

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 spermatophores, sperm-containing structures produced by male accessory glands.

2. The work reported here examined the influence of both spermatophore number and spermatophore size on female reproductive output in two related firefly beetles, *Photinus ignitus* and *Ellychnia corrusca* (Coleoptera: Lampyridae). Based on differences in adult diet, male spermatophores 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 spermatophore 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 spermatophore nutrients to eggs or to other substances transferred within the spermatophore acting as oviposition stimulants.

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

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 spermatophores, 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 spermatophores 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 spermatophore 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 spermatophore 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 spermatophore that undergoes digestion over several days in a specialised structure within the female reproductive tract (van der Reijden *et al.*, 1997; Rooney & Lewis, 1999). *Ellychnia corrusca* and *P. ignitus* females showed distinct allocation patterns when male spermatophores 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 spermatophores, 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

Correspondence: Sara M. Lewis, Department of Biology, Tufts University, Medford, MA 02155, U.S.A. E-mail: slewis1@tufts.edu

nutrients, it was predicted that male spermatophores would increase female fecundity to a greater extent in *P. ignitus* than in *E. corrusca*. Male spermatophores were predicted to increase female longevity in *E. corrusca*.

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 \approx 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 corrusca* adults are diurnally active and lack light organs. Adults overwinter following autumn emergence, and mate in early spring \approx 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. corrusca* females is unknown but females in captivity had a median of four matings over 10 days (Rooney & Lewis, 2000).

Ellychnia corrusca 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 \approx 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 *Plagiomnium* 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 (\approx 15 days). For both *E. corrusca* 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*: $F_{1,36}=1.1$, $P=NS$; *E. corrusca*: $F_{1,66}=2.3$, $P=NS$; for large spermatophore vs. small spermatophore females: *P. ignitus*: $F_{1,26}=0.65$, $P=NS$; *E. corrusca*: $F_{1,25}=0.07$, $P=NS$). 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

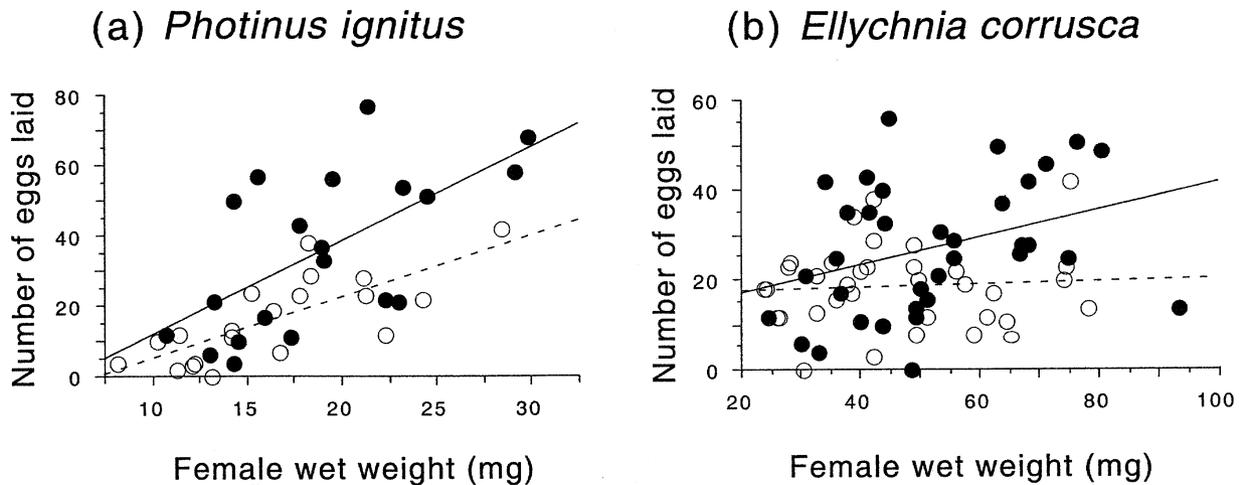


Fig. 1. Relationship between female fecundity and female body weight for singly mated females (---) and triply mated females (—) in (a) *Photinus ignitus* [triply mated females, number of eggs = $-15.1 + (2.7 \times \text{female weight})$; singly mated females, number of eggs = $-12.4 + (1.8 \times \text{female weight})$, $n = 20$ in each mating treatment] and (b) *Ellychnia corrusca* [triply mated females, number of eggs = $11.2 + (0.31 \times \text{female weight})$; singly mated females, number of eggs = $17.0 + (0.03 \times \text{female weight})$, $n = 35$ in each mating treatment].

