

Limits to Nuptial Gift Production by Male Fireflies, *Photinus ignitus*

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*Males of diverse insect species provide females with nuptial gifts, and limits on males' ability to produce these gifts may influence courtship behavior and mating systems. In the firefly *Photinus ignitus*, males transfer a complex spermatophore to females during mating. We provided firefly males unlimited access to responsive females to examine whether spermatophore production limits male mating success. Male spermatophore mass decreased significantly across sequential matings, and the percentage of successful matings declined during the second half of each male's life span. Male body mass explained a significant proportion of variation in size of the first spermatophore produced by *P. ignitus* males, but this relationship disappeared with second spermatophores. This study indicates that males' ability to produce spermatophores declines over their lifetime and that limits on nuptial gift production can constrain male mating success in *Photinus* fireflies.*

KEY WORDS: courtship feeding; male investment; potential reproductive rate; sexual selection; spermatophore.

INTRODUCTION

In many insect species, males supply a nuptial gift to females during courtship and mating. Such nuptial gifts can provide nutrition in the form of captured prey items, male body parts, or male glandular secretions such as

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spermatophores (Thornhill, 1976; Mann, 1984; Zeh and Smith, 1985). The documented benefits accruing to females from nuptial gifts include increased female fecundity and longevity (reviewed by Boggs, 1995; Vahed, 1998). However, less is known concerning limits on males' ability to supply nuptial gifts (but see Boggs, 1995). Because nuptial gifts are so widely distributed, examining how their production might limit male mating success is important to understanding insect mating systems (Svård and Wiklund, 1989; Gwynne, 1991; Simmons, 1995).

Nuptial gift production involves energy and nutrient costs to males, as well as time out from mating (Simmons and Parker, 1989). When males provide only sperm without additional nutrients, male reproductive success is expected to be limited by female availability (Trivers, 1972; Williams, 1975). However, when males provide nuptial gifts, male mating rate is predicted to be further constrained by how quickly males can acquire or manufacture such gifts (Thornhill and Alcock, 1983). Costs of producing nuptial gifts will be mediated by food availability, with higher costs expected in species with nonfeeding adults (Boggs, 1990; Gwynne, 1990). In addition, male investment in nuptial gifts may entail a trade-off between mating frequency and nuptial gift quality (Sakaluk, 1985; Simmons, 1995). Faced with limited resources, males may maintain either constant nuptial gift size or constant intervals between successive matings.

In this study we examined whether nuptial gift production constrains reproduction of male fireflies, *Photinus ignitus* Fall (Coleoptera: Lampyridae). *Photinus* fireflies do not feed as adults (Williams, 1917; Lloyd, 1997), so all reproductive activities must rely entirely on resources acquired through larval feeding. During mating *Photinus* males transfer a complex spermatophore produced by four paired accessory glands (van der Reijden *et al.*, 1997). The male spermatophore is subsequently digested within the female reproductive tract, and spermatophore-derived amino acids are translocated to female oocytes within 2 days after mating (Rooney and Lewis, 1999). Recent work has shown that *P. ignitus* females preferentially respond to longer-duration male flashes and that a longer flash duration is correlated with a larger spermatophore size in early-season males (Cratsley and Lewis, 2003). Females gain a direct fitness benefit from male spermatophores, as triply mated *P. ignitus* females show increased fecundity relative to singly mated females (Rooney and Lewis, 2002). In the present study, we examined possible constraints on male mating success in *P. ignitus* by providing males with a constant supply of responsive females. In addition, we investigated changes in spermatophore size and protein content across successive matings and the relationship between spermatophore size and male body size.

METHODS

Study Organism

Courtship flight and flash dialoging in *Photinus* fireflies are restricted to a 1 to 3 h evening period (Lloyd, 1966). Females mate multiply during their approximately 2-week adult life span, but beetles can mate only once each night, as copulatory mate-guarding follows spermatophore transfer (Lewis and Wang, 1991). Fireflies are generally protandrous, with operational sex ratios shifting from male-biased early in the season to female-biased later on (Lewis and Wang, 1991; Cratsley, 2000). *Photinus ignitus* adults were collected in Lincoln, Massachusetts; because these beetles cannot be reared in the laboratory, age and mating history of these field-collected adults were unknown. However, males were collected during early to midmating season to reduce the likelihood that they had previously mated.

