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Male courtship signals and female signal assessment in *Photinus greeni* fireflies

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The evolutionary dynamic of courtship signaling systems is driven by the interaction between male trait distributions and female preferences. This interaction is complex because females may choose mates based on multiple components of male signals, and female preference functions may vary depending on mate availability, female reproductive state, and environmental conditions. In *Photinus* fireflies (Coleoptera: Lampyridae), flying males emit bioluminescent flash signals to locate sedentary females, which reply selectively to attractive male flash signals with their own response flash. In this study, we first examined temporal variation in the paired-pulse flash patterns produced by *Photinus greeni* males in the field and found significant among-male variation (~70% of total variation) in interpulse intervals (IPIs). There was no significant relationship between male IPI and spermatophore size, suggesting that *P. greeni* male courtship signals do not provide females with reliable indicators of male material resources. In laboratory playback experiments, we presented *P. greeni* females with simulated flash signals to assess how IPI and pulse duration independently affected the likelihood of female flash response. We also examined the effects of female body mass and time during the mating season on female preference functions, hypothesizing that females would be less discriminating when they were heavier (more fecund) and when mate availability declined. We found that *P. greeni* females discriminated among signals within their species' range based primarily on flash pattern IPI. Neither the time during the mating season nor female weight altered female preference functions for IPI, although season did influence female response to pulse duration. These results reveal that *P. greeni* females discriminate among conspecific males based primarily on male IPIs, the same signal character previously shown to be important for firefly species recognition. Field playback experiments indicated that female responsiveness peaked near the average IPI given by males at different ambient temperatures, suggesting that fireflies exhibit temperature coupling similar to that seen in many acoustically signaling animals. *Key words:* bioluminescence, mate choice, multiple cues, preference functions, sexual selection, temperature coupling. *[Behav Ecol 17:329–335 (2006)]*

The direction and intensity of sexual selection on male courtship signals depend on the interaction between male trait distributions and female preference functions (Andersson, 1994; Wagner, 1998). Several hypotheses have recently been proposed for how females might combine information from multiple male signals to recognize or assess potential mates (reviewed in Candolin, 2003; Hebets and Papaj, 2005). For example, multiple signals (sensu Maynard Smith and Harper, 2003) might provide females with redundant information about male quality (backup messages hypothesis) or with information about distinct aspects of male genetic quality or phenotypic condition (multiple messages hypothesis). Additionally, different signals may be used for purposes of species recognition and mate quality assessment. To begin distinguishing among hypotheses concerning multiple signals, we need to examine how females perceive and respond to courtship signal components and determine what information such signals convey.

Theoretical and empirical studies indicate that female preferences are not fixed but rather are phenotypically plastic behaviors that may depend on a variety of factors, including female nutritional status, mating history, mate availability, and temperature (Fawcett and Johnstone, 2003; Gibson and Langen, 1996; Jennions and Petrie, 1997; Lynch et al., 2005; Pitcher et al., 2003; Real, 1990). The phenomenon of temperature coupling represents an example of linked variation between male signals and female preference. In many acoustically signaling ectotherms, temperature dependence of male courtship signals is matched by a concordant change in female preferences (e.g., Gerhardt, 1978; Pires and Hoy, 1992; but see Ritchie et al., 2001). Such temperature coupling allows females to recognize conspecific male signals independently of variation in environmental temperatures. However, few studies have examined whether temperature coupling occurs in the bioluminescent signaling systems of fireflies (but see Carlson et al., 1976).

Because courtship in nocturnal *Photinus* fireflies is based on highly visible bioluminescent signals, these insects are particularly amenable to studies of female choice. Previous work has examined variation in male flash signals and female preference in several *Photinus* firefly species (Branham and Greenfield, 1996; Cratsley and Lewis, 2003; reviewed in Greenfield, 2002; Lewis et al., 2004). In *Photinus ignitus*, males emit a single-pulse courtship signal, and females respond preferentially to longer duration signals. Early in the mating season, *P. ignitus* males with longer signal durations also provide larger nuptial gifts (spermatophores) to females during mating. Therefore, male courtship signals in some *Photinus* may allow females to assess the material resources provided by a potential mate. Previous studies have also demonstrated that female responsiveness in *Photinus* fireflies is context dependent, influenced by both a female's mating history and her nutritional status (Cratsley and Lewis, 2003). *Photinus* fireflies thus offer a useful model for understanding how multiple male signal characters influence female choice as well as for exploring plasticity of female preference functions.

This study was designed to characterize variation in the bioluminescent courtship signals of field-recorded *Photinus greeni* males and to determine whether male flash traits are correlated with direct benefits provided by male spermatophores.

