

Flash Signal Evolution, Mate Choice, and Predation in Fireflies

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animal communication, bioluminescence, courtship, nuptial gifts, sexual selection

Abstract

Many key advances in our understanding of firefly biology and signaling have been made over the past two decades. Here we review this recent research, which includes new phylogenetic results that shed light on the evolution of courtship signal diversity within the family Lampyridae, new insights into firefly flash control, and the discovery of firefly nuptial gifts. We present a comprehensive overview of sexual selection in lampyrids, including evidence from *Photinus* fireflies that females choose their mates on the basis of male flash signals, and discuss the importance of examining both precopulatory and postcopulatory sexual selection in this group. Finally, we review recent findings on firefly chemical defenses, and discuss their implications for flash signal evolution in response to generalist predators as well as specialist predatory fireflies. This review provides new insight into how firefly flash signals have been shaped by the dual evolutionary processes of sexual selection (mate choice) and natural selection (predation), and proposes several exciting directions for future research.

*The fireflies, twinkling among leaves,
make the stars wonder.*

Rabindranath Tagore (1861–1941)

Nuptial gift:

material (other than gametes) provided by males to females during courtship or mating

Signal: any behavior or structure that alters the behavior of another organism, and that has evolved because of this effect

INTRODUCTION

Fireflies (Coleoptera: Lampyridae) are among the most charismatic of all insects, and their spectacular courtship displays have inspired poets and scientists alike. With more than 2000 species in 100 genera, worldwide lampyrid biodiversity is impressive and includes diurnally active as well as nocturnal species (81, 88, 102, 126).

Over the past 50 years, we have gained considerable insight into lampyrid signaling systems, their mating ecology, and their predator-prey interactions. Several earlier reviews have provided comprehensive coverage of many key aspects of firefly biology (13, 14, 18, 23, 30, 68, 73, 74, 81, 83, 84), yet most of these reviews are now more than 20 years old. Several substantive new contributions to our understanding of firefly biology have been made within the past decade. These advances include new insights into lampyrid phylogeny (10, 11, 71, 114, 115), the discovery of firefly nuptial gifts (59, 69, 108, 124), and new evidence that females choose their mates on the basis of male flash behavior (6, 8, 9, 31, 94). Other recent advances include better understanding of the biochemical mechanisms behind firefly bioluminescence and flash control (96, 119, 120, 127), and new findings about the relationship between firefly chemical defenses and predation (37, 43, 52). Finally, many contributions have been made to our understanding of the biodiversity and signaling behavior of lampyrids in Japan, China, Europe, and Brazil (38, 47, 48, 71, 101, 102, 126, 135).

In addition to presenting these new results, our goal in this review is to provide insight into how firefly bioluminescent flash signals may have evolved in response to the dual evolutionary processes of sexual selection (mate choice) and natural selection (predation). We

briefly review courtship signal diversity within the Lampyridae and subsequently limit our scope to bioluminescent signaling systems. We review mechanisms of flash production and energetics and describe lampyrid nuptial gifts and their implications for both sexes. We provide an overview of sexual selection in lampyrids and describe evidence from *Photinus* fireflies that females choose their mates on the basis of male flash signal variation. We explore whether flash signals might act as indicators of male quality, allowing females to choose males with larger nuptial gifts. Finally, we review studies on the chemical defenses used by fireflies to avoid generalist predators, and discuss potential coevolutionary interactions between predatory and prey fireflies. A synthesis of these results provides evidence supporting the idea that firefly flash signals have been shaped by the dual evolutionary processes of sexual selection (through mate choice) and natural selection (through predation). In addition to highlighting these advances in firefly biology, we propose several exciting directions for future research on these fascinating beetles.

DIVERSITY OF COURTSHIP SIGNALS

Pheromones

In most diurnally active lampyrids, adults are incapable of bioluminescence, and these species are generally assumed to rely on pheromones (volatile chemical signals) for their long-range mate attraction. Direct evidence supporting pheromonal signals exists for several diurnal species, including *Lucidota atra*, *Pyropropyga nigricans*, and *Photinus indictus* in North America (75), *Phosphaenus hemipterus* in Europe (38), and *Lucidina biplagiata* in Japan (102). In addition, females of several lampyrid taxa attract males using a combination of pheromonal and bioluminescent signals; these species include *Pyrocoelia rufa* (101), *Pleotomus pallens*, and *Phausis* spp. (11).

Bioluminescent Signals

Among those lampyrids that rely mainly on bioluminescent courtship signals, perhaps the simplest signaling system involves flying males attracted to a continuous or slowly modulated glow emitted by flightless females [these generally have enlarged abdomens, but may be larviform, brachypterous, or apterous (11)]. In North American *Microphotus* as well as in the European glow-worm *Lampyris noctiluca*, males fly and are attracted to glows produced by sedentary females. In *Phausis reticulata* and *Pleotomodes knulli*, glowing males are attracted to sedentary glowing females (74, 82).

Many nocturnally active fireflies use discrete pulses of bioluminescence to locate their mates. In many groups, including North American *Photinus* and *Pyroctomena* (74, 82) and Japanese *Hotaria parvula* (101), both sexes produce flash signals that are precisely timed to encode information concerning species identity and sex. In these taxa, the primary signalers are males, which emit advertisement flashes while in flight. Once a female responds by flashing, a reciprocal courtship dialogue ensues in which males and females exchange flash signals. Females in these species flash mainly in response to male signals and often remain stationary, although they are capable of flying. Courtship dialogues continue until

males contact females and copulation occurs. Thus, flash signaling behavior in these taxa involves males and females exchanging flash signals that contain critical timing elements. Signaling behavior in many *Luciola* species also consists of short bioluminescent flashes but is considerably more complex and highly variable (76, 78, 79, 101, 102, 104). For example, in *L. cruciata* (life stages and sexual dimorphism in lantern size illustrated in **Figure 1**), males flash synchronously while in flight (99, 102). Females flash spontaneously, as well as in response to male signals. Flying males are attracted to flashing females, land nearby, and display diverse flash behaviors before contact and copulation occur. In *Photuris* and other *Luciola* fireflies both sexes also flash spontaneously, and individuals exhibit a wide range of flash behaviors (82, 101); in these taxa, flash timing may be a less critical component of the flash signal.

Perhaps the most spectacular of all animal courtship displays is the mass flash synchrony exhibited by several *Pteroptyx* species in Southeast Asia. Males of *P. malacca* congregate in leks and in unison emit rhythmic courtship flashes that attract females (14, 16, 82, 102). The related yet more ephemeral phenomena of wave and intermittent synchrony among flying males have been documented in several North and South American

Courtship dialogue:

courtship pattern shown by some fireflies in which males and females engage in reciprocal exchange of courtship signals

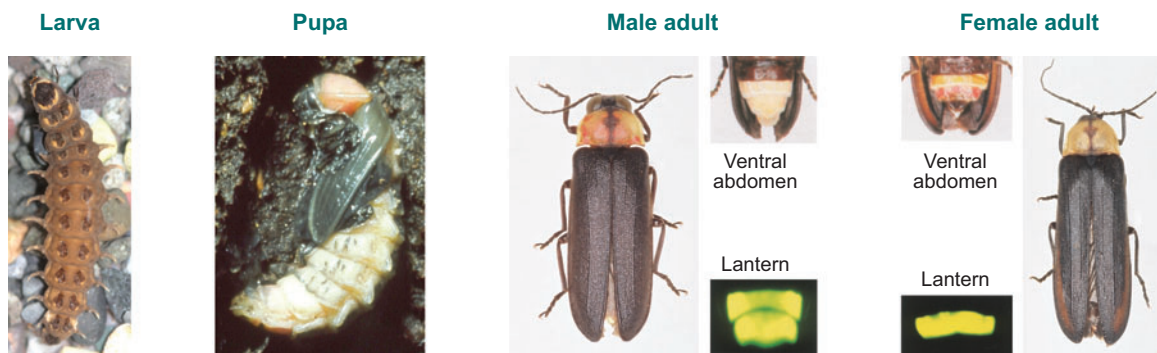


Figure 1

Lampyrid life stages illustrated for the Japanese Genji firefly, *Luciola cruciata*, showing aquatic larva, pupa, and dorsal and ventral views of adult males and females (used with permission from Reference 102; photos by N. Ohba).

fireflies (14, 23, 26–28, 84, 126), as well as in Asiatic *Luciola* (99, 102). Considerable progress has been made in understanding the physiological mechanisms that regulate signal synchrony (55), and detailed observations of flash and mating behavior exist for several synchronous species (22, 78, 79, 102). However, although several plausible hypotheses for the evolution of synchronous flash behavior in fireflies have been proposed (14, 16, 23, 55, 84), testing these hypotheses requires a much more detailed understanding of the factors affecting individual reproductive success in these communal displays than is currently available.

Evolution of Firefly Signaling Systems

Recent phylogenetic studies have elucidated patterns of signal evolution within the Lampyridae (11, 71, 114, 115). Bioluminescence apparently arose in an early cantharoid ancestor (10), in which it may have served as an aposematic (warning) display for unpalatable larvae. Thus, the use of bioluminescence in the courtship signals of adult fireflies likely represents an exaptation from larval warning signals directed toward potential predators (11).

