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Effects of male ejaculate on female reproductive output and longevity in *Photinus* fireflies

A. South and S.M. Lewis

Abstract: In many insects, nuptial gifts in the form of spermatophores have been shown to increase female fecundity and to contribute to female somatic maintenance. Examining how variation in male spermatophore size affects female fitness components can provide insight into the evolution of nuptial gifts, as well as insight into potential conflicts between the sexes. Here we present an experimental study on the firefly *Photinus obscurellus* LeConte, 1851 in which we altered spermatophore size by manipulating male mating history and examined effects on female offspring production and longevity. Females were randomly allocated to one of two mating treatments in which they mated once with a male producing either a large or a small spermatophore. We found that male spermatophore size had no significant effect on lifetime fecundity or daily reproductive rates of female *P. obscurellus*, but females that received a larger spermatophore showed a tendency toward longer postmating life spans. These results suggest a direct benefit to females from nuptial gifts and also reveal the potential for synergistic effects on multiple facets of female fitness.

Key words: Coleoptera, Lampyridae, *Photinus obscurellus*, nuptial gifts, spermatophores, female fecundity, female longevity.

Résumé : Il a été démontré que, chez de nombreux insectes, des cadeaux nuptiaux sous forme de spermatophores accroissent la fécondité et contribuent au maintien somatique des femelles. L'examen de l'incidence de la taille des spermatophores mâles sur des composantes de la valeur sélective des femelles peut mener à une meilleure compréhension de l'évolution des cadeaux nuptiaux ainsi que des conflits potentiels entre les sexes. Nous présentons une étude expérimentale des lucioles *Photinus obscurellus* LeConte, 1851 dans laquelle nous avons modifié la taille des spermatophores par manipulation du rang d'accouplement des mâles et en avons examiné les effets sur la production de descendance et la longévité des femelles. Chaque femelle a subi, au hasard, l'un des deux traitements d'accouplement suivants : un seul accouplement avec un mâle produisant un gros ou un petit spermatophore. Si aucun effet significatif de la taille du spermatophore du mâle sur la descendance finale ou le taux de reproduction quotidien des *P. obscurellus* femelles n'a été observé, les femelles qui ont reçu un plus gros spermatophore tendaient toutefois à présenter une plus longue durée de vie post-accouplement. Ces résultats suggèrent que les femelles tirent un avantage direct des cadeaux nuptiaux et indiquent également qu'il pourrait y avoir des effets synergétiques sur divers aspects de la valeur sélective des femelles.

Mots-clés : coléoptères, lampyridés, *Photinus obscurellus*, cadeaux nuptiaux, spermatophores, fécondité des femelles, longévité des femelles.

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Introduction

In holometabolous insects, nutrients that adult females can allocate to reproduction might come from three sources: larval stores, adult dietary intake, and nutrients that are transferred from males during mating. (Boggs 1990, 2009). Male-derived nutrients are predicted to be of particular economic importance in capital breeders, which include species that lack adult feeding (Boggs 1990, 1997). Spermatophores (sperm-containing packages manufactured by male accessory glands; Mann 1984) comprise a major category of nuptial gifts that are deposited and absorbed in the female reproductive tract (Lewis et al. 2011). Although numerous studies have been conducted in insects to elucidate fitness costs and benefits, considerable debate persists about how such male ejaculates affect female fitness. Some authors have argued that male ejaculates enhance female net fitness (Boggs 1990; Vahed 1998; Gwynne 2008), while others have proposed that male ejaculates produce a net decrease in female fitness by manipulating female reproduction (Arnqvist and Nilsson 2000; Gillott 2003; Vahed 2007; Wolfner 2007). In many insects, nuptial gifts have been shown to increase female fecundity (egg or offspring number), and also to contribute to female somatic maintenance (reviewed by Boggs 1990, 1995; Gwynne 1997, 2008; Vahed 1998). Therefore, examining how variation in male spermatophore size affects female

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reproductive output and longevity provides insight into the evolution of nuptial gifts, as well as insight into potential conflicts between the sexes.

During mating, males of several species of nocturnally active *Photinus* Laporte, 1833 fireflies transfer a spirally coiled, protein-rich spermatophore to the female (van der Reijden et al. 1997). Following sperm release, the male spermatophore disintegrates over several days within a specialized female reproductive organ known as the spermatophore digesting gland. Within a few days after mating, male-derived protein is incorporated mainly into developing oocytes and also into female somatic tissue (Rooney and Lewis 1999). Because adults do not eat in most *Photinus* species, vitellogenesis depends on both larval food stores and input from male spermatophores. Previous work has shown that receiving more male spermatophores increases lifetime offspring production by *Photinus* females (Rooney and Lewis 2002). When female *Photinus ignitus* Fall, 1927 mated with three different males, their lifetime fecundity was 73% greater than that of singly mated females, while female longevity was unchanged.

