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The origin of photic behavior and the evolution of sexual communication in fireflies (Coleoptera: Lampyridae)

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Abstract

Through a phylogenetic analysis using adult morphological characters, we show that the origin of bioluminescence in cantharoid beetles appears to predate the origin of the family Lampyridae. The ability to produce and emit photic signals was first gained by larvae and appears to function as an aposematic warning display; it was subsequently gained in adults and is used as a sexual signal. Our analysis also suggests that while pheromonal sexual signals are used basally in the family, they are used in conjunction with and then subsequently replaced by photic signals in some lampyrid lineages. Both photic signals and the photic organs used to produce them have become greatly elaborated in the fireflies that no longer employ pheromonal sexual signals. In addition, the ability to produce a flashed sexual signal appears to have arisen at least three times in the family Lampyridae. Convergent evolution is also evident in a number of adult male photic organ morphologies. Further, we recommend that individual signal system components be compared rather than overall signal system complexity. The use of this strategy may allow one to recognize and better interpret adaptive correlations despite convergence or loss. We demonstrate that phylogenetic analysis is a powerful tool even for rapidly evolving traits.

Introduction

Sexual selection is often invoked as a particularly powerful evolutionary force (Andersson, 1994; Eberhard, 1985; West-Eberhard, 1979, 1983, 1984). This postulate is generally based on studies of dramatic, apomorphic species or on clusters of species that exhibit remarkable sexual characteristics. Sexually selected characters may be thought to be subject to convergence upon one or a few optimal points in the adaptive landscape, which could be worrisome for phylogenetic analysis. Modern cladistic methods may show more evolutionary paths to the same end points than was formerly suspected (Desutter-Grandcolas, 1997). Of course, all characters are subject to convergence, and what is homoplasy broadly (across genera) is synapomorphy locally (for a given genus). It is possible that sexually selected characters do not behave differently in a cladistic analysis from other characters or that homoplasy in these characters is advantageous for phylogenetic analysis. To some extent, difficulty with sexually selected characters may stem from the definition of the characters themselves, with composite features like "size dimorphism" being used as a single character rather than being explored for the multiple phenomena that they actually are (Hormiga et al., 2000; Nylin and Wedell, 1994).

Fireflies (Coleoptera: Lampyridae) present one of the best systems available for studying sexual selection and phylogeny. The origin of photic organs is now known to precede their use in the context of sexual signaling, originally playing a role as a nocturnal warning signal for unpalatable larvae (Branham and Wenzel, 2000, 2001). Firefly larvae do not use luminous signals for sexual purposes because they cannot reproduce during this life stage, so there has been much speculation as to the function of luminescence in larvae. One of the most widely held hypotheses is that lampyrid larvae use their

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photic emissions as aposematic displays (Belt, 1874; Cowles, 1959; Crowson, 1972; Sivinski, 1981) and it has been shown that fireflies possess distasteful steroids, termed lucibufagins, in their hemolymph (Blum and Sannasi, 1974; Eisner et al., 1978, 1997). Underwood et al. (1997) tested the hypothesis of aposematic display and concluded that a predator, such as a mouse, could associate a bioluminescent glow with a distasteful substance. Additionally, De Cock and Matthysen (1999) found that toads discriminated against glowing prey and found lampyrid larvae to be "disagreeable."

Once co-opted by adults, photic organs in some species are used in sexual display, and sexual selection upon the photic signals has been shown to shape signals in a directional manner. Some characteristics of these sexual signals vary, while others are more or less static, and females discriminate between males on the basis of such signal parameters. Branham and Greenfield (1996) showed that female *Photinus consimilis* prefer higher flash rates, even when such rates exceed the highest rate in natural populations, analogous to phenomena long known in acoustic signal evolution. Lloyd (1997) offered the possibility that certain behaviors, such as males flashing their distinctive signals, were likely convergent among many species. Perhaps this would be the result of similar pressures on different lineages to produce signals that are easy to see from distance, that are unambiguous, and that may indicate something about the quality of the males that females choose. The data available regarding firefly signaling behavior are incomplete but nonetheless adequate to test, through a cladistic analysis, these proposals of behavioral evolution and the evolution of the associated morphological characters.