Field Observations

We determined the maximum mating success of adult *P. ignitus* males by providing them with unrestricted access to females. Males were kept individually in screen cages, with water supplied by a cotton wick. Every night at the beginning of the male flight period, a single field-collected, flash-responsive female was placed in each male's cage. Pairs were observed at intervals throughout the flight period. At the end of the flight period, females were removed if mating had not occurred. When males failed to mate, they generally had not flashed or approached the female. Mating pairs remained in field cages, and females were removed the next morning following natural termination of copulation. The total number of matings achieved and the interval between successive matings were calculated for each male. Percentage of successful matings was calculated as the number of nights each male mated divided by the total number of nights each male was given access to responsive females. Male life span was defined as days from collection until death, and only males that lived >2 days were included in subsequent analyses. A paired *t*-test was used to compare the percentage of successful matings between the first and the second half of each male's life span.

Spermatophore Size and Protein Content

In a separate laboratory study, changes in spermatophore size across successive matings by *P. ignitus* males were examined by interrupting

copulations to retrieve spermatophores from female reproductive tracts. Field-collected *P. ignitus* males were weighed within 24 h of collection to the nearest 0.1 mg and kept in individual containers with moistened filter paper. Each evening a single, field-collected female was added to male containers, and pairs were observed every 15 min until mating occurred. Mating pairs were separated shortly after spermatophore transfer (45 min after beginning of stage II copulation [van der Reijden *et al.*, 1997]), and females were frozen to prevent spermatophore breakdown. Spermatophores were dissected out of female reproductive tracts, rinsed briefly in distilled water, and kept in an airtight container with desiccant for 24 h. These dissections of mated females confirmed that a single, completely formed spermatophore was transferred during each mating. Spermatophore dry mass was measured to the nearest 1 μg on a Mettler MT5 microbalance.

Changes in spermatophore dry mass for first, second, third, fourth/fifth, and \geq sixth matings (numbered from each male's first mating in captivity) were analyzed using one-way ANOVA; unequal numbers of spermatophores produced by different males precluded the use of repeated-measures tests. However, we had measurements of both first and second spermatophore mass for a subset of 21 males, and a paired *t*-test was used to examine the change in spermatophore mass between matings. In addition, the relationship between male body mass and spermatophore mass was examined using separate linear regressions for 85 first and 33 second matings.

After drying, spermatophore proteins were solubilized for a subset of spermatophores ($n = 90$) by homogenizing each in protein solubilization buffer (0.1% Triton X-100, 100 mM Tris, pH 8, 150 mM NaCl) and incubating overnight at 4°C. Spermatophore protein content was determined using a dye-binding protein microassay (Bio-Rad), with sample absorbances read at 595 nm on a microplate reader and compared to a bovine serum albumin standard curve. Linear regression was used to describe the relationship between spermatophore mass and protein content. Changes in protein content among first, second, and third spermatophores produced by field-collected males were analyzed using a nonparametric Kruskal–Wallis test relatively robust to heterogeneous variances.

RESULTS

When *P. ignitus* males were given nightly access to receptive females in the field, they had a median of 2 successful matings (range = 0 to 10 matings; $n = 27$ males) over life spans ranging from 1 to 14 days. For males that mated at least twice, remating intervals ranged from 1 to 4 days, with a mean of 1.4 ± 0.1 (1 SE) days. Some firefly males mated successfully on up to 7 consecutive

nights. The number of matings achieved by a male increased significantly with life span (linear regression $F_{(1,18)} = 17.8, r^2 = 0.58, P = 0.001$) but was not related to male body mass at the start of the experiment (linear regression $F_{(1,18)} = 0.7, r^2 = 0.04, P = 0.41$). Firefly males did not mate every night, even when they were provided with responsive females. The percentage of successful matings averaged $60 \pm 9\%$ during the first half of each male's life span, then declined to $39 \pm 9\%$ of nights during the second half of their life span, a marginally nonsignificant decrease (paired $t = 2.05, 16 \text{ df}, P = 0.057$).

