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MALE COURTSHIP ATTRACTIVENESS AND PATERNITY SUCCESS IN *PHOTINUS GREENI* FIREFLIES

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Although female mate choice and male sperm competition have separately attracted much attention, few studies have addressed how precopulatory and postcopulatory episodes of sexual selection might interact to drive the evolution of male traits. In *Photinus* fireflies, females preferentially respond to males based on their bioluminescent courtship signals, and females gain direct benefits through male nuptial gifts acquired during multiple matings over several nights. We experimentally manipulated matings of *P. greeni* fireflies to test the hypothesis that postcopulatory paternity success might be biased toward males that are more attractive during courtship interactions. We first measured male courtship attractiveness to individual females using field behavioral assays. Females were then assigned to two double-mating treatments: (1) least attractive second male—females were first mated with their most attractive male, followed by their least attractive male, or (2) most attractive second male—females mated with males in reverse order. Larval offspring produced by each female following these double matings were genotyped using random amplified polymorphic DNA (RAPD) markers, and male paternity was determined. Contrary to prediction, firefly males that were more attractive to females based on their bioluminescent courtship displays subsequently showed significantly lower paternity, reflecting possible male trade-offs or sexual conflict. Differences in male paternity were not related to male body condition, testes or accessory gland mass, or to variation in female spermathecal size. Additionally, this study suggests that changes in phenotypic selection gradients may occur during different reproductive stages. These results indicate that it is crucial for future studies on sexual selection in polyandrous species to integrate both precopulatory and postcopulatory episodes to fully understand the evolution of male traits.

KEY WORDS: Courtship signals, female choice, multiple cues, postcopulatory female choice, selection gradients, sexual selection, sperm competition.

Sexual selection is a major force driving the evolution of diverse behavioral, anatomical, and physiological traits that influence reproductive success. However, few studies to date have provided an integrated picture that incorporates both precopulatory and postcopulatory episodes of sexual selection. Considerable empirical and theoretical work has been devoted to identifying male courtship traits and modeling how their evolution is affected by mate choice and competition (reviewed in Andersson 1994; Bonduriansky and Rowe 2003). However, in polyandrous mating systems, male reproductive success depends not only on successful mate acquistion, but also on the paternity share gained by each mating male. Postcopulatory processes that determine male paternity share include sperm competition among mating males for access to fertilizations (Parker 1970; Smith 1984; Birkhead and Moller 1998), as well as postcopulatory female choice of sires (Eberhard 1996; Simmons 2001). Insects have been particularly

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useful study systems providing evidence that females can influence male paternity success through female control over sperm storage (e.g., red flour beetles, *Tribolium castaneum*: Bloch Qazi et al. 1998; Fedina and Lewis 2004; yellow dung flies, *Scatophaga stercoraria*: Ward 1998, 2000).

Although it is clear that male reproductive fitness is ultimately a product of mating success and average paternity share (number of offspring sired per mating), little work has examined whether postcopulatory selection episodes might reinforce, counteract, or be unrelated to female mate preferences. Previous studies that have examined both precopulatory and postcopulatory reproductive episodes have generally found a positive association between these fitness components (Lewis and Austad 1994; Pizzari et al. 2002; Evans et al. 2003; Wagner and Harper 2003; Safran et al. 2005; but see Danielsson 2001). This could arise through concordant female choice of both mates and sires (based, for example, on reliable signals of male quality), or through positive correlations between males' courtship attractiveness and sperm competitive ability. On the other hand, negative associations between female mate choice and male postcopulatory paternity success could arise through male physiological trade-offs or via sexual conflict across reproductive stages (Rice 1998; Arnqvist and Rowe 2005). In any case, determining the relationship between male courtship attractiveness and subsequent paternity success will be fundamental to understanding the evolution of male reproductive traits in any polyandrous mating system.

PRECOPULATORY AND POSTCOPULATORY SEXUAL SELECTION EPISODES IN *PHOTINUS* FIREFLIES

Photinus fireflies (Coleoptera: Lampyridae) are particularly amenable to studies that integrate mate choice and postcopulatory sexual selection. *Photinus* courtship is based on conspicuous bioluminescent flash signals produced by roving males, and stationary females respond by emitting their own flashes (Lloyd 1966). Mate choice in fireflies occurs through females preferentially responding to specific males based on temporal characteristics of the male flash pattern (Branham and Greenfield 1996**;** Cratsley and Lewis 2003, Michaelidis et al. 2006). *Photinus greeni* males produce courtship flashes consisting of paired pulses, and females preferentially respond to male signals based on their interpulse intervals (Buck and Buck 1972; Buck and Case 1986; Michaelidis et al. 2006). Furthermore, it has been shown that when several *Photinus* males court a single female, those males able to elicit the highest female flash response have the highest probability of mating (Lewis and Wang 1991; Vencl and Carlson 1998; Cratsley and Lewis 2005; Demary et al. 2006).