Fireflies have appeared often in poems, songs, and stories of folklore in many diverse cultures (Harvey, 1957), though Osten-Sacken (1861) appears to be the earliest published description of firefly flash patterns as sexual signals. The luminous displays serve to communicate an individual's sex, species, and exact location (Barber, 1951; Lloyd, 1964, 1971; Mast, 1912; McDermott, 1911, 1958; Papi, 1969). Secondary functions for firefly luminescence have been posited, such as illumination of substrate during landing (Lloyd, 1968), but the primary function of luminous behavior in adults is to facilitate pair formation (Buck, 1937; Lloyd, 1966; Mast, 1912; McDermott, 1911, 1912). Some firefly species do not produce light as adults and may rely exclusively on pheromones for pair formation, i.e., *Lucidota atra* (Lloyd, 1972a). In other species, such as *Pleotomus pallens*, the sedentary female produces a pheromone that acts as a long-range sexual signal and emits a glow that males use to locate the source of the pheromone at close range. This type of communication system has been termed "Signal System I" by Lloyd (1971). Some species do not use pheromones at all, such as those of *Microphotus*, of which the sedentary female glows while the nonluminous male flies in search of the glow. Most behavioral research has focused on Nearctic species (*Photinus*, *Photuris*, *Pyractomena*, etc.), most of which posses a signal system in which males fly while broadcasting their species-specific flash pattern and females are sedentary, responding to the male signal with a species-specific signal following a species-specific delay. A short flash dialog commonly ensues until the male either departs or lands near the female, whereupon mating may occur. This type of photic communication system has been termed "Signal System II" by Lloyd (1971), though it was first indicated by F.A. McDermott (Lloyd, 1990). These signals are commonly termed "critically timed signals," because the timing parameters of the males' flash patterns show little variation and the species-specific delay after the male's last flash and the beginning of the female's response is precisely timed. Courtship is compromised when this timing is violated, as signals fail to communicate species identity. Lloyd (1971) argued that "Signal System II" was derived from "Signal System I."

Firefly species in other parts of the world have mating systems that deviate from those of Signal Systems I and II studied in North America. The behavior of many Japanese fireflies has been documented through the work of Ohba (1980, 1986) and Ohba and Goto (1992a,b, 1993). Ohba (1983) proposed a classification scheme for the various mating systems found in Japanese fireflies, designated according to representative taxa. In increasing order of complexity, they are the LB system (for *Lucidina biplagiata*), which is a diurnal signal system composed entirely of chemical signals (pheromones); the CR system (for *Cyphonocerus ruficollis*), in which pheromones are used in conjunction with a weak glow; the PR system (for *Pyrocoelia rufa*), which consists of a continuous glow and pheromones (Signal System I); the LC system (for *Luciola cruciata*), which employs a single long pulse and also synchronous flashing, but does not employ critical parameters; the LL system (for *Luciola lateralis*), wherein males use a single pulse that does not employ critical parameters, though there is a critical timing in the female's response to the male signal; and the HP system (for *Hotaria parvula*), in which both sexes produce photic pulses with critical timing parameters (Signal System II). A dendrogram of eight species of Japanese fireflies representing three types of communication systems (HP, LC, and LL) was subsequently constructed based on electrophoretic analysis of allozymes using Nei's genetic distance by UPGMA (Susuki et al., 1996). This study showed that the LC system was basal to the LL system in one of the two sister clades and the other clade was composed of taxa using the HP signal system. This dendrogram pattern of signal evolution is not very informative. Susuki (1997) later expanded his phylogenetic analyses of Japanese firefly mating systems to combine mitochondrial 16S ribosomal RNA data with allozymes.

Phylogenetic trees were constructed using the neighbor-joining, maximum parsimony, and maximum likelihood methods, but only the tree produced by the neighbor-joining method was presented. While character optimization is ambiguous in several parts of this tree, only one optimization of characters involved in signal system evolution was presented and discussed. The optimization presented shows a trend from (1) diurnal basal taxa that employ only chemical signals (LB), to taxa using chemical signals and photic glows (CR and PR) in one of the two major clades, to (2) taxa using only chemical signals (LB) leading to taxa that use only critically timed photic flashes (HP), which then give rise to taxa that produce discrete flashes while having lost critical timing parameters (LC and LL), in the second clade. The proposals detailed above share certain shortcomings. First, each syndrome (Lloyd's and Susuki's) is a simplification of the true complexity, and analysis of the full range of variation is preferable (Lloyd, 1983). Also, each syndrome is identified as a composite character rather than dissected to its parts, so the evolution of the syndrome cannot be understood any more clearly than by simply optimizing it on a tree (as Suzuki did). Further, there is only a logical argument leading from simple to complex systems, with no evaluation of the potential for loss (reversal) or convergence. Moreover, both of the proposals are regional, restricting themselves either to North America or to Japan, which is an unlikely start for a hypothesis to be generally informative. A much more thorough analysis is necessary to understand the evolution of these signal systems.