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We also used both field and laboratory photic playback experiments to assess female preference for two male signal traits, interpulse interval (IPI) and pulse duration, when these signal traits were varied independently. Finally, we examined the plasticity of female preference functions in response to female body mass, mate availability, and environmental temperatures.

METHODS

Study organism and site

In most *Photinus* fireflies, roving males produce sexual advertisement flashes to locate sedentary females, which respond to attractive male flash signals with their own bioluminescent flashes (Carlson and Copeland, 1985; Lloyd, 1966). Courtship periods typically last 1–2 h each night, during which individuals of both sexes have the opportunity to sample many potential mates, although both sexes mate only once each night (Lewis and Wang, 1991; Wing, 1985). We chose to investigate male courtship signals and female response of *P. greeni* fireflies. Compared to other *Photinus* species (Branham and Greenfield, 1996; Cratsley and Lewis, 2003), relatively little is known about male signal variation or female preferences for *Photinus* species like *P. greeni,* in which males emit courtship signals consisting of paired pulses (but see Buck J and Buck E, 1972; Buck and Case, 1986; Carlson et al., 1976, 1977). In addition, *P. greeni* is part of the *consanguineus* species complex consisting of several morphologically indistinguishable species differentiated only by their courtship flash behavior (Lloyd, 1969). Three species have been described based on the time interval between the paired pulses comprising their male courtship signals: *Photinus consanguineus* has a short, $\sim$0.5-s IPI, followed by *P. greeni* with $\sim$1.0–1.5 s, and *Photinus macdermotti* with $\sim$2.0-s IPI. The *consanguineus* species complex is broadly distributed across the eastern US, with *P. greeni, P. macdermotti,* and *P. consanguineus* showing extensive geographic overlap (Lloyd, 1966, 1969). Female assessment of male flash signals within this species complex may function not only in species recognition (Lloyd, 1966, 1969) but also in mate quality discrimination.

In this study, experiments were conducted and fireflies were collected at the Smith-Andover field in Lincoln, Massachusetts, USA (42° 26′ N, 71° 18′ W) during June–July 2004. *P. greeni,* the only species in the *consanguineus* complex present at our site, was first observed flashing on 8 June (defined as day 1 of the mating season) and was last found on 24 July (day 43). *P. greeni* is a dusk-active species (Buck J and Buck E, 1972; Lloyd, 1969); males begin signaling $\sim$15 min after sunset, and the male flight period lasts $\sim$45 min. By 90 min after sunset, the majority of *P. greeni* dialogs have ceased. As in other *Photinus* species (Cratsley and Lewis, 2005; Lewis and Wang, 1991), *P. greeni* male availability declines over the course of the mating season.

Male flash signal variation

To characterize variation in courtship signals, flash phrases produced by individual *P. greeni* males were video recorded at 30 frames per second (fps) using a Sony TRV80 digital camcorder during days 1–20 of the mating season. Males were videotaped until 15 male phrases were recorded, the male stopped flashing, or the male was lost. After 15 phrases were recorded, males were collected to prevent rerecording. During videotaping, we noted the time and ambient air temperature at the male's flight height.

From the video recordings, we measured two male signal characters, pulse duration and IPI (the time interval between the paired pulses that make up each flash phrase of *P. greeni* males); we did not measure male flash pattern repetition rate because this character is highly dependent on signaling context. IPIs for each male were measured with frame-by-frame analysis of digitized video using iMovie 4. This IPI was determined as the number of frames between the first visible light of the first male pulse and the first visible light of the second male pulse, multiplied by the time span of each frame (33 ms). This method of measuring male *P. greeni* IPI yielded a maximum error of 6.6%. Only males that emitted at least four video recorded flash phrases were included in our analysis, which included 221 male flash phrases from 30 *P. greeni* males.

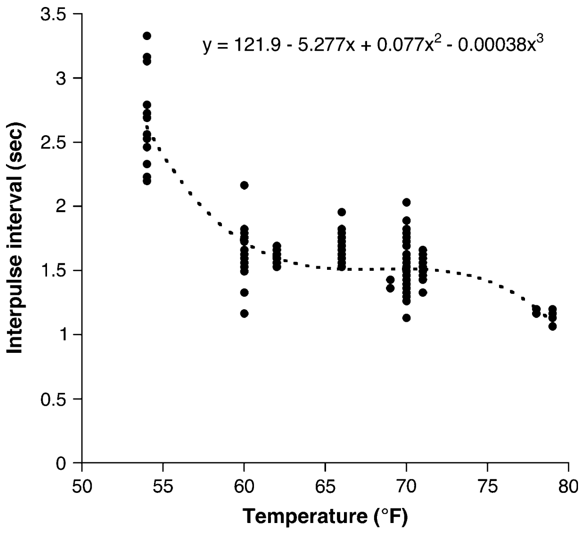

Figure 1
Dependence of field-recorded male *Photinus greeni* courtship IPI on ambient air temperature ($r^2 = .724$). Male IPIs were video recorded in the field ($n = 221$ flash patterns), and a polynomial regression was used to temperature adjust all male IPIs to 70°F (see Figure 2).