Male reproductive success depends on securing both matings and fertilizations, as most *Photinus* fireflies are polygamous; both sexes mate multiple times over a two-week adult life span (Wing 1985; Lewis and Wang 1991; Lewis et al. 2004a). During mating,

males provide a nuptial gift to females consisting of a protein-rich spermatophore that is manufactured by four pairs of male accessory glands (van der Reijden et al. 1997; Lewis et al. 2004a,b). Spermatophore-derived proteins are incorporated into developing oocytes of *P. ignitus* females (Rooney and Lewis 1999), and male spermatophores provide females with a direct benefit in terms of increased female lifetime fecundity (Rooney and Lewis 2002). Nuptial gift production appears to be costly for *P. ignitus* males, as spermatophore mass in sequential matings at 24 h intervals decreases steadily (Cratsley et al. 2003). Mating systems that involve male nuptial gifts may intensify sexual conflict (Arnqvist and Rowe 2005) because females should be selected to mate with multiple males to gain direct benefits (reviewed by Vahed 1998). Thus, the combination in *Photinus* fireflies of female mate choice based on visible courtship signals, male nuptial gifts, and polyandrous females suggest that both precopulatory and postcopulatory episodes of sexual selection will be important in affecting the evolution of male traits.

In this study, we used *P. greeni* fireflies to test the hypothesis that males' courtship attractiveness would be positively related to their paternity success. We first measured male courtship attractiveness in field behavioral assays during which females were allowed to respond to courtship flashes given by three equidistant males. Paternity was determined for offspring produced by doubly mated females assigned to two mating treatments: some females were mated first with the male they found least attractive followed after 24 h by their most attractive male, whereas other females were mated to males in the reverse order. We also examined how phenotypic selection gradients for several male morphological traits (body mass, elytral length, testes, and accessory gland mass) might differ across precopulatory and postcopulatory episodes of sexual selection. Knowledge of how male traits are affected during different sexual selection episodes is necessary for understanding trait evolution.

Materials and Methods
MALE COURTSHIP ATTRACTIVENESS

Photinus greeni males and females were collected in Lincoln, MA (42°26′N, 71°18′W) during days 1–7 of the approximately one-month mating season. In *Photinus,* breeding sex ratios are highly male-biased early during the mating season (Lewis and Wang 1991; Cratsley and Lewis 2005). We used early emerging males in this experiment to reduce the likelihood that they had mated previously. Experimental beetles were housed individually in containers with access to water; in this as in many other *Photinus* species, adults do not feed (Lloyd 1997).

We measured courtship attractiveness of individual *P. greeni* males in field cages, based on the percentage of male courtship flashes that elicited female flash responses. Other studies of

Photinus fireflies have found this measure of courtship attractiveness to be a good predictor of male mating success in competitive ($\geq$ two males courting a single female) courtship interactions (Lewis and Wang 1991; Vencl and Carlson 1998; Cratsley and Lewis 2005). To assay male attractiveness, courtship interactions between a single female ($n = 20$) and three randomly selected males from the population were observed in $30 \times 30 \times 60$ cm screen cages (BioQuip, Gardena, CA). During each trial, the three males were placed individually into optically clear vials that were equidistant (approximately 50 cm) from a female housed in a clear container on the cage floor. Beetles were habituated to cages for 10 min prior to observations, which were conducted during the usual flight period of *P. greeni* (2030–2130 h). During each trial, we recorded the number of flash signals produced by each male, and the number of female flashes given in response to each male. This experimental assessment of male courtship attractiveness mimicked natural *Photinus* courtships in allowing stationary females to simultaneously assess several males based solely on their visible flash signals (Lloyd 1966; Vencl and Carlson 1998; Lewis and Wang 1991; Cratsley and Lewis 2005).

