

Assessing Condition-dependence of Male Flash Signals in *Photinus* Fireflies

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Abstract Females often show a preference for exaggerated male sexual traits or courtship behaviors. Such preferences can benefit females if trait expression is correlated with male genetic quality or phenotypic condition. Previous studies of several *Photinus* fireflies have revealed considerable intraspecific variation in the bioluminescent courtship signals emitted by males, and have also demonstrated that females prefer more conspicuous male signals. Thus, females might gain information about male phenotypic quality if courtship signals reflect male condition. We examined possible condition-dependence of *Photinus* male courtship signals using two complementary approaches. First we experimentally manipulated male mating status, which is expected to affect male condition by depleting resources required for nuptial gift formation, and looked at how individual male flash signals changed with mating status and over time. We used an additional approach to assess condition-dependence by examining whether a relationship exists between flash signal parameters and measures of male condition and body shape. We found that the pulse rate of *P. greeni* courtship signals was not altered by male mating status or age, and that the pulse duration of *P. ignitus* signals was also not affected by male mating status. In *P. pyralis* fireflies, males showed a non-significant trend toward reduced signal pulse duration with age. When we examined the relationship between male flash signals and condition measures, we found no effect of male condition or body shape on courtship signals in *P. greeni* or *P. ignitus*; in *P. pyralis*, males with wider body shapes produced longer duration flash signals. On the other hand, we found no evidence in *P. pyralis* that condition predicted flash duration. Taken together, these

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results indicate that *Photinus* males' flash signals do not reflect adult male condition, and suggest that females are unlikely to use courtship signals as an indicator of male phenotypic quality.

Keywords Bioluminescence · phenotypic condition · firefly · nuptial gift · sexual selection · spermatophore

Introduction

In many animal species, females show preferences for exaggerated male sexual traits or courtship behaviors (Darwin 1871), yet there is persistent debate about how these female preferences have evolved (Andersson 1994; Tomkins et al. 2004). Such preferences could benefit females if male traits act as reliable signals of either male phenotypic condition or genetic quality (Kirkpatrick and Ryan 1991). Because exaggerated male traits are expected to be costly, trait expression should depend on a male's available resources, or condition (Zahavi 1975; Andersson 1986; Kotiaho 2000; Emlen 2001). Although condition-dependence of male sexual traits and behaviors is commonly assumed (Andersson 1994) much of the evidence for this relationship is correlational, and sound experimental evidence remains scarce (Cotton et al. 2004).

In *Photinus* fireflies (Coleoptera: Lampyridae), courtship consists of a bioluminescent signaling dialogue between advertising males and responding females (Lloyd 1966). Previous studies of several *Photinus* species have revealed considerable intraspecific variation in the bioluminescent courtship signals emitted by males, and have also demonstrated that females prefer more conspicuous male signals (reviewed by Lewis and Cratsley 2008). In those *Photinus* species where males emit multiple-pulse courtship signals, females prefer faster pulse rates (Branham and Greenfield 1996; Michaelidis et al. 2006). In *Photinus* species with single-pulse male signals, females prefer longer pulse durations (Cratsley 2000; Cratsley and Lewis 2003). In most *Photinus* fireflies, males provide females with a nuptial gift during mating, which consists of a complex proteinaceous spermatophore (van der Reijden et al. 1997; Cratsley et al. 2003; Lewis et al. 2004). *Photinus* females that receive more spermatophores show higher lifetime fecundity, demonstrating a direct fitness benefit (Rooney and Lewis 1999, 2002). *Photinus* do not feed as adults (Williams 1917; Lloyd 1997) and spermatophore production is costly to males; spermatophores represent about 5% of male body mass and spermatophore size declines monotonically across sequential matings (Cratsley et al. 2003). Thus, if *Photinus* courtship signals reflect male condition, male flash signals are expected to change after mating.