Phylogenetic analysis based on morphological traits of lampyrid taxa worldwide (11) indicates that ancestral lampyrids were most likely diurnally active and that females used pheromonal signals to attract mates. This signaling system occurs in basal taxa such as *Brachylampis* and *Psilocladius*. This analysis also noted that bioluminescent courtship signals subsequently evolved independently at least four times within the Lampyridae. Additionally, the Asiatic genus *Luciola*, whose flash signaling behaviors are mostly asynchronous but include some instances of flash synchrony, is basal to *Pteroptyx*, several species of which show well-developed flash synchrony. A recent molecular phylogeny of North American lampyrids (114) (**Figure 2**) based on a nuclear gene (18S ribosomal) and two mitochondrial

genes (16S ribosomal and cytochrome oxidase I) confirmed that basal taxa rely on pheromonal sexual signals and showed at least two independent origins of courtship signals involving discrete bioluminescent flashes in this group. In addition, an expanded molecular phylogeny including worldwide lampyrids revealed that once bioluminescent courtship flashes had evolved, they were subsequently replaced at least once by bioluminescent glows and reverted at least three times to pheromonal signals (114). Molecular phylogenies have also been constructed using mitochondrial 16S ribosomal sequences for Japanese fireflies (115), as well as for lampyrids from China, Korea, and Japan (71). These analyses show similar trends, with basal taxa using pheromones, many derived taxa employing bioluminescent glows and flashes, and several clades showing secondary reversions to pheromonal signals.

Understanding the ecological context that selects for signaling systems based on pheromones or bioluminescence would contribute greatly to deciphering the evolutionary trajectory of firefly sexual displays. Various advantages and disadvantages of pheromonal versus visual signaling in animals have been extensively discussed (1, 110). Pheromones provide a persistent, long-range, and energetically efficient signal. Owing to the specificity of chemical receptors, pheromonal signals are generally considered to have low susceptibility to eavesdropping generalist predators (1, but see 137). However, because pheromonal signals can be difficult to locate, the receivers of such signals may be subjected to high predation risks while searching. Visual signals, including bioluminescent glows and discrete flashes, are easier for receivers to localize, but they have a shorter range and their propagation requires open habitat without obstacles. Habitats with many obstacles (e.g., forests) might thus select for signaling systems that use a combination of pheromones for long-range attraction and bioluminescence for better short-range localization. While visually orienting predators may select against

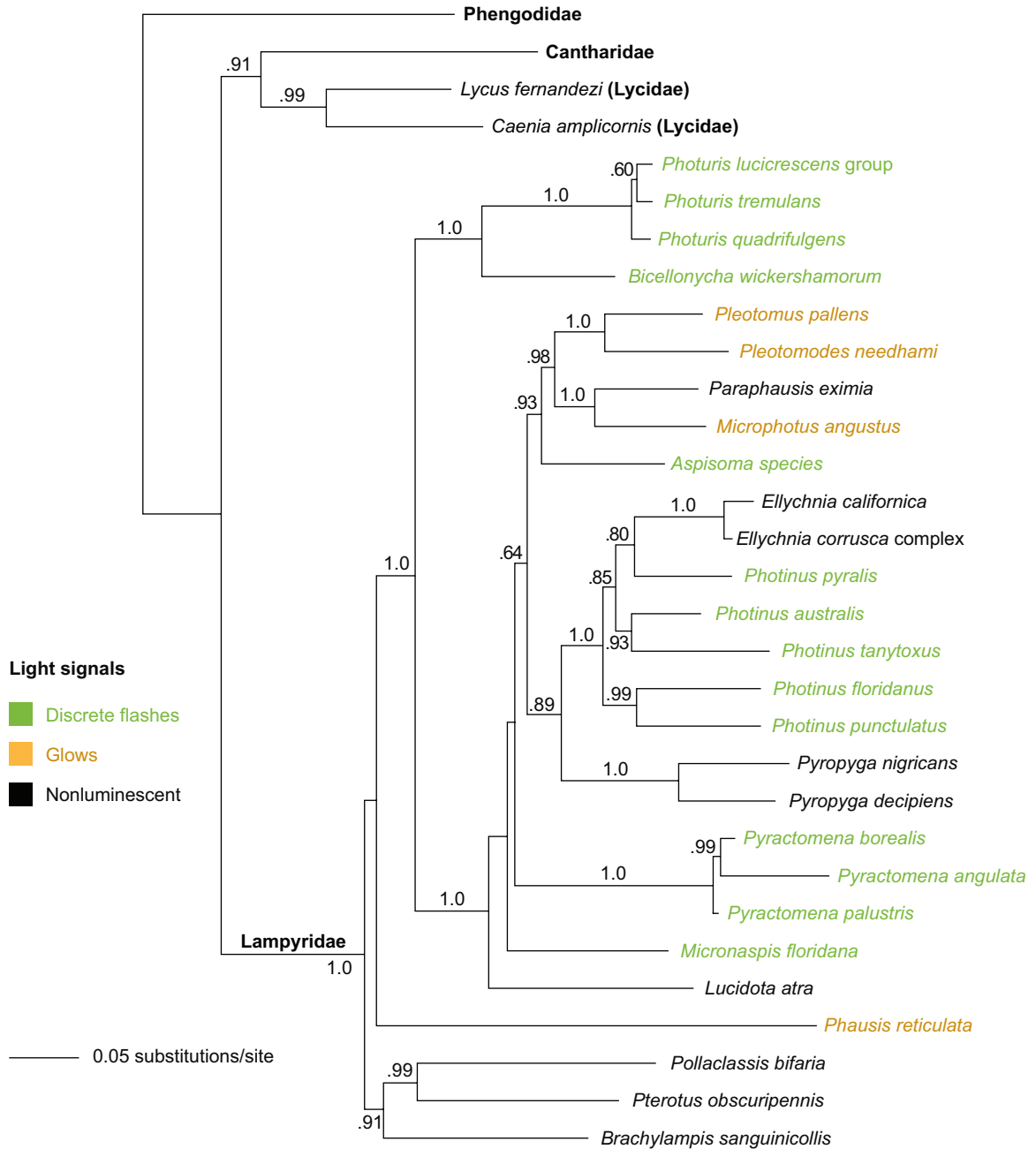


Figure 2

Phylogenetic relationships of North American lamproyrids indicating use of bioluminescence as a sexual signal. Maximum likelihood tree with Bayesian posterior probabilities is based on one nuclear gene (18S ribosomal subunit) and two mitochondrial genes (16S ribosomal and cytochrome oxidase I). Modified, with permission, from Reference 114.

Nitric oxide (NO):
a ubiquitous, small
signaling molecule

conspicuous bioluminescent signals, such selection could be mitigated by prey chemical defenses. In addition, predation could select for discrete flash signals over continuous bioluminescence if flashing fireflies, particularly moving individuals, are more difficult to locate (84). Discrete flashes may also be advantageous because they allow information to be encoded in temporal features of the signal.

Although bioluminescent signals are essential elements of courtship in many derived lampyrid taxa, it is also plausible that multiple signal modalities could be more widespread in firefly courtship than is currently recognized. For example, species differences in cuticular hydrocarbon profiles exist among several Japanese and North American fireflies (111), and studies on other taxa have implicated these contact cues in species and mate recognition (60, 95).

MECHANISMS OF FLASH PRODUCTION

Biochemistry of Firefly Bioluminescence

The detailed biochemical basis of bioluminescence has been elucidated for many organisms (128). Lampyrids capable of bioluminescence produce their light via a two-step reaction catalyzed by the enzyme luciferase. First, luciferase catalyzes the Mg-ATP-dependent adenylation of the substrate luciferin. This step is followed by a sequence of oxygen-dependent reactions that result in light emission. Colors of firefly bioluminescence range widely (66) from yellow-green (~546 nm) to orange (~590 nm) even though the luciferin substrate is identical in all known species. Firefly luciferases show 66%–99% DNA sequence similarity across species, and single amino acid substitutions can alter the bioluminescence emission spectrum (7, 127). Such color differences are caused by changes in the luciferase active site that affect bound substrate conformation (96).

Flash Control Mechanisms

In lampyrids, bioluminescent reactions take place in light-producing organs called lanterns. Although most bioluminescent organisms (including firefly larvae and pupae) produce only slowly modulated glows, adults in many fireflies species control their bioluminescence to emit light in discrete flashes or rapidly modulated flickers. Such precise temporal control of bioluminescence appears to rely on several anatomical and physiological specializations of the adult firefly lantern (13, 25, 49, 67, 98, 105, 113). Lantern light production is initiated by signals originating in the central nervous system, yet in these adult fireflies neural synapses are not located directly on the photocytes (the light-producing cells), but rather they innervate nearby cells that surround branches of the air distribution system. Also, the photocytes of flashing fireflies show an unusual degree of compartmentalization; mitochondria are densely packed into the peripheral cytoplasm, and peroxisomes housing luciferase and luciferin are sequestered in the cell interior.