In addition to multiple matings, females may also encounter considerable variation in ejaculate (spermatophore) size received from a single mating. In many taxa, males transfer spermatophores that decrease in size with successive matings (Davies and Dadour 1989, Svärd and Wiklund 1989; Royer and McNeil 1993, Savalli and Fox 1999; Wilson et al. 1999). In addition, male *P. ignitus* show a mean reduction of 36% in spermatophore mass between a male's first and second mating (Cratsley et al. 2003). Therefore, it is also of interest to see how variation in spermatophore size, not just number, influences female fitness. In a previous study of *P. ignitus*, Rooney and Lewis (2002) found that mated females showed no significant difference in fecundity when they received large vs. small spermatophores from a second male. However, this study included potential extraneous variation because females were collected throughout their mating season, and thus their prior mating history was unknown.

Here we present an experimental study on the firefly *Photinus obscurellus* LeConte, 1851 in which we manipulated male mating history to alter spermatophore size, and then examined effects on female offspring production and longevity. In this study, we maximized our ability to detect any changes by using virgin females. We predicted that females receiving larger spermatophores would exhibit enhanced fecundity and longevity relative to females receiving smaller spermatophores.

Materials and methods

To examine effects of spermatophore size on female reproductive output and longevity, we collected *P. obscurellus* in Lincoln, Massachusetts (42°26′N, 71°18′W), USA, at the beginning of their breeding season. The nightly mating period for this species lasts ~2 h with only one mating occurring per night (Lloyd 1966). To obtain virgin fireflies, the field site was monitored nightly for 1–2 weeks before the anticipated emergence date of *P. obscurellus* (these fireflies are inactive during the day). Once they emerge, both sexes emit easily detected bioluminescent courtship flashes during a courtship flight period that lasts between 45 min and 2 h. Fireflies are protandrous, and over the first several nights, we were able to collect all the emerging males. In the ab-

sence of any signaling males, we could still locate and capture newly emerged females because they will respond to simulated male flashes. This method maximizes the likelihood of obtaining virgins of both sexes. Beetles were weighed to the nearest 0.1 mg and housed individually; no food was provided because *P. obscurellus* has nonfeeding adults, as do most *Photinus* species (Lloyd 1997).

Females collected each night were randomly allocated to one of two mating treatments in which they mated once either with a virgin male (thus receiving a large spermatophore), or with a male that had mated within the previous 24 h (thus receiving a small spermatophore). Males in the latter treatment were collected as virgins (see above for description) but then mated in the laboratory prior to their use in the experimental matings. Previous work in numerous insects has reported that virgin males transfer larger spermatophores compared with previously mated males (Lepidoptera: Oberhauser 1988; Svärd and Wiklund 1989; Royer and McNeil 1993; Hiroki and Obara 1997; Lauwers and Van Dyck 2006; Coleoptera: Eady 1995; Savalli and Fox 1999; Wilson et al. 1999; Orthoptera: Davies and Dadour 1989). A decline in male spermatophore mass after mating has also been confirmed for *P. ignitus* (Cratsley et al. 2003), a sympatric, ecologically similar firefly that also lacks adult feeding. Therefore, based on previous work, it seems reasonable to assume that spermatophore mass is also altered by male mating history in *P. obscurellus*. Spermatophore composition may also change, but this possibility has not been examined.

Most (>70%) females assigned to both treatments had successfully mated within 24 h of collection, and all but one female had mated within 48 h. After mating, females were given moss as an oviposition substrate and eggs were collected every 2 days until females died. Eggs were incubated at 29 °C until they hatched. For each female, we recorded lifetime fecundity (total number of larvae produced), daily reproductive rate (number of larvae produced per day), and postmating life span.