Following each trial, male courtship attractiveness was calculated as the percentage of each male's flashes eliciting a female response, and the three males from each trial were ranked from most to least attractive. In determining courtship attractiveness, any males that produced fewer than three courtship flashes and any females that responded fewer than 15 times were excluded. Courtship attractiveness measured in these trials for different *P. greeni* males ranged from 0 to 100% of male flashes eliciting a response. Differences in courtship attractiveness (male 2 minus male 1) ranged from -69% to -23% in the least attractive second male treatment, whereas in the most attractive second male treatment, this difference ranged from $+25\%$ to $+82\%$.

MATING TREATMENTS

To assess paternity share achieved by least and most attractive males, we mated males with the same female they had interacted with during the courtship attractiveness trials. This controlled for possible differences in female choice associated with age or condition (Jennions and Petrie 1997; Cratsley and Lewis 2005). Immediately following each courtship trial, females were assigned to two experimental double-mating treatments: (1) Least attractive second male treatment ($n = 10$): each female was first mated with her most attractive male, and after 24 h was mated with her least attractive male or (2) Most attractive second male treatment ($n = 10$): females were first mated with their least attractive male, followed 24 h later by their most attractive male. Matings were conducted by placing males and females in close proximity (150 mL containers), where they generally located each other without flashing. Mating pairs were monitored until they reached the second stage of copulation, which is when spermatophore transfer oc-

curs (van der Reijden et al. 1997). After natural termination of copulation, males were removed, frozen at -20°C, and stored in 95% ethanol for later morphological measurements and genomic DNA extraction. The following night, females were paired with their second assigned mate, and again pairs were monitored until copulation was confirmed. We used a 24 h intermating interval because it reflects natural female remating frequencies in other *Photinus* species (Lewis and Wang 1991); one female assigned to the least attractive second male treatment was later excluded from analysis because she had a 72 h mating interval.

Following their second mating, females were maintained in the laboratory on a natural light cycle until their death (five to 17 days following their second mating). Females oviposited at irregular intervals on moss, which was removed every two days and placed in sterile petri dishes with 1X phosphate buffered saline (PBS). Eggs were incubated at 29°C until hatching (about 16 days), and paternity assignment (see below) was conducted on first instar larvae. In previous studies, egg to larval hatching success for *Photinus* was greater than 97% (Rooney and Lewis 2002). To examine female oviposition patterns over time, we calculated the percentage of each female's total offspring production during each two-day period after their second mating. To examine changes in second male paternity share (see below) over time, we excluded five females because all their eggs were laid during a single two-day oviposition period.

MALE PATERNITY SHARE

To determine paternity for larval offspring of doubly mated *Photinus greeni* females, we used randomly amplified polymorphic (RAPD) DNA markers (Welsh and McClelland 1990). These dominant Mendelian markers require no prior genomic sequence information (Lui and Cordes 2004), and have previously been used in numerous systems to assign offspring paternity to identified males (e.g., Goto et al. 2002; Santolamazza Carbone and Cordero Rivera 2003). In addition, this technique has been successfully applied to *P. ignitus* fireflies to determine offspring paternity for females mated with two known males (Rooney 2000).

Genomic DNA (gDNA) was extracted from all adults (each female and both her mates) following removal of elytrae, wings, legs, and antennae; male and female reproductive tracts were also removed for later measurement. The remaining head and thorax were frozen in liquid nitrogen, ground to powder by vortexing with stainless steel balls, and gDNA was extracted with DNeasy tissue kit (Qiagen Inc., Valencia, CA). For larvae, gDNA was extracted with DNeasy kit after larvae were ground individually with sterile disposable pestles.

Each random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) contained (25 μL total volume): 2.5 μL 10X buffer plus magnesium, 2.5 μL 5X Master Taq, 5.0 mL dNTPs (1mM stock), 5 ng gDNA, 3 μL RAPD primer