Until recently, little was known about how signals were carried from the nerve synapses to the light-producing photocytes. Numerous theories of flash control have focused on various mechanisms that might regulate oxygen available to the activated luciferin intermediates stored inside the photocytes' peroxisomes (13, 49, 50, 119). Recent studies demonstrate that nitric oxide (NO) plays a role in the precise temporal control of firefly flashing (2, 120). Studies of whole insects, isolated lanterns, and firefly mitochondria provided evidence that NO synthase is localized near synaptic terminals within the firefly lantern, that exogenous NO induces bioluminescence while the addition of NO scavengers blocks light production, and that NO reversibly inhibits respiration by photocyte mitochondria. These results suggest that NO production within the firefly lantern is a key physiological adaptation that allows firefly flashes to be rapidly switched on and off. The proposed

mechanism involves neural stimulation that triggers the transient release of NO. As NO diffuses into the periphery of the adjacent photocytes, it inhibits mitochondrial respiration and allows oxygen to reach the photocyte interior, where the bioluminescent reactants are housed. This model suggests that NO production should be lacking in larval fireflies as well as those adult fireflies that only glow, but this prediction has not yet been tested. Additionally, although the NO model is consistent with existing data, several other mechanisms have been proposed (13, 50, 119) and may also contribute at various timescales to regulating firefly bioluminescence.

Energetics of Flash Production

The metabolic expenditure required for *Photinus* fireflies to produce a bioluminescent signal has recently been measured using differential open-flow respirometry to compare carbon dioxide production by individual fireflies during flashing, walking, and resting (134). Firefly flashing was surprisingly inexpensive, increasing metabolic rates only 37% above resting values, compared to an increase of 57% during firefly walking. This study additionally suggested minimal costs for maintaining bioluminescence capability, on the basis of similar resting metabolic rates found in bioluminescent and nonbioluminescent lampyrids (134). As other reproductive activities (courtship flight for males and oogenesis for females) are likely to entail considerably higher metabolic expenditures, the energy required for flash signaling appears to represent a relatively minor component of the total energy expended during firefly courtship.

MALE NUPTIAL GIFTS

Male Spermatophore Production

During courtship and mating, males of diverse species provide females with nuptial gifts, defined here as any nongametic mate-

rial that females could subsequently use for somatic maintenance or reproduction. These nuptial gifts, which can include captured prey, spermatophores, or various male body parts, are intimately tied to both precopulatory and postcopulatory sexual selection (5, 123). Although a review of existing evidence suggests that such nuptial gifts often provide net benefits to females by increasing lifetime fecundity (58), this terminology does not preclude some fitness costs associated with nuptial gifts. Male spermatophores, which consist of sperm packaged within a structure manufactured by male accessory glands, may play complex roles in reproductive processes, as in some insects the secretions of male accessory glands reduce female fitness (112, 133).

Firefly spermatophores were first described by van der Reijden et al. (124), and in both *Photinus* and *Ellychnia* spermatophores are produced by four pairs of male accessory glands. During copulation in these firefly species (**Figure 3a**), males transfer a single, spirally coiled, gelatinous spermatophore (**Figure 3b**) to the female, and sperm are released into the female's spermatheca for storage. The remainder of the spermatophore enters a specialized structure within the female

Spermatophore: a sperm-containing package manufactured by male reproductive glands and transferred to the female during mating

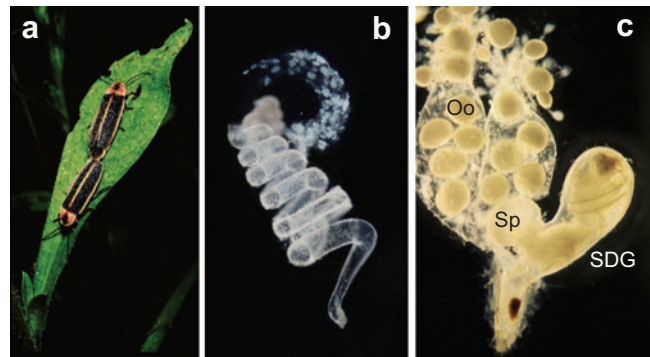


Figure 3

Nuptial gifts in *Photinus* fireflies. (a) During copulation a *Photinus* male (lower) transfers a spermatophore to the female (upper). (b) Coiled spermatophore with sperm rings being released. (c) Female reproductive tract showing coiled male spermatophore located in the spermatophore-digesting gland (SDG). Male sperm are stored in the female's spermatheca (Sp). Spermatophores break down 1–2 days after mating, and spermatophore-derived proteins are allocated to the female's developing oocytes (Oo).

reproductive tract called the spermatophore-digesting gland (**Figure 3c**), where the spermatophore disintegrates over the next few days. Male nuptial gifts may also be important in the mating systems of many other fireflies (59, 69). Spermatophore remnants have been found in females of *Pteroptyx valida* (132), *Photuris* sp. (69), and *Lucidina natsumiae* (59). In an anatomical study of 20 Japanese fireflies, male accessory glands anatomically similar to those responsible for *Photinus* spermatophore production were found in 12 species from 7 genera, including *Hotaria*, *Luciola*, *Pristolycus*, *Curtos*, *Lucidina*, *Cyphonocerus*, and *Drilaster* (59).

Many lampyrids do not feed as adults (87, 131), and thus these beetles must rely entirely on larval resources to fuel adult reproduction. In such capital breeders, nuptial gifts are likely to be of particular economic importance for both sexes (69). Evidence from *Photinus* fireflies indicates that spermatophore production is costly to males (33). In *Photinus ignitus*, male spermatophore size declined over successive matings. Male mating rates also declined over time, even when males were given access to receptive females every night. Thus, male spermatophore production ability is likely to represent a major constraint on male mating success in nonfeeding fireflies.

Fate of Male-Derived Nutrients

In species with nonfeeding adults, male nuptial gifts could provide an important supplement to a female's own resources that can be used for somatic maintenance and reproduction. Rooney & Lewis (106) examined the fate of spermatophore-derived proteins in two lampyrid species with contrasting life histories: *Photinus ignitus* (nocturnal, bioluminescent adults) and *Ellychnia corrusca* (diurnal, nonbioluminescent adults). *P. ignitus* adults have a maximum life span of about two weeks, equivalent to the length of their mating season, and do not feed. In contrast, *E. corrusca* adults live for about 10 months, including a

two-month mating period, and feed on flowers and plant sap (107). Allocation patterns for spermatophore nutrients were examined using ^3H -labeled amino acids, which are incorporated into male spermatophores. Markedly different allocation patterns were revealed for the two species: *Photinus* females allocated spermatophore-derived proteins primarily to their maturing oocytes, whereas the major allocation in *Ellychnia* females was to somatic tissue (mainly fat body). These results suggest that in lampyrids with short-lived, nonfeeding adults, females have evolved to rapidly divert spermatophore-derived nutrients to maximize their current reproductive output. In longer-lived species such as *Ellychnia*, females may use male-derived nutrients for their own somatic maintenance as well as for reproduction.

Direct effects of spermatophores on female reproductive output have been examined for two firefly species (108). Lifetime fecundity was compared between females that were experimentally assigned to mate either with a single male or sequentially with three different males; mean fecundity increased by 73% and 41% in multiply mated *P. ignitus* and *E. corrusca* females, respectively. However, no differences in female fecundity were detected when nonvirgin females were mated to males with assumed differences in spermatophore size (achieved by manipulating male mating history). In these two lampyrid species, male spermatophores appear to benefit both sexes by increasing fecundity. However, the possibility also exists that male nuptial gifts alternatively or additionally serve to manipulate female reproduction in ways that are costly to females. Although it has not been demonstrated in any lampyrid, male accessory gland products might also serve to increase males' paternity success relative to that of other mating males.

Nuptial Gifts and Mating Systems

Females are expected to be polyandrous when nuptial gifts provide material benefits, as they

should continue to forage for nuptial gifts even after they have obtained adequate sperm. Unfortunately, the mating systems of very few lampyrids have been examined in detail. However, the predicted association between nuptial gift presence and polyandry holds for those North American *Photinus* fireflies studied to date (69). In several *Photinus* species, males produce nuptial gifts and both sexes mate multiple times. However, *P. collustrans* lacks nuptial gifts, and in this species females are flightless and monandrous, typically mating with only a single male before they oviposit and die (129, 130). When females mate with multiple males, sexual selection may increase male reproductive allocation. This prediction was confirmed in an interspecific comparison of *Photinus* fireflies (40) that showed that males in the monandrous species *P. collustrans* had 5-fold-lower allocation to sperm production and 100-fold-lower allocation to reproductive accessory glands compared with males from three polyandrous species. Male reproductive glands were also examined in 20 species of Japanese fireflies (59), and these results suggest that the absence of male nuptial gifts might be associated with greater sexual size dimorphism (larger female body size) and female flightlessness.