To investigate the effects of male spermatophore size on female fitness components, we used analysis of covariance (ANCOVA) with female mass included as a covariate. We checked that there was no initial difference in body mass for females assigned to the two treatments (separate variances t test, $t_{[17.9]} = 0.923$, $p = 0.368$). We also checked that the data conformed to assumptions of normality and homogeneity of variances, and checked the ANCOVA assumption of homogeneity of regression slopes (Engqvist 2005). In addition to statistical results, we also report effect sizes, which we calculated as Hedges' d, an unbiased weighted estimate of effect size that is typically used with continuous response variables and categorical predictors (Nakagawa and Cuthill 2007). Hedges' d is calculated as the difference between a control group and an experimental group measured in standard deviation units (Gurevitch et al. 1992). We also conducted parametric survival analysis with female body mass as a covariate to examine how spermatophore size affected female postmating life span (JMP version 9; SAS Institute Inc., Cary, North Carolina, USA).

Results

We found that male spermatophore size had no significant

Fig. 1. Lifetime fecundity as number of larvae produced by female *Photinus obscurellus* that mated once either with a virgin male (thus receiving a large spermatophore, $n = 11$) or with a male that had mated within the previous 24 h (thus receiving a small spermatophore, $n = 10$). Circles indicate data for individual females, while horizontal lines indicate mean $\pm$ 1 SE.

Fig. 2. Postmating life span of female *Photinus obscurellus* that mated once either with a virgin male (thus receiving a large spermatophore, $n = 11$) or with a male that had mated within the previous 24 h (thus receiving a small spermatophore, $n = 10$). Circles indicate data for individual females, while horizontal lines indicate mean $\pm$ 1 SE.
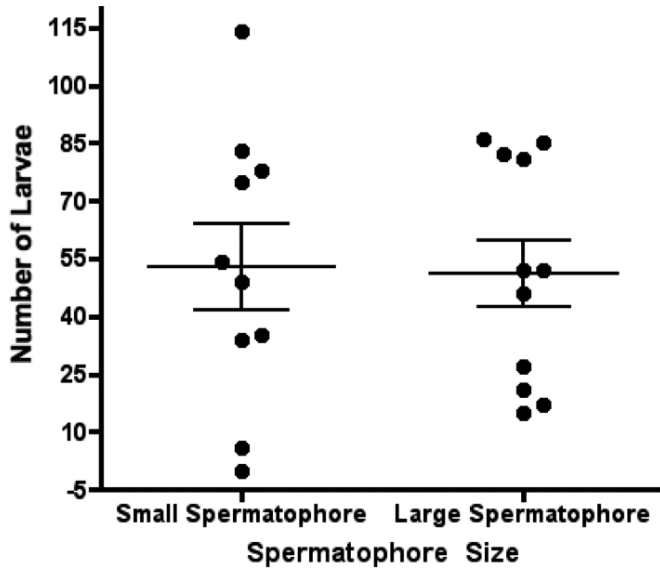

effect on the lifetime fecundity of female *P. obscurellus* (Fig. 1; ANCOVA for spermatophore size: $F_{[1,18]} = 1.527$, $p = 0.232$, effect size = 0.53; ANCOVA for female mass covariate: $F_{[1,18]} = 47.1828$, $p < 0.0001$). Daily reproductive rates also were nearly identical between females that received a large spermatophore (4.16 $\pm$ 0.44 larvae/day (mean $\pm$ 1 SE), $n = 11$) and females that received a small spermatophore (4.16 $\pm$ 0.46 larvae/day, $n = 10$) (ANCOVA for spermatophore size: $F_{[1,18]} = 0.0005$, $p = 0.995$, effect size = 0.0; ANCOVA for female mass covariate: $F_{[1,18]} = 47.6244$, $p < 0.0001$). However, females that received a larger spermatophore had a marginally significant increase in postmating life span, gaining about 2 days in a mean life span of approximately 12 days (Fig. 2; ANCOVA for spermatophore size: $F_{[1,18]} = 4.459$, $p = 0.049$, effect size = 0.87; ANCOVA for female mass: $F_{[1,18]} = 0.7212$, $p = 0.4069$). Parametric survival analysis yielded similar results, showing a marginally insignificant effect of spermatophore size on female longevity (likelihood ratio $\chi^2 = 3.69$, $p = 0.0546$) and no significant effect of female mass (likelihood ratio $\chi^2 = 0.34$, $p = 0.5567$).

Discussion

This study found no difference in lifetime reproductive output between female *P. obscurellus* based on differences in male ejaculate size received during a single mating. This contrasts with the significant 73% increase in female lifetime fecundity documented for triply mated compared with singly mated female *P. ignitus* (Rooney and Lewis 2002). Thus, although variation in male ejaculate quantity or composition may influence female fecundity, at least in *P. obscurellus* this effect appears too subtle to detect based on variation in single spermatophores.