In the present study, we examined possible condition-dependence of *Photinus* male courtship signals using two complementary approaches. First we experimentally manipulated male mating status, which is expected to affect male condition by depleting resources required for nuptial gift formation, and looked at how individual males' flash signals changed with mating and over time. We also used an observational approach to assess condition-dependence by examining whether there was a relationship between flash signal parameters and measures of male condition and body shape.

Methods

Study Organism and Flash Signal Recording

Male courtship signals have been well-characterized for each of three firefly species used in this study. *Photinus greeni* males produce courtship flashes consisting of paired pulses (Buck and Buck 1972). *Photinus ignitus* and *P. pyralis* males produce single-pulsed courtship flashes (Lloyd 1966; Vencl and Carlson 1998). In *P. greeni*, females prefer shorter interpulse intervals (time from the beginning of the first pulse to the beginning of the second pulse; Michaelidis et al. 2006), while *P. ignitus* and *P. pyralis* females preferentially respond to longer pulse durations (Cratsley and Lewis 2003; Cratsley 2000). Males in all three species produce spermatophores that are transferred to females during mating (Lewis et al. 2004).

Actively signaling adult fireflies were collected from breeding aggregations during their respective mating seasons and nightly flight periods. *Photinus greeni* fireflies were collected in Lincoln, MA USA during summer 2008. *Photinus ignitus* fireflies were collected in Lincoln, MA USA during summer 2000 and 2008. *Photinus pyralis* males were collected in Plainsboro, NJ and Bucknell, PA USA during 1998–99 and 2004. For experimental manipulation of male mating status in *P. greeni* and *P. ignitus*, we collected males at the beginning of their mating season (before females emerged) to maximize the likelihood that males were virgins. Fireflies were maintained in individual plastic containers containing moistened filter paper. Females were provided pieces of apple but males were not fed. *P. greeni* were maintained in the laboratory on a 16 L:8D light cycle that was reversed so that, after artificial dusk, courtship flashing could be recorded from 1200 to 1400 h. *Photinus ignitus* and *P. pyralis* were kept on a natural light cycle and their courtship flashing was recorded from 2000 to 2200 h.

About 2 h prior to flash recording, males were transferred to cylindrical mesh cages (20×14 cm) to provide increased opportunity for mobility during flash signaling. Males were visually isolated from one another using cardboard dividers between cages, and male flash behavior was elicited by simulating female courtship signals. We recorded male flashes using a photomultiplier tube attached to a portable data acquisition system (DASport, Intelligent Instrumentation, Tuscon, Arizona). Flash data was recorded at a frequency of 1,000 Hz and streamed to Visual Designer 3.0 software. For all flashes that fell within the range of the characteristic male courtship signal for each species, we used DataView 5.2 to measure temporal flash parameters to the nearest 1 msec. We focused on the particular parameters of male courtship signals that have been previously shown to affect female response: interpulse interval (as defined above) for *P. greeni*, and pulse duration for *P. ignitus* and *P. pyralis*. We measured pulse duration as the time from half-maximal intensity reached during flash rise to half-maximal intensity reached during flash decay (this minimizes variation inherent in measuring waveform tails; Cratsley and Lewis 2003).

Flash timing characteristics are known to be temperature-dependent (Edmunds 1963; Carlson et al. 1976; Lloyd 1966), so ambient temperature was monitored during all flash recordings (range = 71°–80°F) and later male flash traits were adjusted to common temperatures of 77°F, 76°F, and 75°F for *P. greeni*, *P. ignitus*, and *P. pyralis*, respectively, using the following temperature relationships established

in previous studies (Michaelidis et al. 2006; Cratsley and Lewis 2003; Cratsley 2000):

Photinus greeni

$$\text{Interpulse interval (ms)} = 121900 - 5277(\text{temp. } ^\circ\text{F}) + 77.1(\text{temp. } ^\circ\text{F})^2 - 0.3752(\text{temp. } ^\circ\text{F})^3$$