Sexual size dimorphism (and associated changes in female mobility) is likely a major factor influencing the potential for male nutrient contributions to increase female fecundity (Figure 4). Female reproductive investment may be maximized in species with large, larviform, flightless females, which can devote all their resources toward converting larval reserves into egg production. In such species, there should be only weak selection on males to contribute nuptial gifts because of the limited scope for increasing female fecundity. However, highly mobile females have less energy and resources available for oogenesis, and when females fly there are additional biophysical constraints on female fecundity. Hence, in species with flying or otherwise highly mobile females, there should be stronger selection on males to produce nuptial gifts because of their potential to increase female fecundity (Figure 4). This model suggests that male nuptial gift evolution may be influenced indirectly by selection on female mobility and flight. Factors that might influence selection on female mobility include (a) spatial separation between mating and oviposition sites, (b) relative predation pressure, or (c) access to efficient signaling locations. Of course, nuptial gifts may also be subject to

Polyandry: animal mating system in which females mate with multiple males

Monandrous: describes an animal mating system in which females mate with a single male

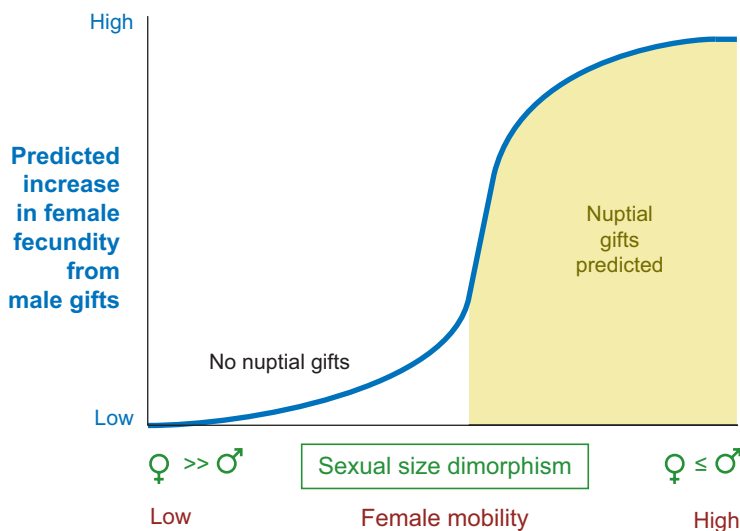


Figure 4
Graphical model predicting the evolution of nuptial gifts in lampyrid beetles based on the relationship between sexual size dimorphism and the potential for male gifts to increase female fecundity.

direct selection if they provide benefits to males in terms of increased paternity success.

FLASH SIGNALING AND SPECIES RECOGNITION

Photinus Flash Codes

Within specific geographic regions, the evolution of distinct flash signal patterns may be driven by their role in species recognition. Among all firefly signaling systems, those of North American *Photinus* fireflies have been deciphered in the greatest detail. This is partly due to the relative simplicity of their courtship signal dialogues, as well as to the pioneering work of Buck (12, 15), McDermott (92, 93), Lloyd (73, 74, 82, 84), and others (17, 20, 21, 23, 24) in decoding the information content of these signals. In *Photinus*, sexual advertisement flashes are produced by roving males while sedentary females generally flash only in response to male signals. Male flash signals in many North American *Photinus* species consist of a single pulse ranging from 100 to 750 ms in duration (pulse duration) that is repeated at specific intervals (73) (Figure 5*a,b*). In other *Photinus* species, males emit multiple-pulsed flash patterns consisting of two to several short pulses delivered at various rates (pulse rate = interpulse interval⁻¹), with such flash patterns again repeated at intervals. When they respond to male signals, *Photinus* females generally emit a single-pulsed response flash, although in a few species female responses contain multiple pulses (Figure 5*a*). These female signals are given at a fixed response delay, timed from the beginning of the last pulse in the male signal (Figure 5*b*).

Sympatric *Photinus* species often differ in their breeding habitats, mating seasons, or time window occupied by their nightly flight periods (73). In addition to these spatial and temporal isolating mechanisms, *Photinus* females rely on male pulse duration, pulse rate, and pulse number to identify males of their own species, and males identify conspecific fe-

males on the basis of the time delay before the female's flash response (6, 15, 73). Flash color, flash kinetics, and spatial gesture are relatively unimportant for species recognition (12, 73). Although most *Photinus* females are capable of flight [a few species have females that cannot fly (73, 131)], during courtship they respond to male signals from perches in vegetation. When a male detects a female reply, he moves toward her and signals again. The male generally lands near the female after a few flash exchanges, and this courtship dialogue continues as he walks toward the female. These courtship dialogues in *Photinus* appear to involve only visual cues, as females in airtight containers engage in normal dialogues (73). However, there is anecdotal evidence that short-range species discrimination in *Photinus* might involve contact chemical or tactile signals (73). Although *Photinus* flash signals are important for species recognition, little is known about the potential for evolutionary divergence as a function of flash signaling behavior.

Geographic Differences in *Luciola cruciata* Flash Patterns

Population differences in flash signaling patterns have been examined in the Japanese Genji firefly, *Luciola cruciata*. In this species, both sexes show complex flash behavior during courtship (100, 102). Geographic differences in both male and female flash patterns have been documented (99, 101, 118): Searching males in populations from eastern Japan show interflash intervals of 4 s (termed slow flash), and those in western Japan show interflash intervals of 2 s (termed fast flash). Although flash timing characters are temperature dependent, these population differences are apparently independent of temperature (118) and reflect distinct populations based on RFLP analysis of the mitochondrial cytochrome oxidase II gene (116). Males preferentially approached simulated female flashes that were typical of their own population (118), which suggests that assortative mating

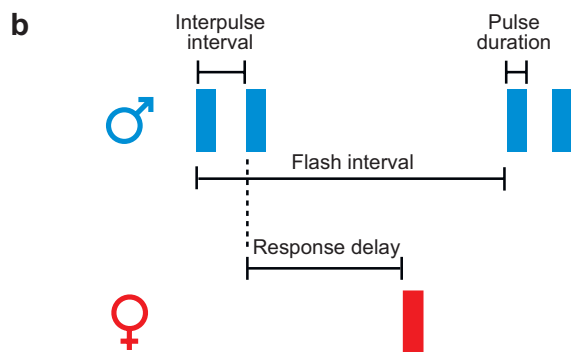
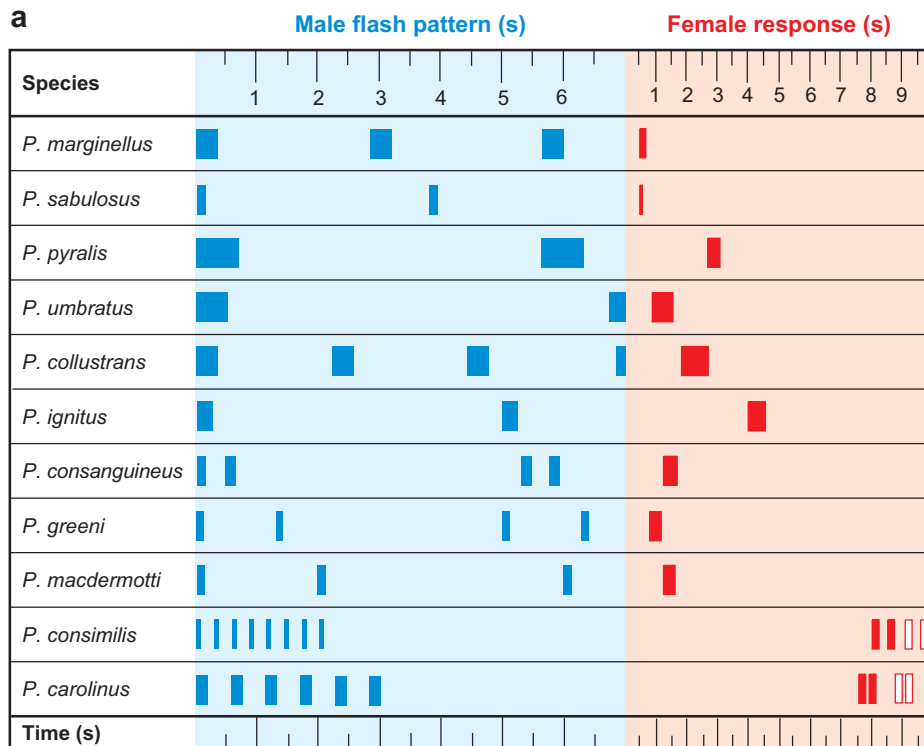


Figure 5

Species differences in the courtship flash signals of *Photinus* fireflies. (a) Examples of male flash signals (blue) and female response flashes (red; note different timescales) for several North American species. Female flash responses are timed from initiation of the last pulse in the male flash pattern (open bars indicate optional responses). Because signal timing is temperature dependent, timing shown is approximate (temperatures ranging from 19°C to 24°C). Modified from Reference 73, with additional data from References 9 and 27. (b) Terminology used to describe *Photinus* flash signals (based on Reference 73).

based on courtship flash timing may eventually lead to speciation. Similar divergence of courtship signals associated with limited gene flow in subdivided populations may have led to speciation in other lampyrid taxa, including North American *Photinus*. However, further research is needed to explore the relative roles of factors such as genetic drift, predation pressure, and sexual selection on the evolution of species-specific flash signaling behavior.

SEXUAL SELECTION IN BIOLUMINESCENT FIREFLIES

When Charles Darwin (34) elaborated his ideas on sexual selection, he envisioned differences in mating success that arise either through competition for access to mates or through mate choice. Recently, behavioral ecologists have recognized that in those species where females mate with multiple

males (this includes the vast majority of insects), sexual selection can also arise through postcopulatory processes (42, 112). Postcopulatory sexual selection includes competition among male gametes for access to fertilizations, called sperm competition, as well as preferential sperm use by females, known as cryptic female choice. Any of these precopulatory and postcopulatory processes can interact to drive evolutionary changes in reproductive behavior, morphology, and physiology. Thus, an integrated view of sexual selection needs to consider differences in reproductive success that arise through both precopulatory and postcopulatory choice and competition.