However, females that had mated with virgin males and
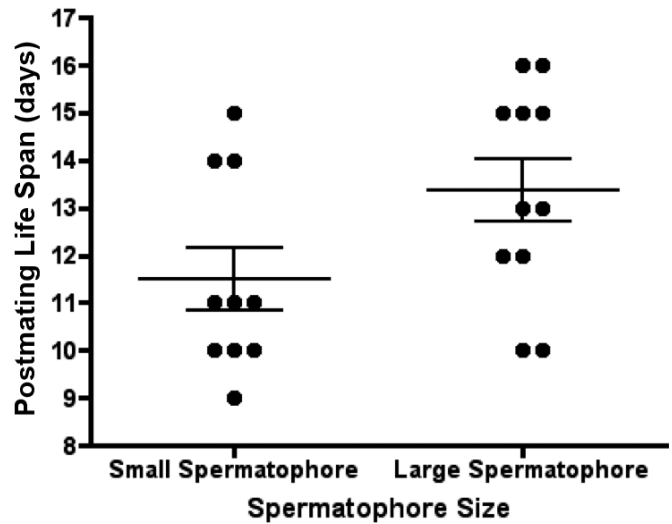
thus had received larger spermatophores showed a ~16% increase in longevity. Although this effect was on the borderline of statistical significance at the $\alpha = 0.05$ level, this possible longevity benefit from male spermatophores is consistent with previous work on *Photinus* fireflies. When female *P. ignitus* that had previously mated were mated again to males with either large or small spermatophores (manipulated via mating history as in this study), there was no significant effect on either female fecundity or female longevity (Rooney and Lewis 2002); however, those females that received larger spermatophores showed a 12% increase in longevity. In addition to studying once-mated females, the current study provided a methodological advantage because we were able to control for differences in prior mating history of both sexes by using virgin females and males. This is likely to have reduced extraneous variation and thus may have improved the ability of the current study to detect potential differences.

These results provide some insight into the possible evolution of nuptial gifts in *Photinus* fireflies. Under natural conditions, the distinct benefits that females derive from larger spermatophores and multiple mating may complement one another. Under artificially created laboratory conditions, the experimental females in our study were not given the opportunity to remate. However, because in field populations female *Photinus* typically remate on subsequent nights (Lewis and Wang 1991), even a small increase in longevity should also increase the number of mates (and thus the number of ejaculates) a female gains, resulting in higher lifetime fecundity (Rooney and Lewis 2002). These combined benefits are expected to select for female choice of males with larger spermatophores, as well as for female polyandry (reviewed in Lewis and Cratsley 2008). Possible benefits to males producing larger spermatophores include increased male paternity share (South and Lewis 2012).

Other experimental studies in a variety of insects demon-

strate a variety of effects of male nuptial gifts on female net fitness (reviewed in Boggs 1995; Vahed 1998; Arnqvist and Nilsson 2000; Gwynne 2008; South and Lewis 2011). It has been proposed that nuptial gifts may have originated via sexual conflict to benefit males at the expense of female net fitness (for a review see Arnqvist and Nilsson 2000; Arnqvist and Rowe 2005; Vahed 2007; Gwynne 2008). Male reproductive accessory glands could have originated to produce compounds that exploit female sensory pathways and induce a greater latency period between matings (Arnqvist and Nilsson 2000; Sakaluk 2000; Fedorka and Mousseau 2002; Arnqvist and Rowe 2005; Engqvist 2007). Females may have then responded by evolving ways to cope with these compounds and derive a direct benefit by using them for somatic maintenance or reproduction. Thus, although it seems likely that the effects of nuptial gifts on the fitness of both sexes will change over evolutionary time, in many insects they currently appear to function to increase female fitness (South and Lewis 2011).

Studies to date on *Photinus* fireflies suggest that male nuptial gifts can exert synergistic effects on multiple components of female fitness: longer female life span suggested by the current study leads to increased probability of females remating and thus obtaining additional ejaculates shown to increase fecundity (Rooney and Lewis 2002). Such fitness benefits would be predicted to favor female choice of males that can provide larger spermatophores, although additional study is needed to explore possible correlated traits that females might use as the basis for choice. Because male gifts are selected through their fitness consequences for both sexes, they may profoundly influence both precopulatory and postcopulatory sexual selection. Additionally, changes in nuptial gift size during male lifetimes in *Photinus* species can potentially alter courtship behavior and mate choice. Further study in this suite of species could reveal unique insights into coevolution between nuptial gifts and other life-history parameters.

Acknowledgements

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