Photinus ignitus

$$\text{Pulse duration (ms)} = 448.1 - 4.8(\text{temp. } ^\circ\text{F})$$

Photinus pyralis

$$\text{Pulse duration (ms)} = 694.9 - 6.67(\text{temp. } ^\circ\text{F})$$

Experimental Manipulation of Male Mating Status

We experimentally manipulated mating status of *P. greeni* and *P. ignitus* males during summer 2008 to determine how individual males' flash signals changed over time. Matings were accomplished by placing an individual male with a female during their normal courtship period. Pairs were monitored continuously until we observed stage II of copulation, which indicates spermatophore transfer (van der Reijden et al. 1997).

For *P. greeni*, flash recordings of each male ($n=9$) were obtained immediately before mating and again 1–2 d later; 5 males were also recorded following their second mating. For *P. ignitus*, after initial flash recordings were obtained, males were randomly assigned to 2 treatments: some males ($n=6$) were mated and their flashes were recorded again 1–2 days later, while other males ($n=5$) remained unmated and were recorded 1–2 d later. These unmated *P. ignitus* males allowed us to determine if male flash characters changed with age independent of mating. Unmated *P. pyralis*, males ($n=12$) were also recorded initially and then again 1–10 days later in 1997, 1998 and 2004.

From the flash recordings for each male, we calculated their mean flash signal parameters (interpulse interval for *P. greeni*, pulse duration for *P. ignitus* and *P. pyralis*) for all flashes recorded on a given night; this ranged from 2 to 5 flashes per male for *P. greeni*, from 1 to 12 flashes per male for *P. ignitus*, and from 2 to 12 flashes per male for *P. pyralis*. We used paired t-tests for each species to determine whether individual male's flash signals changed over time. All statistical tests were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Effects of Male Condition and Body Shape on Flash Signals

We also used another approach to assess condition-dependence by examining whether there was a relationship between male flash signals and measures of male condition and body shape. Although many different measures of physiological condition have been suggested (Jakob et al. 1996; Green 2001), we employed a commonly used condition index calculated as a ratio of body mass to elytral length. Based on our observation that male shape was highly variable, we also measured the ratio of maximum elytral width to elytral length for each male. Fireflies were

weighed to the nearest 0.1 mg within 12 h of collection, and elytral width and length measurements were made on digital photographs taken with a stereomicroscope. Male flash signals were measured as described above, and mean flash signal parameters (interpulse interval for *P. greeni*, pulse duration for *P. ignitus* and *P. pyralis*) were calculated based on all flashes recorded during the initial night of recording for each male. We used linear regressions for each species to examine whether male flash signals were related to male condition index and to body shape.

Results

Effects of Mating Status and Age on Male Flash Signals

For *P. greeni*, the average interpulse interval of male flash signals showed no significant change after mating (Fig. 1; paired $t = 0.884$, $df = 7$, $P = 0.406$). There was also no apparent change in interpulse interval for 5 males that were measured again after two matings (Fig. 1). Similar results were found for *P. ignitus*, as there was no significant change in average pulse duration of males before and after mating (Fig. 2a; paired $t = -0.726$, $df = 4$, $P = 0.508$). For unmated males, average pulse duration also did not change significantly between two nights on which they were recorded (Fig. 2b; paired $t = -0.332$, $df = 5$, $P = 0.753$). Finally, for *P. pyralis* there was a trend showing a decrease in male pulse duration from an initial night on which they were recorded to a subsequent night, although this was not significant (Fig. 3; paired $t = 2.022$, $df = 11$, $P = 0.068$).

Effects of Male Condition and Body Shape on Flash Signals

In *P. greeni*, neither condition index nor body shape explained a significant proportion of the variation among males in the interpulse interval of their courtship signals (Fig. 4a: condition index regression $r^2 = 0.088$, $F = 0.580$, $df = 1, 6$, $P = 0.475$; Fig. 4b: body shape regression $r^2 = 0.123$, $F = 0.981$, $df = 1, 7$, $P = 0.355$). Similarly,

Fig. 1 Changes in mean interpulse interval of the flash signals produced during courtship by *P. greeni* males when male condition is manipulated by mating. Individual males (represented by lines) were recorded before mating ($n = 10$), after 1 mating ($n = 9$), and after 2 matings ($n = 5$).