Within many lampyrid genera, species are often differentiated by anatomical differences in male genitalia (54) or by behavioral differences in courtship signals (73), suggesting the importance of sexual selection in driving evolutionary diversification within this group. Much of the work described below was inspired by ideas Lloyd (82) presented in his 1979 review of sexual selection in luminescent beetles. In this section we review recent developments in this area, again with a focus on *Photinus* fireflies of North America, because most work on firefly sexual selection to date has been done in this group.

Female Mate Choice

Precopulatory female choice in dialoguing fireflies is most likely to occur through differential female response based on male flash signals. Male *Photinus* fireflies require female responses in order to locate and mate with a female; therefore females can choose their mate by preferentially responding to the flash signals of a particular male. Field studies of several *Photinus* species demonstrate that in competitive courtships involving several dialoguing males, those males that elicit more female flash responses are more likely to locate and mate successfully with females (32, 41, 70, 125). Female response behavior also provides a mechanism for choice, as *Photinus* females aim their lanterns toward specific males when

emitting their response flashes (15, 24, 73, 125). While females could reject males after contact by moving away, in *Photinus* fireflies such postcontact discrimination appears uncommon (32; S. Lewis, personal observation).

Demonstrating female preferences based on male signals requires (a) quantifying intraspecific variation in male signals, and (b) showing that female response changes across this signal range (while controlling for any other potential differences among males). Early work on *Photinus* fireflies established reliable methods for measuring male flash traits, generating artificial flash signals, and testing female responses (12, 15, 20, 21, 73). Artificial male flash signals can be generated that vary in a single characteristic, such as pulse duration, pulse rate, or pulse number. In addition, female response to such artificial signals can be easily monitored because *Photinus* females respond with highly visible bioluminescent signals of their own. In most *Photinus* species, females respond to a male flash pattern with a single flash pulse, but in other species the female response may include two or more flash pulses (Figure 5). Thus, female preference can be measured as the proportion of male flashes to which a female responds, and in some cases the number of pulses within a female response (6, 8, 9). Recent work has also incorporated random presentation sequences of signals to statistically remove possible presentation order effects (6, 9, 31, 94).

Intraspecific variation in male flash signals has been documented in many *Photinus* species. Male pulse duration varies in several species, including two single-pulse species [*P. pyralis* (29) and *P. ignitus* (31)], a double-pulse species [*P. greeni* (94)], and three multiple-pulse species [*P. consimilis* (9), *P. carolinus*, and *P. ardens* (6)]. Male pulse rate also varies in *P. greeni* (15, 94) and *P. macdermotti* (20), as well as in *P. consimilis* (9), *P. carolinus*, and *P. ardens* (6). In three multiple-pulse species, variation has also been documented in the number of pulses per flash pattern (6, 8). Pulse duration and pulse rate are repeatable signal characteristics for an individual male on a given night

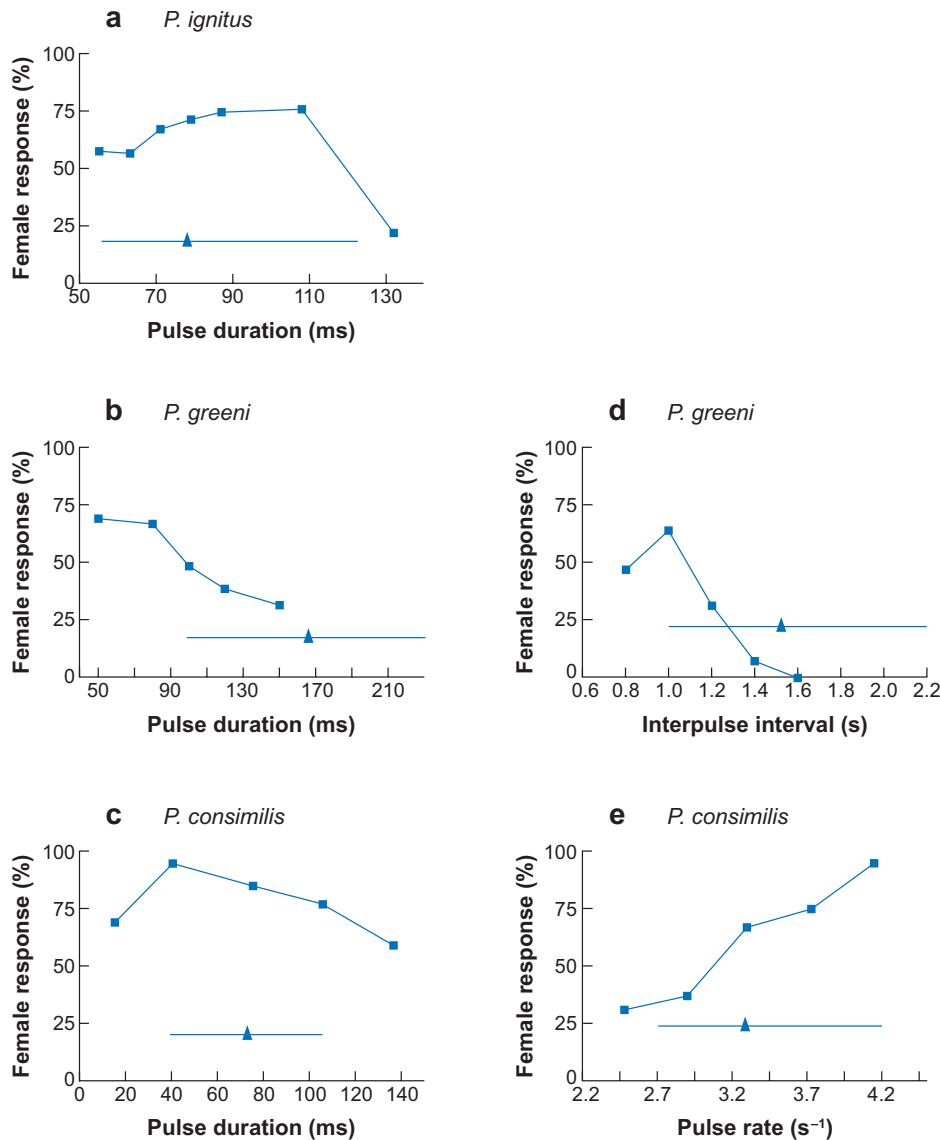


Figure 6

Photinus spp. female preferences (measured as % response) for male flash characters tested using artificial flash signals. (a) *P. ignitus* pulse duration. (b) *P. greeni* pulse duration. (c) *P. consimilis* pulse duration. (d) *P. greeni* interpulse interval. (e) *P. consimilis* pulse rate. The ranges (horizontal lines) and means (triangles) are indicated for male flash characters measured in corresponding field populations. Modified from References 9, 68, and 94.

of recording (29, 31, 94), but no studies to date have examined how a male's flash signals might change over longer time periods.

Photinus females preferentially respond to different signals when flash characteristics are varied within the observed range of conspecific males (Figure 6). In the single-pulse species, *P. ignitus* (Figure 6a) and *P. pyralis*, females show increased response to longer-duration pulses across the range observed in

conspecific males (29, 31). In contrast, in the multiple-pulse species, *P. greeni* (Figure 6b), *P. consimilis* (Figure 6c), *P. ardens*, and *P. carolinus*, females show higher flash response to pulses that are shorter than the mean male pulse duration (6, 9, 94).

However, in most of the multiple-pulse species studied to date, female responsiveness is influenced more strongly by variation in pulse rate than by variation in pulse

Operational sex ratio: ratio of breeding females to reproductively active males in a population

duration (9, 20, 21, 94). Females in many *Photinus* species prefer faster pulse rates (generated by decreasing the interpulse interval while holding pulse duration constant) than the mean observed among conspecific males (6, 9, 94) (**Figure 6d** shows interpulse interval, which is the inverse of pulse rate, **Figure 6e**). This preference appears most pronounced in species with more pulses per flash pattern. *P. consimilis* and *P. carolinus* male flash patterns each consist of four to nine pulses (73), and these females prefer faster pulse rates (6, 9) (**Figure 6e**). In contrast, the flash pattern of *P. ardens* males consists of three to four pulses, and these females prefer pulse rates approximating the observed mean among conspecific males (6). In *P. greeni*, females tested in the laboratory prefer shorter than average interpulse intervals (**Figure 6d**), although field tests suggest female preference for average interpulse intervals (94). Whereas there is also some evidence that female preference changes as a function of pulse number in the multiple-pulse species *P. consimilis* and *P. carolinus*, these preferences are not as pronounced as those for faster pulse rates (6, 9).

Thus, in *Photinus* fireflies the same temporal components of male flash signals that function in species recognition (68) also play a role in intraspecific female choice. Females show directional preferences for these flash traits in some *Photinus* species, suggesting the potential for signal evolution through female choice. Across *Photinus* species, there appears to be a general female preference for more conspicuous courtship signals; this includes female preference for longer pulse duration in species with single-pulse patterns, and for higher pulse rate and pulse number in species with multiple-pulse patterns. The preference of *Photinus* females for higher flash rates is similar to female choice for rapid rhythms in other visual and acoustic courtship signals (9). Sexual selection through female preference for these temporal characteristics of male flash patterns may play an important role in the evolution of firefly flash signals.

