References


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