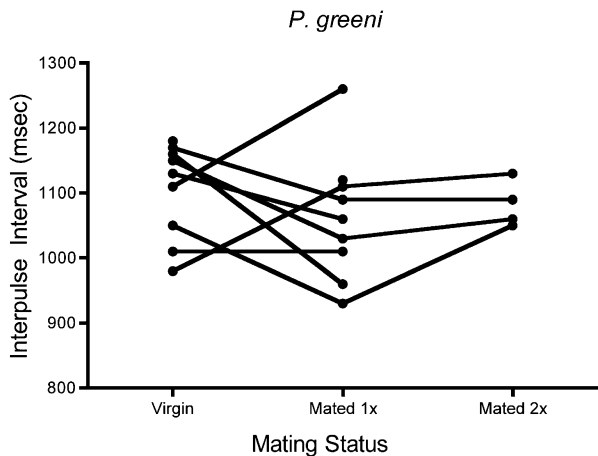
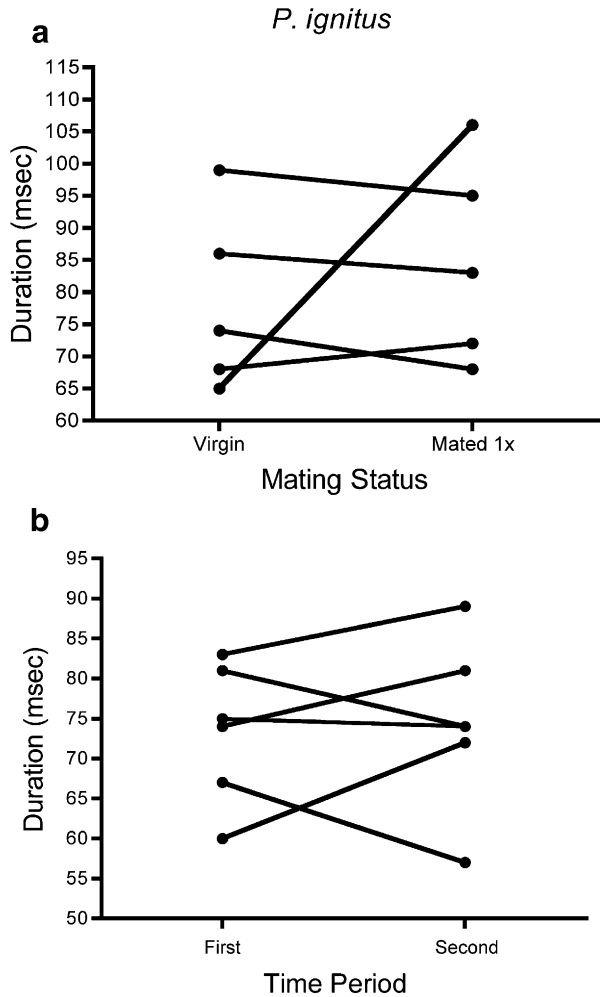


Fig. 2 Changes in mean pulse duration of the flash signals produced during courtship by *P. ignitus* males as a function of a) male condition, manipulated by mating ($n=5$) and b) male age ($n=6$) Each line represents one male.