Male-Male Competition

Differential mating success in male fireflies may reflect both mate preferences and mate competition (125); the latter can include scramble competition, endurance rivalry, and direct contests (1). Many authors have noted that firefly mating aggregations show male-biased operational sex ratios, particularly early in the mating season (32, 62, 70, 93), which can lead to intense indirect male-male competition for access to females. Indirect male competition includes scramble competition to reach females first and endurance rivalry to remain reproductively active for longer periods. Several morphological and behavioral traits of male fireflies may have evolved in response to such indirect selection. Many nocturnal lampyrids exhibit marked sexual dimorphism in lantern size (82, 102) (**Figure 1**), and larger male lanterns may reflect sexual selection to produce signals that are more readily detected by females. Similarly, eye size in *Photinus* fireflies is also sexually dimorphic (23, 68), suggesting that scramble competition to quickly locate females may produce stronger sexual selection for signal detection by males than by females. Several male behaviors observed in fireflies may also reflect indirect mate competition. As firefly males search for females, they are attracted to ongoing male-female flash dialogues (82, 104, 125). Several types of flash competition have been described between males engaged in dialogues with the same female (19, 22, 82). Males that have been unsuccessful in finding females have been observed to emit pseudofemale flashes in *Luciola lusitanica* (104) and *P. macdermotti* (82).

Direct male-male contests may also occur in some firefly species. In several *Photinus* fireflies, males attempt to physically disrupt copulating pairs (70, 82), leading to the formation of highly competitive mating clusters called love knots in *P. pyralis* and *P. carolinus* (45, 90, 125). A morphological trait that may have evolved in response to such direct competition is the hooked wing covers that characterize *Pteroptyx* males; during

mating these hooked elytral tips form a copulatory clamp around the female's genitalia (132), which may prevent intrusions by rival males. Finally, some *Photinus* fireflies show prolonged copulations lasting up to 9 h (70, 130), and such mate-guarding behavior likely prevents females from remating on the same night.

Male Competition Interacting with Female Choice

Male competition may interact with female signal preferences to generate differential mating success. Both *P. pyralis* and *P. ignitus* females prefer brighter male flashes (31, 125). However, flash intensity varies with distance from the receiver, and *P. ignitus* females cannot discriminate between more distant flashes produced by larger simulated lanterns and closer flashes produced by smaller simulated lanterns (31). Therefore, mate choice through female preference for flash intensity can depend in part on male scramble competition to approach females more quickly, thereby increasing their perceived flash intensity.

Male flash competition may also generate differential mating success through the relative timing of male flashes. *P. pyralis* females prefer preceding flashes when paired flashes from different directions are presented in quick succession (125). However, asynchronous male flashes from divergent directions prevent female response in *P. pyralis* (24). This pattern of female response may have played an important role in the evolution of male flash synchrony in some lampyrid taxa, as this may be an adaptation to female preference for preceding flashes or to reduce jamming from competing signals (55).

Field studies investigating which morphological and behavioral traits might best predict male mating success have yielded conflicting results. In *P. pyralis*, males that were successful at mating in noncompetitive courtships (a single male dialoguing with each female) had larger body and lantern sizes compared

with the population mean (125). However, in competitive courtships (defined here as three or more males simultaneously dialoguing with each female), successful males had smaller body and lantern sizes (125). In contrast, successful males in *P. ignitus* competitive courtships had wider lanterns (32). In competitive courtships in *P. greeni*, male flash behavior was a better predictor of mating success than was any aspect of male morphology (41). In particular, successful *P. greeni* males showed higher flash pattern repetition rates (number of flash patterns per minute) and were also more likely to elicit female responses. Thus, female choice based on male flash traits is likely to interact with male flash and scramble competition to determine male mating success in natural populations.

Courtship Role Reversal

Whereas the male-biased operational sex ratios that appear characteristic of many lampyrids can generate intense male competition, sex ratios tend to become female-biased during the late mating season in some *Photinus* species (32, 70, 93). In *P. ignitus* this shift in operational sex ratio was accompanied by a late-season decrease in both male body mass and spermatophore mass (32). Nuptial gift donation can represent a substantial reproductive investment by males and has the potential to select for males to discriminate among potential mates. Such male mate choice has been documented in *P. ignitus*, in which males selectively rejected less fecund females after contact (32).

Under some circumstances, courtship role reversal may also include females competing for access to males. In some *Photinus* species, female responsiveness increased as mate availability declined (32, 70). When sex ratios become female-biased during the late mating season, multiple *Photinus* females often respond synchronously to any male courtship signal (70). This may reflect female competition for access to males' nuptial gifts. Thus, both seasonal changes in operational sex ratios

and changes in nuptial gift availability have the potential to influence both the direction and the intensity of sexual selection.

Therefore, it appears that precopulatory sexual selection in *Photinus* fireflies acts through female mate choice via preferential response to particular males, in concert with male-male competition to quickly find and contact responsive females. However, two key factors that should alter the relative importance of these different sexual selection mechanisms are differences among lamproyrid taxa in male nuptial gifts and seasonal changes in operational sex ratios. Future work on sexual selection and mating ecology in other lamproyrid taxa should continue to explore how each of these processes contributes to generating differences in mating success.

Postcopulatory Sexual Selection

In polyandrous mating systems, a male's reproductive success depends not only on his successful mate acquisition, but also on the paternity share he gains with each mating. Postcopulatory processes that determine male paternity share include sperm competition among mating males for access to fertilizations and female choice of sires (42, 112). Postcopulatory sexual selection is likely to be particularly important in *Photinus* fireflies, as females are polyandrous in most of the species studied to date. In addition, the presence of male nuptial gifts is expected to select for females to mate with multiple males to gain material benefits and to later exercise postcopulatory choice. However, studies of postcopulatory sexual selection require morphological or molecular markers that can be used to trace paternity of several mating males; the absence of convenient markers for most lamproyrid taxa has slowed progress in this area.

Recent work examining postcopulatory sexual selection in fireflies has used PCR-based RAPD (randomly amplified polymorphic DNA) genetic markers to assign paternity to offspring of doubly mated females (39). This study traced the paternity success

of *P. greeni* males that were more attractive versus less attractive to females based on their courtship flash signals (39). Although it is often assumed that males highly successful in courtship interactions will also gain high postmating paternity share, this prediction has rarely been tested. Contrary to expectation, more attractive *P. greeni* males showed lower paternity success compared with less attractive males. These results suggest that *Photinus* females might choose among potential mates on the basis of their flash signals, and subsequently choose among potential sires on the basis of some short-range tactile or chemical signals. Further study is needed to distinguish male and female roles in biasing paternity, as well as to identify specific cues that females might use to assess the quality of mating males.

Flash Signals as Mate-Quality Indicators

Female preferences for male courtship signals are selected when females can gain a fitness benefit by discriminating among potential mates (1). Females could choose males on the basis of courtship signals that indicate either the material resources a male can provide (females benefit directly via increased fecundity or survival) or a male's genetic quality or compatibility (females benefit indirectly via enhanced offspring fitness) (64). In taxa where males provide nuptial gifts that increase females' lifetime fecundity, choice is most likely based on direct benefits, and females should be selected to choose males that provide larger nuptial gifts.

In three *Photinus* firefly species, the relationship between males' courtship flash timing and spermatophore size has been examined to explore whether male courtship signals act as indicators of nuptial gift quality. As discussed above, females in *P. ignitus* prefer conspecific male signals with longer pulse durations, and the pulse duration of a male's courtship signal was a good predictor of that male's spermatophore mass and protein

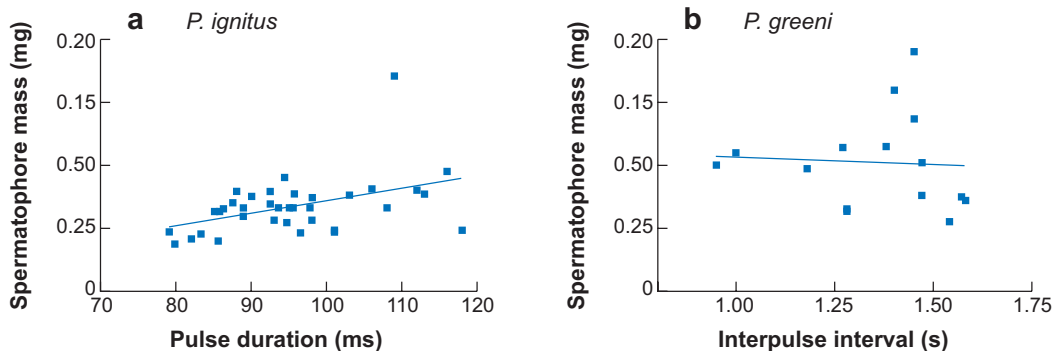


Figure 7

Male flash characters as predictors of nuptial gift size in *Photinus* fireflies. (a) *P. ignitus* pulse duration predicts a significant proportion of the variation in spermatophore mass ($r^2 = 0.191$, $n = 36$; modified from Reference 31). (b) *P. greeni* interpulse interval does not predict spermatophore mass ($r^2 = 0.004$, $n = 15$; data from Reference 94).

content (31) (**Figure 7a**). However, this relationship only held during the early mating season, possibly because male spermatophore mass decreases over successive matings (30, 33). In *P. pyralis* fireflies, females also prefer longer duration signals, yet in contrast male pulse duration did not predict spermatophore mass in this species (29). Similarly, although *P. greeni* females show a preference for courtship signals with shorter interpulse intervals, these preferred male flash characteristics also did not predict spermatophore mass in this species (94) (**Figure 7b**). One possible explanation for these conflicting results is that the courtship signaling systems of different lampyrid species may vary in their location along an evolutionary continuum from reliable to uninformative to manipulative courtship signals.