For measurements of pulse durations, we increased temporal resolution by deinterlacing video recordings to yield a frame rate of 60 fps. This allowed us to measure male pulse durations in 16.67-ms increments. Because this results in a possible overestimate of up to 50% for the shortest pulse durations ($\sim$70 ms), we report only the range of *P. greeni* pulse durations measured at 70°F, which represents the testing range used in our laboratory studies of female preference.

Temperature affects the mating signals of many ectotherms, including fireflies (Carlson et al., 1976; Lloyd, 1966). To assess the variation in IPI among males recorded in the field at different ambient temperatures, we temperature adjusted each IPI to a common temperature of 70°F, the ambient temperature used during laboratory testing of female preferences. Field-recorded IPIs were temperature adjusted by fitting a polynomial regression (Figure 1, $r^2 = .724$) and adding the residual from this regression to the mean IPI at 70°F. We examined variation within and among *P. greeni* males in their temperature-adjusted IPI using one-way ANOVA. We also examined the possible relationship between male signal characteristics by comparing each male's average temperature-adjusted IPI to his average temperature-adjusted pulse duration. To avoid including pulse duration in both variables, we first subtracted pulse duration from the IPI measurements and then examined the correlation between signal traits.

We also assessed variation in flash signals produced by *P. greeni* males during three distinct stages in their nightly activity period: warm up, patrolling, and dialoguing. Warm-up flashes were defined as those flashes produced by males perched at the top of grass blades at the onset of the male flight period (approximately up to 30 min after sunset). Patrolling flashes were defined as advertisement signals given by flying males. Dialoguing flashes were defined flashes as given by males that were engaged in courtship dialogs with females. We compared male IPI (temperature adjusted as described above) across the three stages using a Kruskal-Wallis nonparametric test.

We examined whether courtship signals in *P. greeni* are correlated with the mass of males' spermatophores, which provide a direct fecundity benefit to females (Rooney and Lewis, 2002). Due to the difficulty of measuring both male signals and spermatophore size, for this analysis we combined data from 2004 and 2005 mating seasons. In both years, males were collected during the first 10 days of the mating season; we focused on early mating season because spermatophore size is known to decline across successive matings (Cratsley et al., 2003). After each male's flash pattern was video recorded (2–15 flash phrases for each male), males were allowed to mate with assigned females, and spermatophores were dissected from female reproductive tracts shortly after transfer (van der Reijden et al., 1997). Spermotophores were rinsed briefly in distilled water, dried in a desiccator for 24 h, and then weighed to the nearest 1 μg on a Mettler MT5 microbalance. In *P. ignitus,* spermatophore mass has been shown to be influenced by male body size (Cratsley and Lewis, 2003; Cratsley et al., 2003), so we accounted for possible covariation by including male mass in a multiple linear regression analysis to see if male flash IPI predicted male spermatophore mass.

Female preference functions: laboratory experiments

Flash characteristics affecting female response were identified through experiments in which *P. greeni* females were presented sequentially with courtship signals differing in either IPI or pulse duration. Field-collected females were weighed to the nearest 0.1 mg and kept on a reversed 8:16 h light:dark cycle. To examine seasonal changes in female responses, we compared *P. greeni* early-season females (collected on days 7–11 of the mating season under male-biased sex ratios) to late-season females (collected on days 21–39 under female-biased sex ratios). Females' previous mating histories were unknown, although late-season females had likely mated at least once.