The cladistic analysis offered here aspires to answer several questions. How does the use of pheromones relate to the use of photic signals? Is the use of photic signals a synapomorphy for Lampyridae, with loss in those taxa that use only pheromones? Do some signal systems lead to others as proposed by authors? Are there multiple origins of the use of photic signals in sexual context? Is there evidence that sexual selection has driven species to converge on certain signaling systems?

Materials and methods

Our analysis was conducted using 85 exemplar taxa, selected to represent maximal diversity within the family Lampyridae and taxa from each subfamily within each of the outgroup families when possible (Appendix A), based on Lawrence and Newton (1995). We refer loosely to the taxa used in this analysis as "cantharoids," as they have generally been thought of as a monophyletic group within the Elateroidea (Lawrence, 1988). Some families closely related to Lampyridae are luminescent in at least one life stage (Rhagophthalmidae, Phengodidae, and Omalisidae). Wittmer and Ohba (1994) elevated the luminous genus *Rhagophthalmus* Motschulsky to family status, whereas it was previously classified either as Lampyridae (McDermott, 1964, 1966) or Phengodidae (Crowson, 1972). Branham and Wenzel (2001) removed *Dioptoma* Pascoe and *Diplocladon* Gorham from Phengodidae to Rhagophthalmidae. All known larvae of Lampyridae, Phengodidae, and Rhagophthalmidae, as well as the genus *Omalisus* Geoffroy (= *Omalysus*, *Homalisus*) in Omalisidae, are luminous. Omalisidae is the only one of the four luminous families in which bioluminescent adults are lacking. The glowing click beetles, Pyrophorinae and Elateridae, are also bioluminescent as both larvae and adults but are excluded from this analysis because they are too distant (Beutel, 1995; Lawrence, 1988) to be informative regarding the topology of Lampyridae. Plastoceridae on the other hand is more closely related to the families of interest—those comprising the old superfamily Cantharoidea—and seemed the most logical choice for a distant outgroup (Lawrence, 1988). For further discussions of outgroup taxa used in this analysis and the evolution of bioluminescence in these outgroup taxa, see Branham and Wenzel (2001).

The ingroup includes all subfamilies within Lampyridae but one (no specimens of *Ototretadrilus*, Ototretadrilinae, were available for study), based on the classification schemes of Crowson (1972) and Lawrence and Newton (1995).

There has been some confusion regarding what segments bear photic organs, so a few words are necessary to explain our character coding. Because the first abdominal segment is ventrally internalized in adults, larval abdominal segments do not correspond directly to visible adult segments (Branham and Archangelsky, 2000). Visible adult abdominal sclerites are called "ventrites," with ventrite 1 corresponding to larval segment 2, ventrite 2 corresponding to segment 3, and so forth. In some individuals, usually females, as seen in *Photuris* species, the adults bear "normal" adult photic organs on the fifth and sixth ventrites and, in addition, sometimes bear a functional larval-type photic organ on the seventh ventrite (Hess, 1922). In the case of still other fireflies, those species employing sophisticated photic signals produce them from morphologically advanced photic organs that possess increased amounts of tracheation, innervation, and a reflective layer. Such organs are found in adult *Photinus*, *Photuris*, *Bicellonycha*, etc. (Buck, 1948), and are generally confined to the fifth and sixth ventrites (Branham and Archangelsky, 2000; Hess, 1922). Adults in the subfamily Luciolinae (*Luciola*, *Colophotia*, *Pteroptyx*, etc.) also produce complex photic signals, though the abdomen has undergone reduction of the seventh ventrite in this clade of fireflies (Ballentyne, 1992).

Characters used in the analysis were 74 male morphological characters with a total of 212 character states. Inapplicable characters were coded as "-," while missing characters were coded as "?." All characters were run equally weighted with 20 characters being

additive and 54 nonadditive (Appendix B). The Parsimony Ratchet was implemented in Nona (Goloboff, 1993), run within Winclada (Nixon, 2000). Plastoceridae was secondarily designated as the root of the tree (Nixon and Carpenter, 1993), based on Lawrence's (1988) phylogenetic analysis of the Elateriformia. Bremer support was evaluated using Nona and the search was set to a Bremer support level of 5, with four runs (each holding 100 trees) and a total hold of 5000 trees.