Theoretical models generally assume that for honest signaling systems to be evolutionarily stable, the costs of signaling must depend on signaler quality such that only high-quality males can afford to produce the most attractive signals (91, 109; in such models, male benefits of signaling are assumed to be independent of male quality). Signaling costs include the energy required for signal production as well as predation costs associated with signaling, but relatively few studies have addressed this differential cost assumption (65). Measurements of energetic expenditures dur-

ing flash production by *Photinus* fireflies (134) indicate that bioluminescent signals have low energy requirements and thus are unlikely to generate differential costs. Substantial predation costs associated with *Photinus* courtship signals have been quantified (134; discussed below), but it remains unknown whether such costs are differential, i.e., whether the costs of attractive flash signals are higher for low-quality males.

Honest signaling systems can also be stable if male signal benefits are dependent on male quality (61; in such models signal costs are assumed to be independent of male quality). Under these model assumptions, low-quality males need not be constrained by the high costs of producing a more attractive signal; instead low-quality males might gain a smaller benefit from the same signal relative to high-quality males. In this context, it is critical to consider such benefits not just in terms of increased male mating success, but also in terms of increased male postcopulatory success. In many insects where males provide nuptial gifts, larger gifts lead to higher male paternity share (112, 123). Thus, if in addition to providing a greater benefit to females, males with larger nuptial gifts also gain increased paternity share, this differential benefit could lead to male courtship signals that are reliable indicators of nuptial gift size.

Lucibufagins

(LBG): steroidal pyrones found in some lampyrids that are distasteful to many generalist predators, yet attract and are sequestered by specialist *Photuris* predators

In summary, the relationship between firefly courtship signals and mate quality is just beginning to be explored. Among the key questions that remain to be addressed for lampyrid taxa are (a) What aspects of male quality are most important to females? (b) How do male courtship signals vary across taxa in the costs they incur and the benefits they provide to signalers? (c) Do differential costs and/or benefits exist that would alter the degree of reliability or deception observed in firefly signaling systems? Additional studies are needed in other lampyrid taxa with nuptial gifts to determine the costs and benefits of these nuptial gifts for males, and the relationship between courtship signals and both direct and indirect benefits provided by males.

PREDATION AND CHEMICAL DEFENSE

Although mate choice in many animals favors the most conspicuous visual, acoustic, or olfactory signals (1), such signals may also attract attention from illegitimate eavesdropping predators (109, 137). Sexual selection theory predicts that runaway selection for increasingly conspicuous courtship signals will be balanced against some fitness cost of signaling, perhaps in the form of increased predation risk (1). However, conspicuous animal displays may also evolve as aposematic signals that warn potential predators of the signalers' unpalatability (57). Firefly flash signals are highly conspicuous and therefore may be subject to selection through their role in attracting or deterring a wide variety of potential predators. The emerging story of firefly chemical defenses is filled with enough subterfuge, poison, and intrigue to rival the most exciting spy fiction, and there are many fascinating avenues of research still remaining to be explored.

Chemical Defenses and Aposematism

Many lampyrid taxa are distasteful, and even toxic, to a variety of generalist predators, in-

cluding arthropods, birds, amphibians, reptiles, and fish (36, 37, 43, 44, 48, 53, 56, 63, 77, 117, 122). Such unpalatability is associated with secretions that many lampyrids produce through reflex bleeding when they are disturbed (4, 46, 103). Adult *Photinus* fireflies are protected against predation by thrushes (genus *Hylocichla*) and jumping spiders (genus *Phidippus*) by a class of steroidal pyrones known as lucibufagins (LBG) (44, 51), which have also been isolated from adults of *Lucidota atra*, a diurnally active firefly (56). Firefly larvae possess specialized defensive glands (48, 121). Recent work has shown that larvae of *Luciola leii* gain protection against several aquatic and terrestrial predators by emitting volatile terpenoid compounds from these glands (48).

Both chemical defense and larval bioluminescence are considered ancestral in lampyrids (10, 11), and it has been proposed that such a combination of chemical and visual cues represents multimodal aposematic signals that help reinforce aversion learning by diverse predators (48). In addition, the remarkably similar color patterns exhibited across lampyrid genera (adults are generally black, red, and yellow) may also serve as aposematic signals to potential diurnal predators (93).

Although the adaptive value of aposematic displays depends on a predator's ability to learn, only a few studies have explored the potential for firefly predators to develop a learned negative response to fireflies (36, 37, 122). De Cock & Matthysen (36) demonstrated that starlings (*Sturnus vulgaris*) find *Lampyris noctiluca* larvae distasteful and learn to avoid them on the basis of coloration. When presented with glowing prey, wild-caught toads (*Bufo bufo*) showed lower attack rates and longer latency to attack (35). Toads also became more reluctant to attack after they had encountered *L. noctiluca* larvae, which they find distasteful (37). House mice (*Mus musculus*) also rejected *Photuris* firefly larvae as prey, and mice learned to avoid distasteful food that was positioned next to a simulated larval glow (122).

Thus, it appears that generalist predators can learn to associate both color and bioluminescence of firefly larvae with their unpalatability. However, the relative importance of this response in the evolution of bioluminescence within the Lampyridae needs to be explored further. In particular, more studies are needed to establish the distribution and diversity of chemical defenses across lampyrid species and to examine how these defenses affect the palatability of fireflies to various predators.

Specialist Predators

Most fireflies in the North and South American genera *Photuris* and *Bicellychonia* are specialist predators of other fireflies (3, 85, 87) (Figure 8). Within the *Photuris pennsylvanica-versicolor* complex, females use aggressive flash mimicry to attract prey (72, 80, 85, 97, 136). These *Photuris* fireflies do not appear to produce LBG on their own, but rather they rely on the trophic acquisition of these compounds through consumption of *Photinus* prey (43). LBG were absent in *Photuris versicolor* females reared from larvae without access to *Photinus*, but LBG content of *Photuris* females increased following consumption of two *Photinus* males (Figure 9a). Only *Photuris* females that had consumed *Photinus* prey were protected from predation by *Phidippus* jumping spiders, and the degree of protection gained by field-collected *Photuris* females depended on their LBG content (43) (Figure 9b).

Photuris versicolor adults and larvae endogenously produce another defensive compound called betaine (53), and females transmit both their own betaine and some of the acquired LBG to their eggs (52) (Figure 9c). When offered to predators, eggs laid by *Photuris* females that had eaten two *Photinus* males were consumed slightly less often, although this difference was not significant. When chemical extracts were applied to normally palatable insect eggs, both LBG and betaine reduced egg mortality in laboratory and field tests (52).

Females of many *Photuris* species (although not those in the *P. congener* group) engage in aggressive flash mimicry (85, 87). Detailed field observations have provided considerable insight into the behavioral interactions between these *Photuris* predators and their *Photinus* prey. These *Photuris* “femmes fatales” attract male prey by responding to males’ courtship signals with flashes resembling the response delays and flash patterns appropriate for females of their prey species (72). Field experiments have shown that individual *Photuris versicolor* females have signal repertoires, facultatively switching their responses when exposed to simulated male flash patterns of different *Photinus* species (80). In addition, Lloyd & Wing (89) described field observations showing that *Photuris* females attack aerial decoys containing light-emitting diodes. Recent controlled field experiments have quantified the predation costs of firefly signaling by measuring *Photuris* attraction to simulated signals



Figure 8

Predatory *Photuris* female in the process of consuming a *Photinus* male she has attracted through aggressive mimicry of response flashes typically produced by *Photinus* females (photo by J.E. Lloyd).