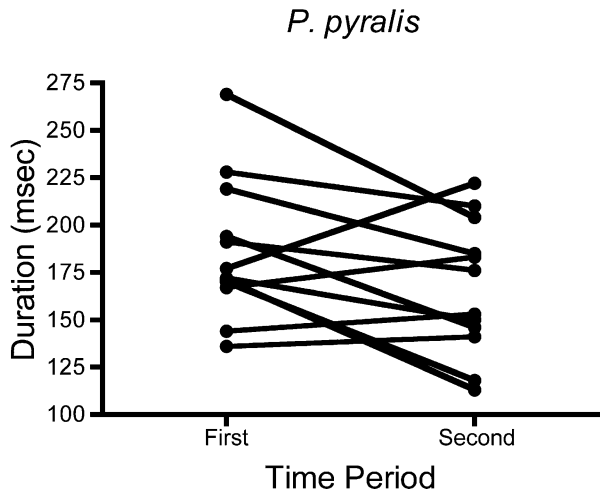


for *P. ignitus* there was no significant relationship between male pulse duration and either male condition index or body shape (Fig. 5a: condition index regression $r^2 = 0.047$, $F=2.407$, $df = 1, 49$, $P=0.127$; Fig. 5b: body shape regression $r^2 = 0.020$, $F=0.997$, $df = 1, 49$, $P=0.323$). Likewise, the pulse duration of *P. pyralis* males was not related to condition index (Fig. 6a: regression $r^2 = 0.034$, $F=0.869$, $df = 1, 25$, $P=0.360$). However, *P. pyralis* body shape accounted for a significant proportion of the variation in male pulse duration (Fig. 6b: $r^2 = 0.179$, $F=5.453$, $df = 1, 25$, $P=0.028$), such that wider-bodied males had longer duration signals.

Discussion

In this study, we found little experimental or observational evidence across three *Photinus* species to support the idea that firefly male courtship signals are condition-dependent. Although we predicted that reduced male energy reserves after mating

Fig. 3 Changes in mean pulse duration of the flash signals produced during courtship by *P. pyralis* males as a function of male age ($n=12$, each line represents one male).



(and associated spermatophore production) would reduce the pulse rate or pulse duration of male flash signals, we found no change in male courtship signals before and after mating for either *P. greeni* or *P. ignitus* fireflies. In these non-feeding adults we also expected that as males got older, declining nutritional resources might alter male flash signals. However, we observed no consistent change in male signals with increased age for *P. ignitus* fireflies, although there was a non-significant trend over

Fig. 4 Relationship between the interpulse interval of courtship signals produced by *P. greeni* males and a) condition index (male mass [mg]/elytral length [mm]); least-squares regression line: $1257.506 - 54.135 * \text{condition index}$, $n=8$ males) and b) male body shape (maximum elytral width/elytral length); least-squares regression line: $1757.653 - 1533.083 * \text{body shape}$, $n=9$ males).