Females were tested individually shortly after artificial dusk (lights dimmed to ~20 lux), at temperatures maintained between 70°F and 72°F. Flash pulse duration and IPI were varied separately, with five levels of each presented to females in a latin square design to control for presentation order effects. Females were positioned 24 cm from the output LED (570 nm, Ledtronics Inc., Torrance, CA) of a programmable flash generator that produced square pulses near the peak wavelength of male *P. greeni* flashes (572 nm; Case, 1984). To examine the effects of different pulse durations on the likelihood of female response, each female ($n = 56$) was presented with 20 flash phrases, consisting of four replicates each of five different pulse durations: 50, 80, 100, 120, and 150 ms, keeping a constant 1.0-s IPI, with 10 s between flash phrases. Using a similar design to examine the effects of different IPIs on female response, we presented females ($n = 49$) with 20 flash phrases, consisting of four replicates each of five different IPIs: 0.8, 1.0, 1.2, 1.4, and 1.6 s, with a constant 100-ms duration and 10 s between flashes. Most females ($n = 45$) were given both trials on the same night, in random order (either duration or IPI first) with 40 min between trials. As flash responses of *P. greeni* females are all or nothing, females were scored as responding if they produced a response flash at the characteristic 0.8-s delay following the simulated male phrase. Females that failed to respond to at least one stimulus phrase were omitted from the analysis.

To analyze female preference functions (Wagner, 1998), we modeled female response as a correlated binary response variable (repeated measures on each female) with generalized estimating equations (GEE) using PROC GLM (SAS version 9.1) and an exchangeable correlation structure (Quinn and Keough, 2002). Because firefly courtship matches assumptions of mate choice models involving sequential search with time constraints (Fawcett and Johnstone, 2003; Johnstone, 1997; Real, 1990), we tested the prediction that females will show lower mate acceptance thresholds later in the mating season as mate availability declines. We also tested the prediction that females with lower residual reproductive value (fewer eggs) would have lower acceptance thresholds by examining how preference functions changed with female body mass (*Photinus* female mass is correlated with egg count; Cratsley and Lewis, 2005). Separate GEE models were used for duration and IPI trials to examine how each signal characteristic, presentation order, female body mass, and time during mating season (early versus late season) affected the probability of female flash response.

Female response to simulated courtship flashes: field experiments

To investigate whether laboratory measurement of female preference functions reflected female behavior under field conditions, we presented *P. greeni* females with simulated flash phrases differing only in IPI. These trials were conducted during the latter part of the mating season (days 22–38) on females in situ that were located immediately prior to testing. The output LED of the flash generator was positioned in view of and ~30 cm away from perched females ($n = 34$), and the presence or absence of a female response was noted for 20 simulated courtship flash phrases (four replicate flash phrases for each of five different IPI levels, with constant 100-ms pulse duration and 10 s between consecutive phrases). The levels presented were adjusted to ambient air temperatures recorded near each female and bracketed the mean male IPI observed at that temperature (Figure 1): $\overline{X}$ minus 0.6, 0.4, and 0.2 s, $\overline{X}$ and $\overline{X}$ plus 0.2 s. During testing, any males that came within 2 m of the female were removed, and after testing females were collected for body mass measurements as described above.

Response of these *P. greeni* females tested in situ was again modeled as a correlated binary response variable using GEE to examine the effect of IPI, presentation order, female body mass, and ambient temperature on the probability of female flash response. Only females that were tested between 60°F and 71°F were included in our analysis as the pulse duration of *P. greeni* male signals remains fairly constant across this temperature range (unpublished data). Again, any female that failed to respond to at least one stimulus phrase was omitted from the analysis.

RESULTS

Male flash signal variation

When flash patterns of *P. greeni* males were measured in the field, IPIs averaged 1.51 ± 0.18 s (mean ± 1 SD, temperature adjusted to 70°F; Figure 2). IPIs differed significantly among males (ANOVA: $F_{29,191} = 17.88$, $p < .0001$), with among-male variation representing 69.8% of the total variation. When we compared flash signals given by males during different courtship stages, there was no significant difference in the IPI of signals used by males during warm-up, patrolling, and dialoguing stages (Figure 3; Kruskal-Wallis $\chi^2 = 1.81$, 2 df, $p = .40$). For *P. greeni* courtship signals measured at 70°F, pulse durations

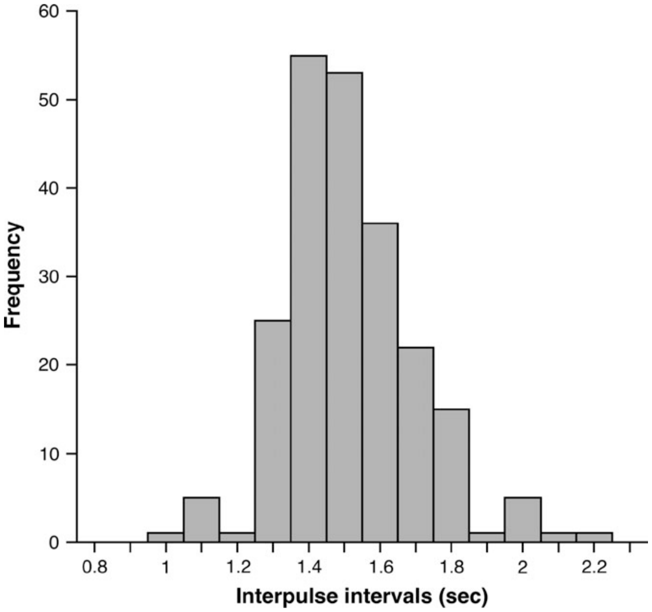

Figure 2
Frequency distribution of male IPIs (temperature adjusted as described in methods) from field-recorded *Photinus greeni* males ($n = 221$ two-pulsed flash phrases recorded from 30 males).