had significantly increased fecundity compared with singly mated females (ANCOVA for mating frequency, $F_{1,36} = 8.8$, $P = 0.005$). *Ellychnia corrusca* females also showed increased fecundity following three matings compared with a single mating (ANCOVA for mating frequency, $F_{1,66} = 6.8$, $P = 0.01$). 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 \pm SE = $87.8 \pm 5.5\%$ and $93.0 \pm 1.6\%$ respectively) and *E. corrusca* ($92.7 \pm 1.6\%$ and $90.9 \pm 1.5\%$), and was unaffected by number of matings (t -tests on ln-transformed data, *P. ignitus*: $t = 0.2$, d.f. = 36, $P = \text{NS}$; *E. corrusca*: $t = 0.8$, d.f. = 66, $P = \text{NS}$).

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, $P = \text{NS}$).

Effects of spermatophore size

Spermatophore size did not affect female reproductive output in either *P. ignitus* or *E. corrusca* (Table 2). Fecundity

Table 1. Female fecundity (adjusted least-square means \pm 1 SE) for *Photinus ignitus* and *Ellychnia corrusca* females that were singly or triply mated (n = number of females per treatment).

	<i>P. ignitus</i> ($n = 20$)	<i>E. corrusca</i> ($n = 35$)
Singly mated females	18.5 ± 3.2	18.7 ± 2.0
Triplicly mated females	32.0 ± 3.2	26.3 ± 2.0

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) (ANCOVA for mating frequency: *P. ignitus*, $F_{1,26} = 0.4$, $P = \text{NS}$; *E. corrusca*, $F_{1,25} = 0.2$, $P = \text{NS}$; both tests had power $\approx 70\%$ 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 (ln-transformed data: *P. ignitus*, $t = 0.1$, d.f. = 28, $P = \text{NS}$; *E. corrusca*, $t = 1.3$, d.f. = 27, $P = \text{NS}$), and no difference was found in female lifespan (*P. ignitus*, $t = 1.3$, d.f. = 28, $P = \text{NS}$; *E. corrusca*, $t = 1.1$, d.f. = 27, $P = \text{NS}$).

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 *Ellychnia corrusca* females assigned second mates that were either recently mated males (small spermatophore) or control males (large spermatophore). Means \pm 1 SE based on 15 females per treatment, except 14 in the *E. corrusca* small spermatophore treatment.

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

detected in either species, perhaps due to the fact that only second male spermatophore size was manipulated in this study.

Increased female fecundity following multiple mating does not necessarily imply that male spermatophore nutrients are directly responsible for increased reproductive output, as male accessory gland products are known to act as oviposition stimulants in other species (Leopold, 1976; Gillott, 1988; Kaulenas, 1992; Eberhard, 1996). While radiolabelling experiments demonstrate direct incorporation of spermatophore-derived nutrients into oocytes, such techniques allow tracing of small nutrient quantities that may not represent a substantive contribution to total reproductive output (Eberhard, 1996). Other spermatophore components may contribute to increased fecundity in multiply mated females, as the soluble protein contained in each *P. ignitus* spermatophore is small (average 11.4 \pm 0.8 μ g) compared with the protein content of each mature oocyte (600 \pm 150 μ g; J. Rooney, unpublished).

Several additional factors have been proposed to explain interspecific differences in spermatophore effects on female reproductive output and longevity, including relative spermatophore size and protein content, female nutritional status, female age (Boggs, 1995), and predictability in diet quality (Leimar *et al.*, 1994). Relative spermatophore weight may be important for the two species considered here, as spermatophore wet weights in *P. ignitus* represent \approx 11% of male body weight, while *E. corrusca* spermatophores represent only \approx 5% of male body weight (J. Rooney, unpublished).

These results indicate that female fireflies do not remate simply to replenish their sperm supply because hatching success was high even for singly mated females. In addition to the direct fitness advantage of multiple mating demonstrated in this study, it is possible that multiple mating may have indirect effects on offspring fitness. Further studies of how such male-derived nutrients influence both female and male fitness should provide insight into nuptial gift evolution.

Acknowledgements

We thank E. Schlüter for moss identification, and C. Cratsley and two anonymous reviewers for comments on the manuscript. This work was supported by National Science Foundation grant IBN-9816432.