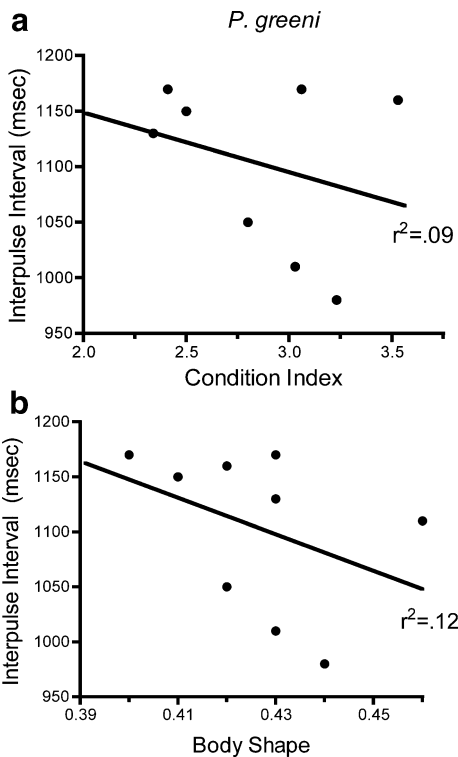
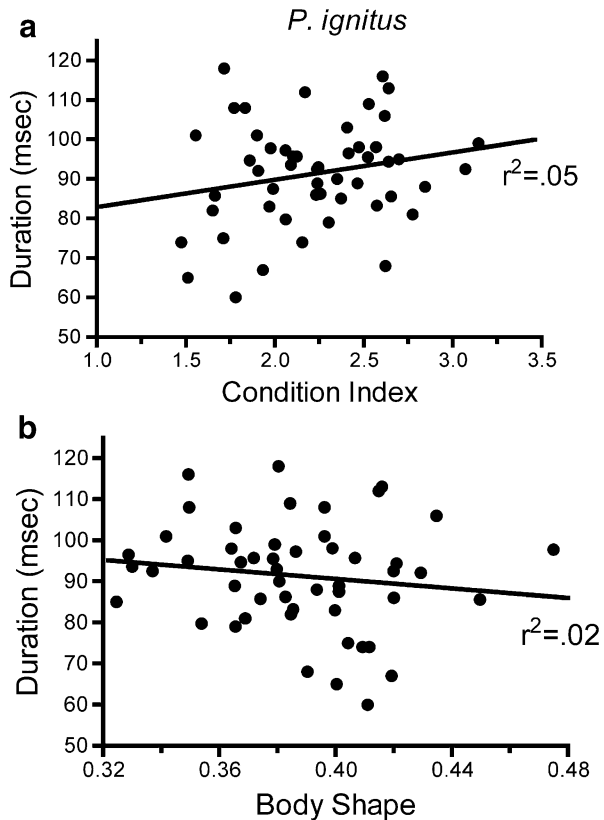


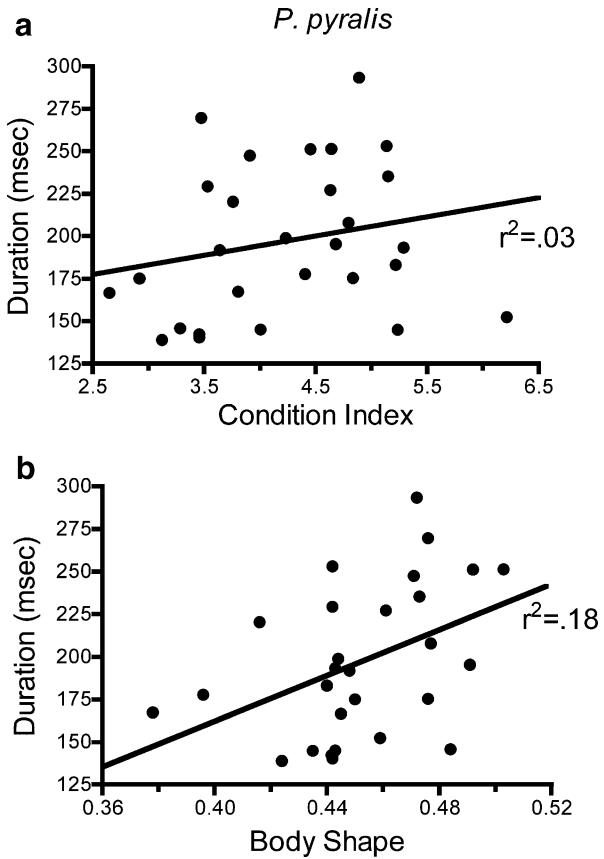
Fig. 5 Relationship between the pulse duration of courtship signals produced by *P. ignitus* males and a) condition index (male mass [mg]/elytral length [mm]); least-squares regression line: $75.828 + 7.013 * \text{condition index}$, $n=51$ males) and b) male body shape (maximum elytral width/elytral length) least-squares regression line: $114.182 - 58.761 * \text{body shape}$, $n=51$ males).



time for *P. pyralis* males to emit shorter duration signals. In another approach to investigate condition-dependence, we found no dependence of male flash signals on condition index for any of these *Photinus* species. We did observe that male *P. pyralis* with relatively wider bodies emitted longer duration courtship signals. Although it is not clear what this pattern means, our findings suggest that it is unlikely that variation in adult firefly condition generates this relationship between body shape and flash signal duration.