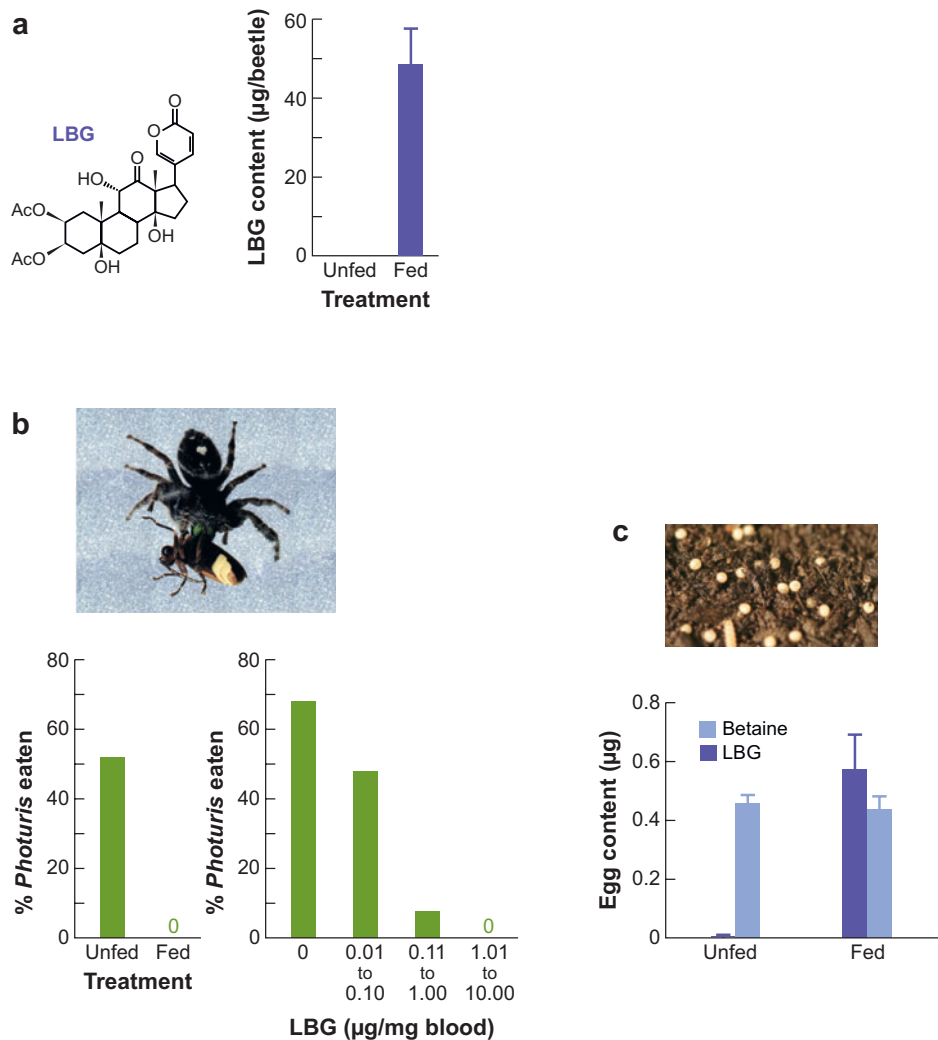


Figure 9

Trophic acquisition of defensive steroids by *Photuris* predatory fireflies. Comparisons of *Photuris* sp. females that were reared without access to *Photinus* prey (unfed) versus females that had recently consumed two *Photinus ignitus* males (fed): (a) Total lucibufagin content (LBG, structure shown) of *Photuris* sp. females (redrawn with permission from Reference 43). (b) Percentage of *Photuris* sp. females that were eaten by *Phidippus audax* jumping spiders (photo by T. Eisner) depending on either experimental treatment (left) or hemolymph LBG content of field-collected *Photuris* females (right) (redrawn, with permission, from Reference 43). (c) Amounts of two defensive compounds, LBG and betaine, in eggs laid by *Photuris* sp. females (redrawn, with permission, from Reference 52; photo by B. Chan).

that matched the courtship flashes of *Photinus greeni* males (134). This study demonstrated that bioluminescent courtship signals significantly increased predation risk on *Photinus* fireflies, and that higher flash pattern repeti-

tion rates increased the likelihood of *Photuris* predation.

Field observations of *Photinus* courtships in the presence and absence of *Photuris* predators provide some insight into how such

predation may have shaped courtship signaling in prey species. *Photinus greeni* females reduced their flash responses to courting males in the presence of predatory *Photuris*, but males that retained high levels of flash activity in the presence of these predators were more likely to secure mates (41). This study also found that male mating success in *P. greeni* was associated with higher flash pattern repetition rates by males and higher female response rates (41). Because higher flash pattern repetition rates also increase the likelihood of *Photuris* predation, these results indicate conflicting selective pressure on male courtship signals from sexual selection versus natural selection.

Recent work has thus provided considerable evidence supporting the idea that *Photuris* predation represents an important force shaping the evolution of bioluminescent signals in North American fireflies (83, 86, 87). It has also been suggested (56, 87) that the evolutionary reversion to diurnal activity and pheromonal signals observed in several North American lampyrid clades (114) may be related to *Photuris* predation. Further research is needed to explore how predation pressure from *Photuris* and other specialist predators might vary across lampyrid species, and how prey signaling behavior has evolved to reduce such predation risks.

Finally, the information content of firefly flash signals seems to be fundamentally different depending on whether the receivers are generalist versus specialist predators. Firefly bioluminescence may serve as aposematic signals for generalist predators, yet the same signals are highly attractive to specialist predatory fireflies. Because of the complex interactions between chemical defenses, aposematic signals, and predation, fireflies provide an outstanding system for future studies to explore hypotheses about aposematism, Batesian mimicry, and aggressive mimicry. For example, it is possible that Batesian mimicry might account for some of the remarkable similarities in body coloration and repeated evolution of bioluminescent signals

within the Lampyridae. Research to date on predation and chemical defenses in fireflies has provided a strong foundation for this future research.

CONCLUSIONS

This review provides a comprehensive synthesis of recent work on firefly signal evolution, mate choice, and predation. The studies reviewed here have provided many new insights, allowing us to develop a much more detailed understanding of how sexual selection and natural selection may act together to shape the evolution of courtship flash signals. In addition, recent work elucidating lampyrid phylogenetic relationships has laid a foundation for rigorous comparative tests that will help answer many outstanding questions about the evolution of firefly courtship signals.

Sexual selection has been a major force in firefly evolution. Studies of sexual selection in North American *Photinus* fireflies demonstrate that females preferentially choose males on the basis of their flash signals, although for most lampyrids we still know little about how and why females choose their mates. Male nuptial gifts (spermatophores transferred to females during mating) have been discovered in many lampyrids, and these are likely to play an important role in firefly mating systems. Additional studies across lampyrid taxa are needed to better understand the fitness benefits such gifts might provide to individuals of both sexes. Finally, the apparent prevalence of promiscuous mating systems in fireflies indicates that it is essential for future studies to integrate both precopulatory and postcopulatory stages of sexual selection. For example, it would be worthwhile to explore the possibility that females could use male flash signals to choose their mates and subsequently use contact chemicals or copulatory behaviors to choose the best sires for their offspring.

Studies covered in this review have also revealed the importance of predators in driving the evolution of firefly chemical defenses and

bioluminescent signals. Generalist predators of ancestral lampyrids likely created selection for chemical defenses along with bioluminescent glows that continue to serve as warning signals for firefly larvae. Thus, the familiar bioluminescent courtship signals used by adults in so many firefly species have likely evolved from aposematic signals used by firefly larvae. Several distinct chemical compounds that deter generalist predators have been isolated from the adults of some North American lampyrids, but the distribution and diversity of chemical defenses across lampyrid taxa remain largely unexplored. Specialist predatory fireflies in the genus *Photuris* have been shown to rely on the trophic acquisition of defensive chemicals from their prey. These predatory fireflies are geographically restricted to North and South America, and their signal exploitation may have been an important selective force driving evolutionary reversions to diurnal activity and pheromonal signals observed in some lampyrid clades. Conversely, their absence may have allowed the evolution of highly conspicuous signaling systems shown by Southeast Asian *Pteroptyx* fireflies, with highly synchronous flashes emitted by aggregations of sedentary males.

In conclusion, recent research provides new evidence supporting the idea that firefly courtship signals are shaped by the evolutionary processes of sexual selection through female choice acting in combination with natural selection through predation. Studies on several *Photinus* fireflies indicate that females prefer more conspicuous male signals, including flash patterns with longer pulse durations and faster pulse rates. However, predatory *Photuris* fireflies are also attracted to bioluminescent signals, and current evidence indicates that more conspicuous signals result in higher predation risks. Thus, sexual selection on *Photinus* courtship signals in favor of more conspicuous flashes appears to be balanced against natural selection acting through signal-exploiting predators. Because predator abundances are likely to vary both spatially and temporally, the resulting geographic mosaic will create differing selection regimes among prey populations. One implication is that this shifting balance between natural and sexual selection could promote evolutionary diversification of firefly courtship signals. Much remains to be learned, and we have no doubt that future studies will greatly enhance our understanding of the evolution of firefly flash signals.

SUMMARY POINTS

1. Extant fireflies (family Lampyridae) use diverse sexual signaling modalities, although ancestral lampyrids likely relied on pheromones to attract their mates. Within this family bioluminescent courtship signals evolved multiple times, and reversion to pheromonal signals also occurred several times.
2. The ability to produce precisely timed bioluminescent flashes (versus slowly modulated glows) is associated with several anatomical and physiological adaptations of the adult lantern in derived lampyrid taxa, and involves the signaling molecule nitric oxide.
3. In the well-studied North American genus *Photinus*, females preferentially respond to male flash signals based on pulse duration or pulse rate. These female preferences generally favor more conspicuous courtship signals.
4. In numerous firefly taxa, males transfer spermatophores to females during mating. Such male reproductive investment has the potential to reverse the typical sexual selection pattern of female mate choice and male mate competition.

5. Females are expected to choose males that offer better nuptial gifts, but it remains to be determined whether courtship signals act as honest indicators of mate quality across most firefly species.
6. Firefly bioluminescence and coloration likely originated as aposematic displays. Lucibufagins protect *Photinus* fireflies against generalist predators, but not against specialist *Photuris* fireflies. These predators rely on the trophic acquisition of lucibufagins from *Photinus* prey for use in their own defense.
7. Directional sexual selection for more conspicuous flash signals in *Photinus* fireflies is balanced against natural selection via predation by eavesdropping predatory fireflies. Differing predation pressure within and across firefly taxa may play an important role in flash signal evolution.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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