There was a significant decline across sequential matings in the dry mass of spermatophores produced by *P. ignitus* males (Fig. 1: ANOVA $F_{(4,137)} = 9.5, P < 0.0001$). When spermatophore mass was compared between a male's first and his second matings, there was an average decline of 36% (Fig. 2; paired $t = 3.1, 20 \text{ df}, P = 0.006$).

The relationship between a male's body size and his spermatophore mass differed between the first and the second matings (Fig. 3). For the first spermatophore collected from *P. ignitus* males, spermatophore mass increased linearly with male body size (regression $F_{(1,83)} = 69.9, r^2 = 0.46, P < 0.0001$). However, by the second mating, male body size no longer explained a significant proportion of variation in spermatophore mass (Fig. 3: second spermatophore regression $F_{(1,31)} = 3.2, r^2 = 0.09, P = 0.08$). Based

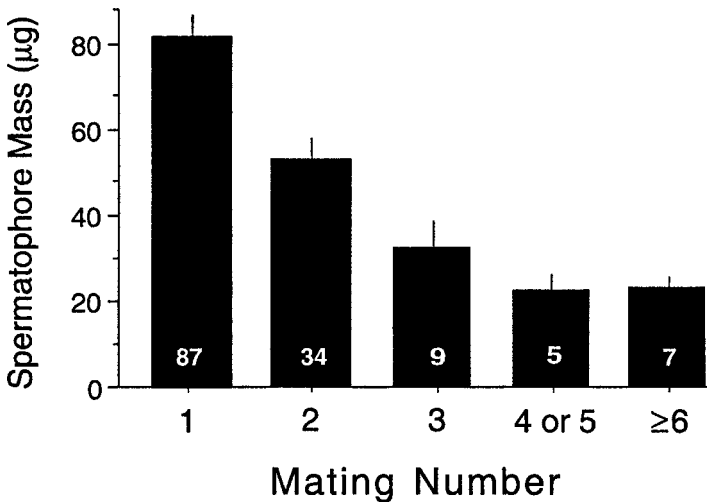


Fig. 1. *P. ignitus* male spermatophore size (dry mass; mean + 1 SE) across sequential matings. Sample size (# spermatophores) given inside bars for each group.

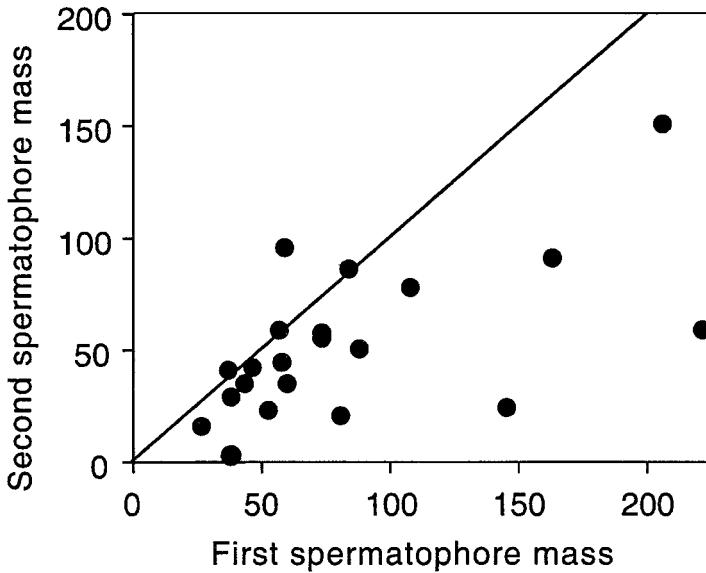


Fig. 2. Comparison of spermatophore dry mass for the first and second spermatophores produced by field-collected *P. ignitus* males ($n = 21$ males). Diagonal line represents null hypothesis of equality of first and second spermatophore mass.

on wet mass, *P. ignitus* spermatophores ($n = 9$) averaged $5.3 \pm 1\%$ of male body mass.