Signal costs are responsible for generating condition-dependence of male sexual signals, because males in poor physiological condition should be unable to produce exaggerated signals (Kotiaho 2001; Searcy and Nowicki 2005). Condition-dependence has been demonstrated in numerous species with acoustic courtship signals, and production of such male signals generally requires high energetic expenditure (Kotiaho 2001). For example, rates of substrate drumming in wolf spiders depends on male condition, and signal production is energetically costly and reduces male longevity (Kotiaho 2000). Our finding that the bioluminescent signals of *Photinus* fireflies are not condition-dependent is consistent with recent work that documented low energetic costs of firefly flash signals (Woods et al. 2007). This low signal cost may explain the observed lack of condition-dependence in *Photinus*

Fig. 6 Relationship between the pulse duration of courtship signals produced by *P. pyralis* males and a) condition index (male mass [mg]/elytral length [mm]); least-squares regression line: $159.313 + 9.355 * \text{condition index}$, $n=27$) and b) male body shape (maximum elytral width/elytral length); least-squares regression line: $-92.675 + 644.738 * \text{body shape}$, $n=27$).



courtship signals, as males would expend little energy producing flash signals with longer pulse durations or faster pulse rates.

However, signal costs need not be incurred at the time of signal production for these costs to maintain signal honesty (Searcy and Nowicki 2005). Developmental costs of producing the firefly lantern and the associated neural pathways necessary for generating conspicuous courtship flashes could make male flash signals dependent on larval nutritional conditions. Variation in larval resources obtained to invest in male lanterns, body size, and even the accessory glands necessary for spermatophore production, could help explain the relationship we observed between male body shape and flash duration in *P. pyralis* as well as the covariance between spermatophore mass and flash duration observed in early season *P. ignitus* (Cratsley and Lewis 2003). This possibility merits further study, as honest signaling of male larval condition represents one of many possible explanations that need to be explored to understand the evolution of female preferences for conspicuous male flashes in *Photinus* fireflies.

Photinus females have been shown to prefer more conspicuous male flash signals; they are more likely to respond to faster pulse rates in species whose males emit multiple-pulse courtship signals and to longer pulse durations in species whose males emit single-pulse signals (reviewed by Lewis and Cratsley 2008). Results of the

present study suggest that such preferences based on male flash signals would not allow *Photinus* females to identify mates having better adult phenotypic condition.

Previous studies have examined whether male flash characteristics might provide reliable information about direct benefits provided by a male's spermatophore. However, contradictory results have been found concerning the relationship between male flash characteristics and nuptial gift size (reviewed by Lewis and Cratsley 2008). In *P. ignitus* fireflies, early-season males with longer duration flash signals also had larger spermatophores, but this relationship disappeared later in the mating season (Cratsley and Lewis 2003). In *P. greeni*, no relationship was detected between interpulse interval of early-season males and their spermatophore size (Michaelidis et al. 2006). Thus, the direct benefits provided by male nuptial gifts are unlikely to provide a general explanation for how female preferences for conspicuous male flash signals are maintained.

Signal preferences of *Photinus* females could have evolved through sensory bias (Endler and Basolo 1998; Fuller et al. 2005) or because conspicuous signals are honest indicators of other aspects of male phenotypic or genetic quality (Andersson 1994; Searcy and Nowicki 2005). Female preference greater flash intensity has been demonstrated in *P. ignitus* (Cratsley and Lewis 2003) and *P. pyralis* fireflies (Vencl and Carlson 1998); this may represent sensory bias because signal intensity varies with distance and thus seems unlikely to provide reliable information about male quality (Cratsley and Lewis 2003). The temporal characteristics of male flashes on the other hand may yet prove to serve as honest indicators of some aspect of male quality. While we found little to suggest that adult male condition influences male flash characteristics, further studies are needed to elucidate the relationship between firefly female preferences, male courtship signals, and mate quality.

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