(10 μM stock; University of British Columbia RAPD primer set 100/7), 0.5 μL extra magnesium (25 mM stock), and 0.5 μL Eppendorf Master Taq polymerase. The final reaction concentrations were 1X Eppendorf buffer, 1.2 μM random primer, 0.2 mM dNTPs, 2.0 mM magnesium, 0.5X Master Taq solution, and 0.1 unit of Eppendorf Master Taq polymerase. A ThermoElectron Px2 thermocycler was programmed for one cycle at 94°C, 20 cycles at 94°C for 30 sec, 36.4°C for 1 min, and 72°C for 1 min followed by a 4°C soak. PCR products were run out on 1.5% agarose gels (Fisher Biotech DNA grade) and stained with ethidium bromide. We were able to definitively assign paternity using one to four RAPD primers per family; paternity assignments were confirmed for a subset of larvae using duplicate runs with the same or with different RAPD primers. All 21 offspring rerun with the same primer yielded identical paternity assignments, and additionally two different RAPD primers yielded identical paternity assignments for 20 of 21 offspring; in the single discrepant case, paternity assignment was determined with a third run. Gels were digitally photographed and Kodak 1.D software was used to estimate molecular weights of each band. Paternity was determined for each larval offspring based on the presence of polymorphic bands unique to either of the two potential fathers. Paternity share for most or least attractive second males (depending on treatment) was calculated as the proportion of offspring sired by the second mating male (P_2). Any larval offspring that could not be positively attributed to either of the two males was excluded from P_2 calculations (the highest proportion of such larvae was seven out of 37 larvae in a single family). Final calculated P_2 values were based on six to 103 larvae per family; a total of 1069 offspring were genotyped for this study.

To determine if second male paternity success (based on females' total lifetime offspring production) differed between the two mating treatments, two complementary statistical approaches were used: a generalized linear model approach where P_2 was modeled as a binomial response variable with a logit link function using SAS PROC GenMod (SAS Inc., Cary, NC) corrected for overdispersion, as well as a separate variance t-test (Systat 10.2: Systat Inc., Chicago, IL). Distributions of P_2 values within each mating treatment are presented as box plots; in addition, because family sizes varied widely we present both medians and the weighted means (weighted by the number of larvae per family) for these data.

FEMALE REPRODUCTIVE ANATOMY

In *Photinus* fireflies, sperm are stored within the female reproductive tract in a highly expandable, spherical spermatheca (Wing 1985; van der Reijden et al. 1997). *Photinus greeni* females have an additional, non-expandable structure for sperm storage closer to the site of fertilization; the size of this secondary spermatheca has been shown to be highly variable among conspecific fe-

males (Demary 2005). It has been hypothesized that additional sperm storage sites might allow multiply mated females to exert greater control over paternity (Hellreigel and Ward 1998; Parker and Partridge 1998; Snow and Andrade 2005). Here we tested a related prediction that larger secondary sperm storage compartments might provide *P. greeni* females with greater control over fertilizations. We measured each female's secondary spermatheca size as described in Demary (2005) by dissecting their reproductive tracts after death. We hypothesized that larger secondary spermathecae might allow females to store more sperm from preferred males, and thus bias paternity in their favor.

MALE MORPHOLOGICAL TRAITS AND REPRODUCTIVE ALLOCATION

For each *P. greeni* male in the mating experiment, we measured body mass to the nearest 0.1 mg within 24 h of collection. Male elytra length was also measured, and an index of physiological condition was calculated as wet mass (in mg) divided by elytral length (in mm). As body length is determined at eclosion and adults do not feed, this condition index is likely to reflect resources available for reproductive activities (relative merits of using body weight:length ratios versus using residuals of body mass on length have been discussed by Darlington and Smulders 2001).

We also measured male reproductive allocation to testes and accessory glands used to manufacture male spermatophores (van der Reijden et al. 1997). Considerable variation in male reproductive allocation has been found both between and within *Photinus* species (Demary and Lewis 2006). Male reproductive allocation was measured for each male by removing both testes, spiral, long and medium accessory glands from the reproductive tract. Reproductive tissue dry mass was measured to the nearest 1 μg on a Mettler MT5 balance, and percentage allocation to each tissue was calculated based on each male's total body dry mass. To determine if there were trade-offs between a male's courtship attractiveness to females and other male reproductive traits, we examined correlations between each individual male's courtship attractiveness and his accessory gland or testes mass, and body condition index (Systat 10.2: Systat Inc., Chicago, IL).

We examined whether paternity share of males that females found most attractive during courtship was influenced by mating order, body condition differences, the comparative reproductive allocation of the two males that mated with each female, or female secondary spermathecal size. Comparative reproductive allocation was calculated as ratios of the two males' accessory gland and testes masses (most preferred divided by least preferred), and condition index difference (most preferred minus least preferred). Again we employed a generalized linear model approach where preferred male paternity share was modeled as a binomial response variable with a logit link function using SAS PROC Genmod (SAS Inc., Cary, NC).