ranged from 67 to 233 ms ($n = 81$ pulses). For individual males ($n = 23$), there was no correlation between average pulse duration (temperature adjusted) and average IPI (minus duration, temperature adjusted; $r^2 = .319$, $p = .14$).

P. greeni male spermatophore mass ranged from 55 to 191 μg and was not significantly related either to male IPI (multiple regression, $n = 15$ males, $t = 0.19$, $p = .853$) or to male mass ($t = 1.10$, $p = .295$).

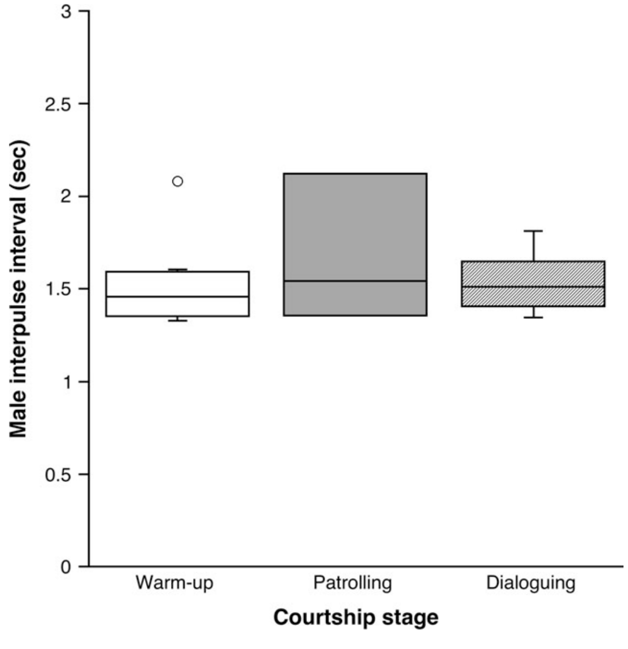

Figure 3
Box plots comparing mean IPIs from field-recorded *Photinus greeni* males during three courtship stages (warm up, $n = 12$ males; patrolling, $n = 4$ males; dialoguing, $n = 14$ males). In each box plot, the mid line shows the median, the box represents the middle 50% of data values, whiskers extend to 10th and 90th percentiles, and the circle indicates an outlying data point.

Female preference functions: laboratory experiments

Females showed highly significant differences in how responsive they were to simulated courtship signals with differing IPIs ranging from 0.8 to 1.6 s presented in a latin square design in the laboratory (Figure 4a, Table 1). Females showed maximum flash responses to signals with IPIs of 1.0 s, with female responsiveness declining below 10% for signals with 1.4- and 1.6-s IPIs. The likelihood of female response was not affected by presentation order, by whether females were collected early versus late in the mating season, or by female body weight (Table 1).

Females also showed highly significant differences in responsiveness to different pulse durations, although responsiveness remained between 32% and 70% for signals with pulse durations ranging from 50 to 150 ms (Figure 4b, Table 1). In these duration trials, early-season females were significantly more responsive than late-season females, and female responsiveness was also influenced by presentation order. Female body mass did not influence female response to pulse duration.

Female preference functions: field experiments

Although there was no effect of temperature on female responsiveness, there was a significant interaction between temperature and IPI (Figure 5, Table 2). Females tested at low ambient temperatures (60°F–65°F) were much more responsive to longer IPIs compared to females tested at high ambient temperatures (66°F–71°F). Neither female body weight nor presentation order (increasing or decreasing IPI) affected female response.

Differences in female response profiles to signal IPI between laboratory experiments (Figure 4) and field experiments (Figure 5) could reflect differences in female body mass or time during the mating season. Laboratory-tested females were collected earlier during the mating season and weighed significantly more (27.4 ± 1.0 mg) than field-tested females (20.8 ± 1.0 mg) (Kruskal-Wallis $\chi^2 = 16.96$, 1 df, $p < .0001$).