The protein content of *P. ignitus* spermatophores increased directly with spermatophore mass (Fig. 4: regression $F_{(1,88)} = 557.5$, $r^2 = 0.86$, $P < 0.0001$). Protein represented about 15% of spermatophore dry mass, with no change between first spermatophores ($15.1 \pm 0.5\%$ protein, $n = 61$), second spermatophores ($14.2 \pm 0.7\%$ protein; $n = 26$), and third spermatophores ($15.8 \pm 5.6\%$ protein; $n = 3$; Kruskal–Wallis $H = 2.4$, $P = 0.30$).

DISCUSSION

This study indicates that the ability of *Photinus ignitus* males to produce spermatophores declines over their lifetime and that limits on nuptial gift production can constrain male mating success. Even when they were provided with responsive females, and thus needed to expend little energy on courtship flight and flash signaling, *P. ignitus* males mated less often than nightly. Males showed a marginally nonsignificant reduction in the percentage of successful matings during the latter half of their life span. Because

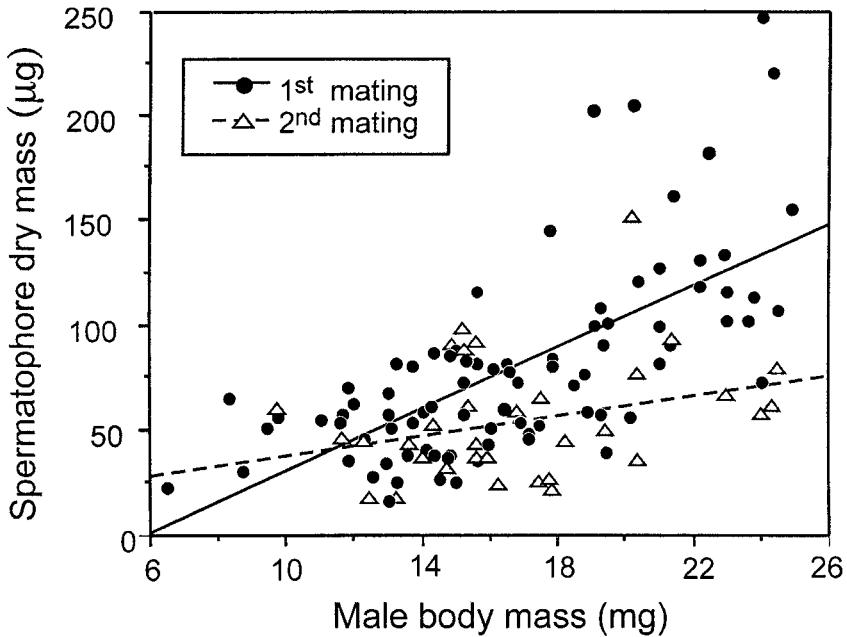


Fig. 3. Relationship between male body size (wet mass) and spermatophore size (dry mass) for first vs. second spermatophores of field-collected *P. ignitus* (least-squares regression lines: first spermatophore mass = $-42.5 + 7.3 * \text{male mass}$, $n = 85$; second spermatophore mass = $14.5 + 2.4 * \text{male mass}$; $n = 33$).

males that failed to mate generally produced no courtship flashes (which indicate readiness to mate), it is unlikely that the reduction in successful mating is due to females rejecting older males. In addition, *P. ignitus* nuptial gift size declined across successive matings, with spermatophore mass decreasing by one-third between a male's first and his second matings. Previous studies on orthopteran and lepidopteran insects have suggested a trade-off between spermatophore size and mating rate, based on observed changes in either male mating frequency or spermatophore size when adult nutritional regimes have been manipulated (Gwynne, 1990; Sakaluk, 1985; Boggs, 1995; Simmons, 1995; Bissoondrath and Wiklund, 1996). In *Photinus* males, reductions in both spermatophore size and percentage of successful matings likely reflect diminishing reserves remaining for spermatophore production, due to the absence of adult feeding in this group of fireflies. These results support the prediction (Marshall, 1982; Boggs, 1995) that limits on nuptial gift production should represent an important constraint on male reproduction in species with nonfeeding adults.