PHENOTYPIC SELECTION ON MALE TRAITS ACROSS REPRODUCTIVE FITNESS COMPONENTS

We estimated phenotypic selection on male traits (body weight, elytral length, testes mass, and male accessory gland mass) during two episodes of sexual selection: precopulatory male courtship attractiveness and postcopulatory male paternity measured as the number of offspring sired by each male (log transformed). Directional selection gradients for each male trait were estimated by standardized partial regression coefficients from least-squares multiple regressions of each fitness component on male traits (Arnold and Wade 1984; Conner and Hartl 2004).

Results

The two mating treatments differed significantly in the paternity share gained by second males that were least versus most attractive (Fig. 1; separate variance $t = 2.28$, df $= 15$, $P = 0.038$; generalized linear model, treatment estimate $= 1.87$, likelihood ratio $\gamma^2 = 9.77$, $P = 0.0018$). However, contrary to predictions, *P. greeni* males that had been least attractive to females during their courtship interactions subsequently fertilized a greater proportion of females' offspring than did the most attractive males. Male courtship attractiveness did not predict the total number of progeny (log transformed) subsequently sired by each male (linear regression, $r^2 = 0.031$, $F_{1,36} = 1.162$, $P = 0.288$).
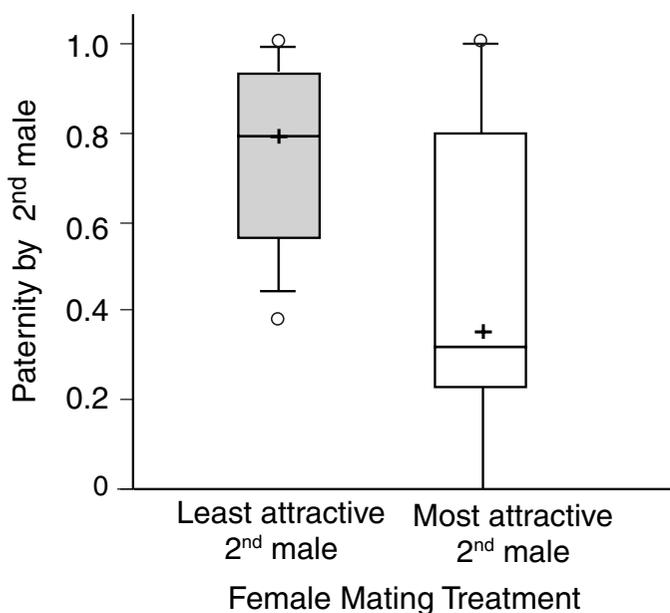
P. greeni females varied widely in how second-male paternity changed across oviposition periods (days after second mating), but there was no consistent difference between the two mating treatments (Fig. 2A). In both treatments, some females produced offspring primarily sired by either the first male (least or most attractive) or the second male across all oviposition periods, whereas other females showed a decline in second-male paternity over time. For females in both treatments, the highest progeny
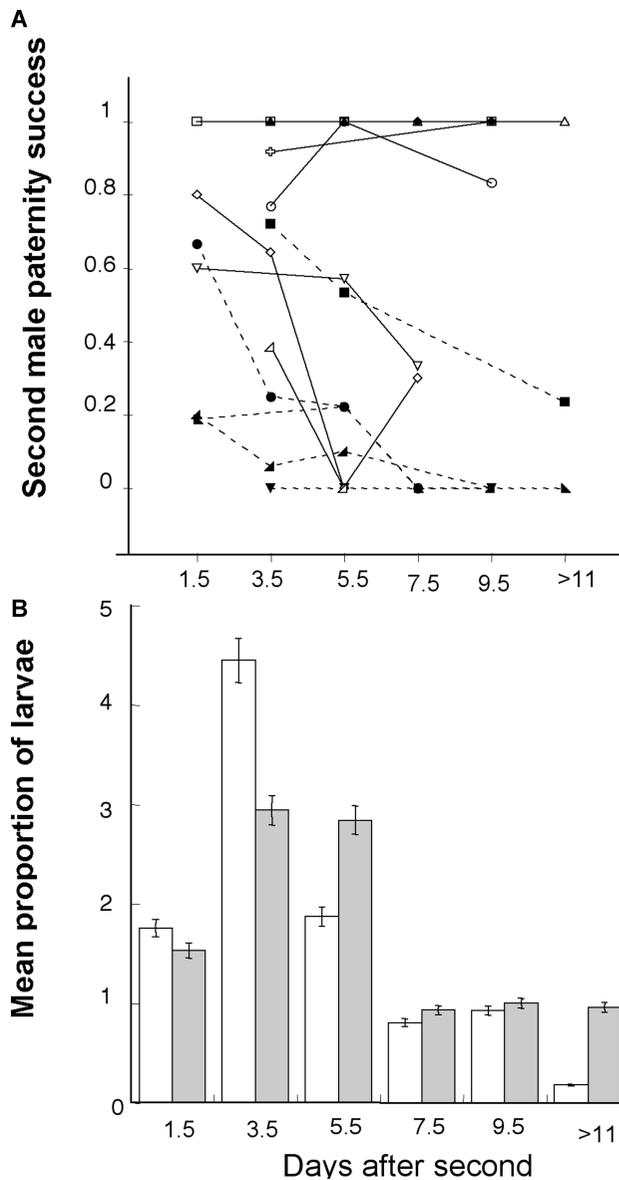