DISCUSSION

This represents the first comprehensive study of intraspecific variation in male bioluminescent courtship signals, female preference functions, and possible benefits of female choice for fireflies in the *P. consanguineus* complex. We found considerable among-male variation in both temporal characteristics of *P. greeni* flash patterns, IPI and pulse duration. Although males in the sibling species *P. macdermotti* emit different flash patterns while patrolling and courting (Carlson et al., 1976), we found that signal IPIs were consistent across three courtship stages for *P. greeni* males. Our results also demonstrate that female *P. greeni* fireflies discriminate among conspecific males based mainly on their IPIs. When we independently varied signal characteristics of male courtship flashes in laboratory photic playback experiments, the slope of the female preference function to different IPIs (Figure 4a) was steeper than the slope of the female preference function to different pulse durations (Figure 4b). However, although *P. greeni* females were less sensitive to pulse duration, this characteristic may still play a role in female signal assessment.

In *Photinus consimilis* fireflies, where male signals consist of multiple pulses, females were also less sensitive to male pulse duration compared to pulse rate (inverse of IPI, Branham and Greenfield, 1996; Greenfield, 2002). However, females responded to differences in pulse duration in *P. ignitus*, where

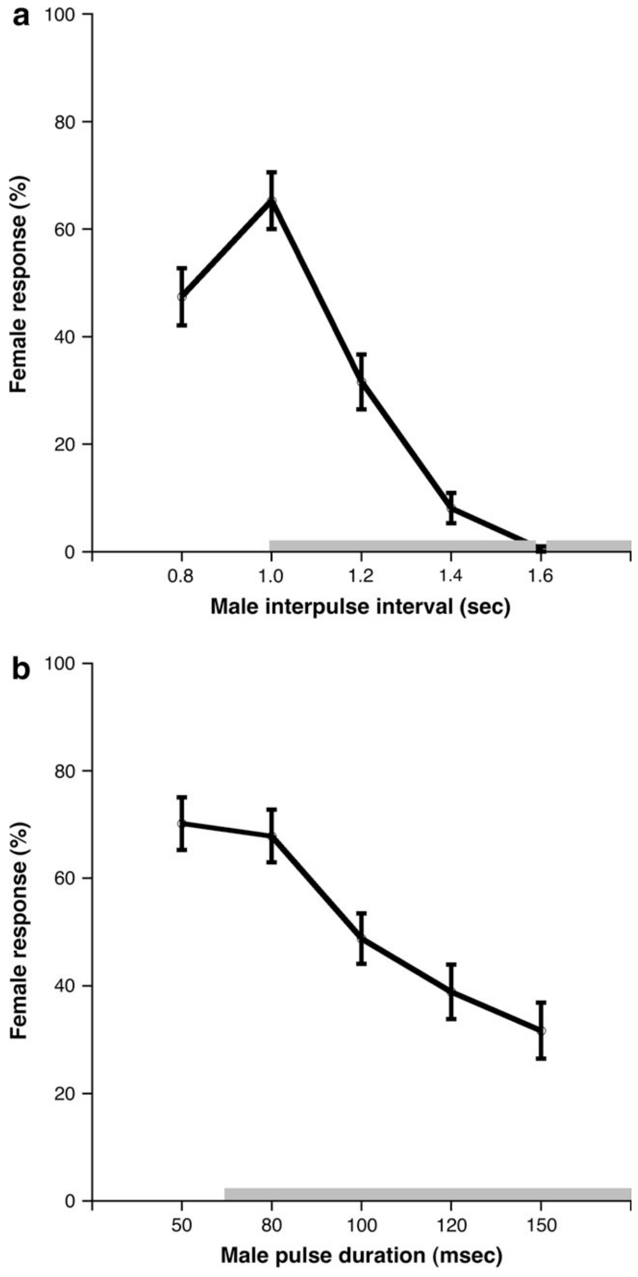

Figure 4
Preference functions of *Photinus greeni* females to simulated male signals presented in a latin square design in laboratory tests conducted at 70°F–72°F. Each female was presented with 20 flash phrases (four repetitions of each of five levels, with 10 s between consecutive flash phrases). Data shown as mean (±1 SE) percent of flashes to which females responded during (a) IPI trials ($n = 49$ females tested with five different IPIs and constant 100-ms pulse duration) and (b) pulse duration trials ($n = 56$ females tested with five different pulse durations and constant 1.0-s IPI). Shaded blocks indicate observed range of male IPIs and pulse durations for this *P. greeni* population.