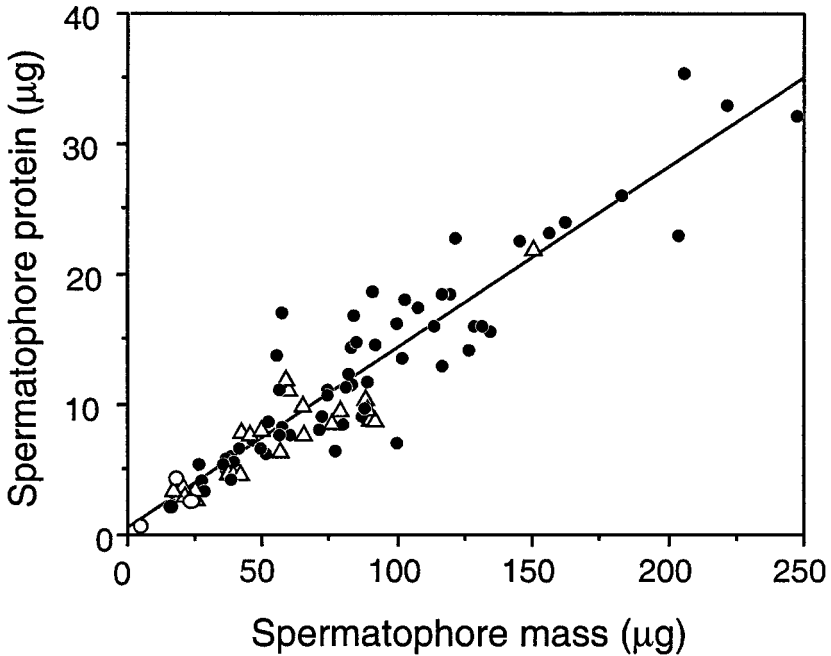


Fig. 4. Protein content of *P. ignitus* male spermatophores as a function of spermatophore dry mass. Data are shown for spermatophores from males' first matings (filled circles; $n = 61$), second matings (triangles; $n = 26$), and third matings (open circles; $n = 3$). Least-squares regression line for combined data: $\text{protein} = 0.603 + 0.138 * \text{mass}$.

Female availability is likely to limit mating success of *Photinus* males under some conditions, particularly with male-biased sex ratios that prevail early in the flight season. However, later in the flight season when operational sex ratios approach unity or become female-biased (Lewis and Wang, 1991; Cratsley, 2000), constraints on male spermatophore production are likely to become increasingly important. Seasonal changes in operational sex ratios coupled with declining nuptial gift availability are associated with reversal of traditional courtship roles in orthopterans (Gwynne, 1981; Gwynne and Simmons, 1990; Simmons and Bailey, 1990). Limits on firefly nuptial gift production demonstrated here may be responsible for shifting courtship behavior from male competition and female choice early in the mating season to female competition (and possibly male choice) prevailing later in the season (Lewis and Wang, 1991; Cratsley, 2000).

Although spermatophore size is often correlated with male body mass (e.g., Sakaluk, 1985; Svård and Wiklund, 1986; Rutowski *et al.*, 1987), in the present study this relationship is shown to depend on a male's mating

history. In *P. ignitus*, the significant correlation between spermatophore size and male size that was observed for first matings disappeared in subsequent matings. This may influence *Photinus* mating strategies, as male body size will be a useful indicator of spermatophore size only for virgin males. As a result, females are expected to exhibit choice based on male body size only during the early mating season (Cratsley, 2000). Additional studies of how nuptial gifts affect both male and female reproductive success will contribute to a more synthetic understanding of life-history evolution, foraging ecology, and sexual selection.

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