Figure 1. Boxplots showing distributions of second-male paternity by *Photinus greeni* males that were least attractive ($n = 9$) to females during their flash courtships interactions compared to males that were most attractive ($n = 10$) to females. Box edges indicate 1st and 3rd quartiles, and horizontal lines within boxes show medians; vertical lines extend out to closest data values within (1.5 × interquartile range) of box edges, open circles represent outside values, and crosses indicate weighted means based on number of offspring (family sizes ranged from six to 104).

Figure 2. (A) Changes in second-male paternity success (P_2) over time (days after second mating) for individual *Photinus greeni* females whose second mates were least attractive (open symbols) compared to females whose second mates were most attractive (closed symbols). (B) Proportion of total number of offspring produced during each two-day interval (means ± 1 SE) by *P. greeni* females whose second mates were least attractive (open bars) compared to females whose second mates were most attractive (dark bars).

Table 1. Generalized linear model with paternity share achieved by *Photinus greeni* males that were more attractive during courtship as the dependent variable. Effects included mating order, female secondary spermathecal size, magnitude of courtship attractiveness difference, and morphological differences between female's most preferred versus least preferred mates. Morphological differences were calculated as ratios of the two males' accessory gland and testes masses (most preferred divided by least preferred), and condition index difference (most preferred minus least preferred).

Source	Estimate (SE)	Likelihood ratio χ^2	P
Mating order	-1.68 (0.96)	3.19	0.074
Female spermathecal size	-6.81 (20.11)	0.11	0.737
Courtship attractiveness difference	0.01 (0.02)	0.42	0.517
Male accessory gland ratio	1.49 (1.80)	0.71	0.399
Male condition index difference	-0.67 (0.49)	1.84	0.175
Male testes mass ratio	0.86 (1.35)	0.41	0.521

production was seen three to four days after their second mating (Fig. 2B), with more than 75% of offspring produced within the first six days after mating. Thus, a male's paternity share during these first six days postmating largely determined the total number of offspring that he sired.

Other possible factors affecting the paternity share achieved by preferred *P. greeni* males were examined using a generalized linear model (Table 1), which indicated that paternity share was unaffected either by the magnitude of differences in courtship attractiveness, by the ratio of male accessory gland weights or testes weights, or by differences in condition indices. In addition, paternity share achieved by a female's most preferred male was not influenced by the size of her secondary sperm storage compartment. There was a trend indicating that preferred males mated in

the first position (versus second) were less likely to sire progeny, although this did not reach the 0.05 level of significance.

No evidence was found for trade-offs between male courtship attractiveness and reproductive traits. For individual males, there was no relationship between courtship attractiveness and either absolute testes weight (Pearson $r^2 < 0.001$, $n = 38$, $P = 0.995$) or relative testes weight (percentage of total body dry weight, $r^2 = 0.02$, $n = 38$, $P = 0.398$). Also, male courtship attractiveness was unrelated to the total weight of reproductive accessory glands ($r^2 = 0.01$, $n = 38$, $P = 0.533$), relative accessory gland weight ($r^2 < 0.001$, $n = 38$, $P = 0.855$), and male body condition index ($r^2 = 0.027$, $n = 38$, $P = 0.321$).

Phenotypic selection gradients were used to estimate directional selection acting on male morphological traits (Table 2). Of the measured traits, male body weight was influenced most strongly during both precopulatory and postcopulatory selection episodes. Although no significant selection gradients were identified, reversed signs in the observed selection gradients on male body weight between episodes of sexual selection were noted; a negative selection gradient for male weight was found for male courtship attractiveness, whereas male body weight was positively associated with male paternity (Table 2).