Table 1

Statistical results from separate GEE analyses examining how the flash responsiveness of *Photinus greeni* females is influenced by courtship signal characteristics of IPI and pulse duration, female body weight, season (early versus late mating season), and presentation order

Source	df	Chi square	*p*
IPI trials			
IPI	4	95.53	<.0001
Female weight	1	2.06	.1517
Season	1	2.01	.1559
Presentation order	4	10.86	.1303
Pulse duration trials			
Pulse duration	4	37.46	<.0001
Female weight	1	0.11	.7448
Season	1	8.29	.0040
Presentation order	4	18.45	.0010

In both experiments, *P. greeni* females ($n = 49$ for IPI trials, $n = 56$ for pulse duration trials) were each tested with 20 flash phrases (four each of five signal parameter levels) in the laboratory at 70°F–72°F.

males emit a single-pulse courtship signal (Cratsley and Lewis, 2003). Only a few previous studies have examined female preferences within those *Photinus* species where males emit two-pulsed courtship signals. Our finding that *P. greeni* females discriminate among male signals mainly on the basis of IPI is consistent with results from earlier studies of *P. macdermotti* (Carlson et al., 1976, 1977) and *P. greeni* (Buck J and Buck E, 1972; Buck and Case, 1986; Lloyd, 1969), although these studies did not control for the effects of presentation order. Thus, although *Photinus* species differ in which temporal aspects of male courtship signals are important, each species' females appear to rely primarily on a single aspect of male flash signals, rather than multiple flash characteristics, to assess males as potential mates. Because this study focused only on flash signals, we cannot rule out the possibility that females might also assess male traits using other sensory modalities. For example, in addition to assessing flash IPIs, after contacting a male females might use chemical signals, such as cuticular hydrocarbons, to gain further information about mate quality.

Sexual signals can function in both species recognition and mate choice (reviewed in Andersson, 1994), and these might involve either the same or different signals. For example, in the acoustic signals of *Hyla versicolor* and *Hyla chrysoscelis* tree frogs, females use the pulse rate of male calls for species recognition, while call duration is used for mate assessment (Gerhardt, 2001). In contrast, *P. greeni* females appear to discriminate among conspecific males based mainly on their IPI, a signal character that Lloyd (1966, 1969) has shown to be also important in species recognition within the *consanguineus* complex. When *P. consanguineus* and *P. macdermotti* females were tested in the field, they failed to respond to flash signals with IPIs outside the range of their own species (Lloyd, 1966). Thus, within the *consanguineus* species complex, female preference functions for male IPI are likely to be shaped simultaneously by the dual processes of species recognition and mate quality assessment.

Many models of sexual selection are based on male mating signals that act as reliable indicators of male phenotypic or genetic quality (Andersson, 1994). During mating, *Photinus* males transfer a protein-rich spermatophore to females (van der Reijden et al., 1997), and these spermatophores have been shown to increase female fecundity (Rooney and Lewis, 2002). In *P. greeni*, we found no relationship between male IPI and spermatophore size, which suggests that in this species, male IPIs are uninformative with respect to male material resources. However, we cannot eliminate the possibility that other flash characteristics (pulse duration) may indicate male resources, as has been shown for the congener *P. ignitus* (Cratsley and Lewis, 2003). It is also possible that *P. greeni* courtship signals reflect other aspects of male phenotypic or

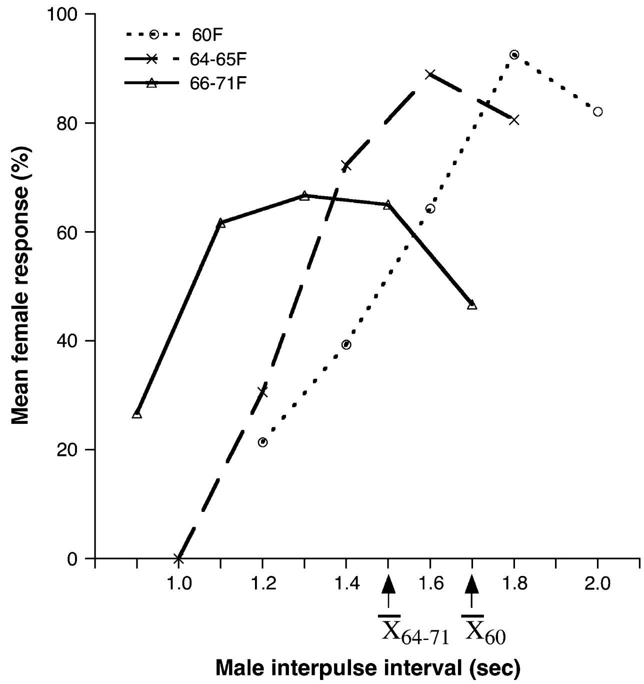

Figure 5
Preference functions of *Photinus greeni* females tested in the field at different ambient temperatures: 60°F (n = 7 females), 64°F–65°F (n = 9), and 66°F–71°F (n = 15). Each female was presented with 20 flash phrases (four flash phrases at each of five different IPIs with constant 100-ms pulse duration). Mean female response is shown for females within each temperature range (error bars omitted for clarity of presentation). Arrows indicate observed population means for male IPIs for the temperature ranges at which females were tested.

genetic quality or that females assess additional traits during or after mating to correct mate choice errors (as proposed by Lewis et al., 2004).