References

- Boggs, C.L. (1990) A general model of the role of male-donated nutrients in female insects' reproduction. *American Naturalist*, **136**, 598–617.
- Boggs, C.L. (1995) Male nuptial gifts: phenotypic consequences and evolutionary implications. *Insect Reproduction* (ed. by S. R. Leather and J. Hardie), pp. 215–242. CRC Press, New York.
- Dillon, E.S. & Dillon, L.S. (1972) *A Manual of Common Beetles of Eastern North America*. Dover, New York.
- Eberhard, W.G. (1996) *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, New Jersey.
- Gillott, C. (1988) Arthropoda – Insecta. *Reproductive Biology of Invertebrates* (ed. by R. G. Adiyodi and K. G. Adiyodi), pp. 319–471. John Wiley & Sons, New York.
- Gwynne, D.T. (1997) The evolution of edible 'sperm sacs' and other forms of courtship feeding in crickets, katydids, and their kin (Orthoptera: Ensifera). *The Evolution of Mating Systems in Insects and Arachnids* (ed. by J. C. Choe and B. J. Crespi), pp. 110–129. Cambridge University Press, Cambridge.
- Kaulenas, M.S. (1992) Insect accessory reproductive structures: function, structure, and development. *Zoophysiology*, **31**, 1–301.
- LeConte, J.L. (1881) Synopsis of the Lampyridae of the United States. *Transactions of the American Entomological Society*, **9**, 15–72.
- Leimar, O., Karlsson, B. & Wiklund, C. (1994) Unpredictable food and sexual size dimorphism in insects. *Proceedings of the Royal Society of London, Series B*, **258**, 121–125.
- Leopold, R.A. (1976) The role of male accessory glands in insect reproduction. *Annual Review of Entomology*, **21**, 199–221.
- Lewis, S.M. & Monchamp, J. (1994) Sexual and temporal differences in phorid parasitism of *Photinus marginellus* fireflies (Coleoptera: Lampyridae). *Annals of the Entomological Society of America*, **87**, 572–575.
- Lewis, S.M. & Wang, O.T. (1991) Reproductive ecology of two species of *Photinus* fireflies (Coleoptera: Lampyridae). *Psyche*, **98**, 293–307.
- Lloyd, J.E. (1966) Studies on the flash communication system in *Photinus* fireflies. *University of Michigan Miscellaneous Publications*, **130**, 1–95.
- Lloyd, J.E. (1997) Firefly mating ecology, selection and evolution. *The Evolution of Mating Systems in Insects and Arachnids* (ed. by J. C. Choe and B. J. Crespi), pp. 184–192. Cambridge University Press, Cambridge.
- Mann, T. (1984) Spermatophores: development, structure, biochemical attributes and role in the transfer of spermatozoa. *Zoophysiology*, **15**, 1–217.
- McDermott, F.A. (1964) The taxonomy of the Lampyridae (Coleoptera). *Transactions of the American Entomological Society*, **90**, 1–72.
- van der Reijden, E.D., Monchamp, J.D. & Lewis, S.M. (1997) The formation, transfer, and fate of spermatophores in *Photinus*

- fireflies (Coleoptera: Lampyridae). *Canadian Journal of Zoology*, **75**, 1202–1207.
- Ridley, M. (1988) Mating frequency and fecundity in insects. *Biological Reviews*, **63**, 509–549.
- Rooney, J.A. (2000) *Male reproductive investment in two fireflies, Photinus ignitus and Ellychnia corrusca: effects on male and female reproductive success*. PhD Dissertation, Tufts University, U.S.A.
- Rooney, J.A. & Lewis, S.M. (1999) Differential allocation of male-derived nutrients in two lampyrid beetles with contrasting life-history characteristics. *Behavioral Ecology*, **10**, 97–104.
- Rooney, J.A. & Lewis, S.M. (2000) Notes on the life history and mating behavior of *Ellychnia corrusca* (Coleoptera: Lampyridae). *Florida Entomologist*, **83**, 324–334.
- Simmons, L.W. & Parker, G.A. (1989) Nuptial feeding in insects: mating effort versus paternal investment. *Ethology*, **81**, 332–343.
- Thornhill, R. (1976) Sexual selection and paternal investment in insects. *American Naturalist*, **110**, 153–163.
- Vahed, K. (1998) The function of nuptial feeding in insects: a review of empirical studies. *Biological Reviews*, **73**, 43–78.
- Wedell, N. (1993) Spermatophore size in bushcrickets: comparative evidence for nuptial gifts as sperm protection devices. *Evolution*, **47**, 1203–1212.
- Williams, F.X. (1917) Notes on the life-history of some North American Lampyridae. *Journal of the New York Entomological Society*, **25**, 11–33.
- Wing, S.R. (1989) Energetic costs of mating in a flightless female firefly, *Photinus collustrans* (Coleoptera: Lampyridae). *Journal of Insect Behavior*, **2**, 841–847.
- Zeh, D.W. & Smith, R.L. (1985) Paternal investment by terrestrial arthropods. *American Zoologist*, **25**, 785–805.

Accepted 17 October 2001