Discussion

This study demonstrates that contrary to prediction, male *P. greeni* fireflies that elicited the highest percentage of female responses to their courtship signals subsequently sired a lower percentage of female offspring. These results are important because they indicate that those males successful in attracting mates will not necessarily gain greater paternity share when females mate with multiple males. Additionally, this study suggests that changes in phenotypic selection gradients may occur during different reproductive stages, and highlights the need to extend selection measurements to include both precopulatory and postcopulatory stages to better understand the evolution of male sexual traits.

Table 2. Estimated phenotypic selection gradients ($\pm$ SE) for *Photinus greeni* males ($n = 38$) across precopulatory and postcopulatory components of sexual selection. Selection gradients are based on multiple regressions of each fitness component on standardized (mean $= 0$, $s = 1$) male trait values. Reported *P*-values test H$_0$: $\beta_\iota = 0$.

Male traits	Fitness components			
	Male courtship attractiveness		Male paternity (no. of offspring)	
	Selection gradient (b $\pm$ SE)	P	Selection gradient (b $\pm$ SE)	P
Body weight	-0.176 ± 0.100	0.087	0.169 ± 0.175	0.339
Elytral length	0.066 ± 0.069	0.346	0.145 ± 0.121	0.238
Testes weight	0.053 ± 0.068	0.441	-0.010 ± 0.120	0.934
Accessory gland weight	0.057 ± 0.085	0.504	-0.098 ± 0.149	0.516

Female preferences for male courtship traits are maintained when females gain material and/or genetic benefits from distinguishing among potential mates (Andersson 1994). If male courtship signals provide reliable indicators of male quality, then more attractive males would also be predicted to gain higher relative paternity when mating with previously mated females. A number of previous studies have found evidence supporting this predicted positive correlation between precopulatory and postcopulatory episodes of sexual selection (Lewis and Austad 1994; Pizzari et al. 2002; Evans et al. 2003; Wagner and Harper 2003; Pilastro et al. 2004; Safran et al. 2005). In *Tribolium castaneum* flour beetles, males that were more attractive to females based on their olfactory signals subsequently gained higher paternity success (Lewis and Austad 1994). Pizzari et al. (2002) found that female mating preferences for socially dominant males continued postcopulation in the fowl, *Gallus gallus domesticus*. Similarly, higher paternity was achieved by male barn swallows (*Hirundo rustica)* whose plumage color had been experimentally enhanced (Safran et al. 2005). Such concordance between sexual selection episodes might arise if male courtship signals covary with male traits affecting either male sperm competitive ability or postcopulatory (cryptic) female choice. For example, in the variable field cricket *Gryllus lineaticeps,* males' courtship calls were correlated with ejaculate traits, such that males producing longer and faster chirps transferred more sperm to females, which increased female fertility (Wagner and Harper 2003). Studies in *Poecilia reticulata* guppies have demonstrated that enhanced postcopulatory success of the most attractive males (based on their carotenoid coloration) arises both through enhanced male sperm competitive ability and through females allowing increased sperm transfer by more attractive males (Pilastro et al. 2002, 2004; Evans et al. 2003).

In contrast to these previous studies, we found a strongly negative association between male courtship attractiveness and paternity share in *P. greeni* fireflies. Similar results have been found in *Gerris lacustris* water striders, where large males have higher mating success, but small males have longer copulations and gain higher fertilization success (Danielsson 2001). One plausible explanation for these observed negative associations between reproductive fitness components is that male courtship signals may be subject to energetic trade-offs with other traits influencing postcopulatory paternity share. Trade-offs among various male sexual traits have been found in other species. Moore et al. (2004) identified genetic trade-offs between male sperm viability and testes mass in *Nauphoeta cinerea* cockroaches. Evans et al. (2003) found a possible phenotypic trade-off in *Poecilia reticulata* guppies between male body size and sperm competitive ability. Such proposed phenotypic trade-offs could be especially important in *Photinus* fireflies because these adults do not feed (Lloyd 1997), so all reproductive activity must be fueled by resources acquired during larval stages (Cratsley et al. 2003; Lewis et al. 2004a).