Female preference functions are predicted to vary as costs of mate choice change with female condition or mate availability (Fawcett and Johnstone, 2003; Jennions and Petrie, 1997; Real, 1990). Previous studies of other *Photinus* species have measured female response to conspecific males in the field and have shown that the overall level of female response increases as the availability of potential mates decreases later in the mating season (Cratsley and Lewis, 2005; Lewis and Wang, 1991). In this study, we found no seasonal change in *P. greeni* female preference functions for different IPIs, although females collected early in the mating season were more responsive when tested with different pulse durations. In laboratory experiments, *P. ignitus* females that had mated or had been fed an artificial diet showed reduced response levels to simulated male signals (Cratsley and Lewis, 2003). However, our results indicated that female preference functions were unaffected by differences in female body weight. Thus, while changes in mate availability and female mass may affect the overall level of female responsiveness, this study suggests that the shape of female preference functions for male IPIs remains relatively constant even as costs of mate choice change.

Table 2

Statistical results from GEE analysis for the effects of courtship signal IPI, female condition index, ambient temperature, and presentation order on the flash responsiveness of *Photinus greeni* females measured in the field at temperatures ranging from 60°F to 71°F (n = 34 females each tested with 20 flash phrases)

Factor	df	Chi square	p
IPI	4	17.00	.002
Female weight	1	1.03	.310
Ambient temperature	1	1.54	.215
Presentation order (increasing or decreasing IPI)	1	0.12	.730
Temperature × IPI	4	15.13	.004

The acoustic signals of many ectotherms show strong temperature dependence in their temporal components (Gerhardt, 1978; Greenfield, 2002; Pires and Hoy, 1992). In contrast, we found that IPIs of *P. greeni* males were roughly constant across a relatively wide range of ambient temperatures. Our results differ from the linear temperature dependence found for the temporal features of other *Photinus* signals noted by Carlson et al. (1976) and Lloyd (1966), perhaps reflecting geographical differences between study populations. However, our results do indicate that the preference functions of *P. greeni* females for male flash signals change with ambient temperatures as we found a significant temperature × IPI interaction for females tested in the field across temperatures ranging from 60°F to 71°F (Figure 5, Table 2). Females tested in the field at different ambient temperatures showed highest responses near the average IPI displayed by males at that temperature. As suggested by Carlson et al. (1976), these results indicate that *P. greeni* fireflies exhibit some temperature coupling between male signals and female preference, as has previously been shown for acoustically signaling insects (e.g., Pires and Hoy, 1992).

Different preference functions for IPIs were found when females were tested in the laboratory (Figure 4) versus in the field (Figure 5). Females tested in the laboratory (70°F–72°F) showed their highest response to shorter IPIs compared to females tested in the field at similar temperatures (66°F–71°F). Possible explanations for this discrepancy include that field-tested females were tested later in the season and thus were more likely to have mated multiple times. Field-tested females also had significantly lower body mass, reflecting lower egg loads. It is also possible that female preference functions may involve an interaction between IPI and pulse duration. For instance, given the demonstrated female preference for shorter pulse durations and the increase in male pulse durations at lower temperatures, it is possible that the 100-ms pulse duration used in our field testing of females may have been more attractive to females at colder temperatures. Overall, the observed plasticity in female mating preferences may contribute toward the maintenance of variation in male signals.

In conclusion, these studies have demonstrated variation in two temporal characteristics of courtship flashes among male *P. greeni* fireflies. *P. greeni* females appear to discriminate among conspecific males based primarily on male IPIs, the same signal character previously shown to be important for species recognition. There was no significant relationship between male IPI and spermatophore size, suggesting that *P. greeni* male courtship signals do not provide females with reliable indicators of male material resources. Female preference functions changed significantly across temperatures, with female responsiveness peaking near the average male IPI at different ambient temperatures. Further studies of the relationship between male courtship signals, direct and indirect benefits, and female preference functions in other firefly species will provide additional insight into the evolution of complex signaling systems.

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