Thus, males could potentially allocate resources toward producing attractive (longer or faster) flash signals rather than investing in ejaculate components that influence male paternity share. Male spermatophore size has been shown to affect paternity in many insects (reviewed in Vahed 1998; Simmons 2001). In a related firefly species, *P. ignitus*, males transferring larger spermatophores gained increased paternity (Rooney 2000); whether this was due to greater sperm number or differences in male accessory gland products is unknown. However, in this study we did not detect any evidence of trade-offs between individual males' courtship attractiveness and either testes or male accessory gland size. It is possible that differences in male paternity might depend on more subtle aspects of sperm transfer, sperm viability, or differences in the composition and quantity of particular male accessory gland products.

Another plausible explanation for the negative association between male courtship attractiveness and paternity success involves female influence over paternity. It has been suggested that females can use multiple signals to assess male quality at sequential mating stages (reviewed by Candolin 2003). For example, in bitterlings, *Rhodeus sericeus*, females use male coloration, courtship behavior, and resource quality to assess males during different stages of courtship (Candolin and Reynolds 2001). Similarly, we suggest that females might use different signals, perhaps in different sensory modalities, for mate choice versus postcopulatory choice of sires. The chase-away theory of sexual selection (Holland and Rice 1998) predicts that male courtship signals will often evolve to become uninformative or misinformative with respect to male quality, which could lead to sexual conflict taking place across reproductive stages (Brown et al. 1997; Rice 1998). If males give unreliable or deceptive courtship signals, it is possible that females subsequently use other signals to correct initial mate choice errors (Lewis et al. 2004a); for example, females might bias paternity toward those males providing larger spermatophores. For species with internally transferred nuptial gifts, it is possible that females could assess male gift size within their reproductive tracts, for example by stretch receptors in the bursa copulatrix (Sugawara 1979). Although *Photinus* females are known to rely on visual signals for long-range mate assessment, there is some evidence that after contact they can switch to other, perhaps tactile and/or short-range chemical signals (Lloyd 1966). Recent studies of two *Photinus* species reveal variation in how reliably male flash signals indicate male nuptial gift size. In *P. ignitus*, males that produced more attractive courtship signals (longer pulse durations) also transferred larger spermatophores (Cratsley and Lewis 2003). However, Michaelidis et al. (2006) found no relationship in *P. greeni* between male interpulse interval (the flash trait preferred by these females) and spermatophore mass. Both of these studies focused exclusively on early season males to assess size of males' first spermatophores. However, because *Photinus* male

spermatophore size is known to decline with successive matings (Cratsley et al. 2003), it is possible that the reliability of male signals fluctuates over the mating season. In addition, it is possible that these two species may represent different stages in the evolution of *Photinus* signaling systems, moving from reliable to non-informative or manipulative courtship signals. Additional work is needed to determine the relationship between male courtship signals, nuptial gift size and quality, and male genetic quality.

Studies in other taxa have documented extremely bimodal P_2 distributions (e.g., Simmons 2001; Hockham et al. 2004), yet causes of such variation remain unknown. In the present study, several *Photinus* females produced only offspring sired by the first male ($P_2 = 0$) or by the second male ($P_2 = 1$). In our experiment we visually confirmed successful mating by both males, and numerous dissections have previously confirmed that progression to the second stage of copulation always indicates successful spermatophore transfer (unpubl. data). It has been suggested that multiple sperm storage organs might provide females greater control over paternity (Hellreigel and Ward 1998; Snow and Andrade 2005), but we found no correlation between the size of a particular female's secondary spermatheca and the paternity success of the males that were most attractive to that female during courtship interactions. Differences in where a particular male's sperm is stored could potentially generate extremes in second-male paternity. In *P. greeni* females, sperm viability differed among storage sites; sperm stored in the secondary spermathecae retained higher viability than sperm stored in the primary spermatheca (Demary 2005). Further studies are needed to identify the proximal mechanisms responsible for paternity bias in this species.

Under polyandrous mating systems, male reproductive fitness is a product of mating success and average paternity share achieved with each mating. Although many studies have addressed how courtship traits influence male mating success, little attention has been given to whether postcopulatory selection episodes might reinforce, counteract, or be unrelated to precopulatory selection. This study demonstrates a significant discrepancy between female mate choice based on the bioluminescent flash displays of firefly males and subsequent male paternity share. These results suggest that an integrated approach to understanding the evolution of male sexual traits will need to incorporate both precopulatory and postcopulatory episodes of sexual selection.

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