Results

The Parsimony Ratchet consisted of 100 iterations, weighting 12% of characters to get 52 most parsimonious trees (tree length 818 steps, consistency index (CI) = 0.16, retention index (RI) = 0.59). Starting from these 52 most parsimonious trees and using the "max*" command produced 336 trees of 818 steps. The "best" command then identified 56 of the 336 most parsimonious trees as suboptimal and these were removed from the tree file, thus leaving 280 most parsimonious trees of 818 steps. A strict consensus of these 280 trees collapsed 13 nodes and produced a consensus tree of 848 steps (CI = 0.16, RI = 0.57). The consensus tree (Fig. 1) is fully resolved except for the four phengodid taxa plus *Stenocladius* sp. and a trichotomy involving *Malthinus occipitalis* (Cantharidae), the adjacent clade of *Pseudotelegeusis* and *Telegeusis*, and the aforementioned clade of Phengodidae plus *Stenocladius* sp. The ingroup of the consensus tree is fully resolved except for a polytomy of lampyrid taxa plus two trichotomies: (a) *Bicellonycha amoena*, *Photuris divisa*, and *P. brunnipennis* and (b) *Luciola lateralis* and *L. salomonis* plus the adjacent *Luciola*/*Colophotia*/*Pteroptyx* clade (Fig. 1). Bremer values for the ingroup, listed in Fig. 2, indicate the number of steps that are required, up to 5, to derive a tree that does not include the particular node of interest.

Discussion

Taxonomy of Lampyridae

McDermott (1964) created the modern classification for the family Lampyridae. McDermott clearly believed the classification was not natural, though he regarded it as "perhaps being of some utility." McDermott revised his classification of Lampyridae a few years later (McDermott, 1966). Crowson (1972) revised McDermott's (1966) classification through a "possibly improved scheme of subfamilies" by removing some taxa from Lampyridae and placing them in Omethidae and Phengodidae (thereby eliminating the Matheteinae and Rhagophthalminae of McDermott's Lampyridae) and moved *Ototretadrilus* out of Drilidae and into Lampyridae (thereby creating Ototretadrilinae). Additionally, Crowson added Cyphonocerinae and Ototretinae to Lampyridae by further subdividing previously existing subfamilies. Lawrence and Newton (1995) have since adopted Crowson's classification scheme for Lampyridae in their work on the higher level classification of Coleoptera.

Comparing our phylogenetic analysis to McDermott's (1966) and Crowson's (1972) classifications, our analysis supports the monophyly of only two subfamilies, Luciolinae and Photurinae, the generic composition of which has been stable since McDermott (1964). A recent comparison of the larval morphology of several genera of lampyrids (Archangelsky and Branham, 2001) suggested the need to revise the current classification system of Lampyridae in general, and this conclusion is reinforced here.

Our analysis shows that the historical Lampyridae, largely Lampyridae *sensu* Lawrence and Newton (1995), is not monophyletic: *Drilaster*, *Harmatelia*, *Pterotus*, and *Stenocladius* do not belong within Lampyridae. Interestingly, these four genera have a history of being moved between various families that are close to Lampyridae (Branham and Wenzel, 2001). Our phylogeny places *Drilaster, Harmatelia*, and *Pterotus* outside of Lampyridae but also does not place them within any existing family groups. *Stenocladius* is placed outside of Lampyridae, but in an unresolved polytomy that is composed of taxa currently in the family Phengodidae (Fig. 3). Given this pattern, our solution is to formally move *Drilaster*, *Harmatelia*, and *Pterotus* to "Elateroidea *incertae sedis*" status and to move *Stenocladius* into "Phengodidae *incertae sedis*" (Branham and Wenzel, 2001). Expanded studies of these groups is necessary to place them definitively. Rhagophthalmidae is shown in this analysis to be monophyletic and forms a sister clade to Lampyridae in our sense. *Brachylampis sanguinicollis* and *Psilocladus* sp. compose the basal stem of the lampyrid clades. The family is composed of two major clades, one with *Ellychnia corrusca* in a basal position and the other composed of two sister clades with *Macrolampis acicularis* and *Robopus* sp. defining the basal position of each.

Origin of bioluminescence

Our phylogenetic analysis indicates at least two origins and one loss of luminescence in the cantharoid lineage (Fig. 3). The first origin was ancient within this lineage and applies to Omalisidae, Rhagophthalmidae, and Lampyridae, plus the "Elateroidea *insertae sedis*" genera *Harmatelia*, *Drilaster*, and *Pterotus* (Branham and Wenzel, 2001). All of these have luminous larvae, and all but Omalisidae glow in at least one adult sex (Crowson, 1972). This suggests that luminescence arose first in the larvae and then subsequently in the adults and that the origin of luminescence precedes the origin of Lampyridae (Fig. 3). This finding supports Crowson's

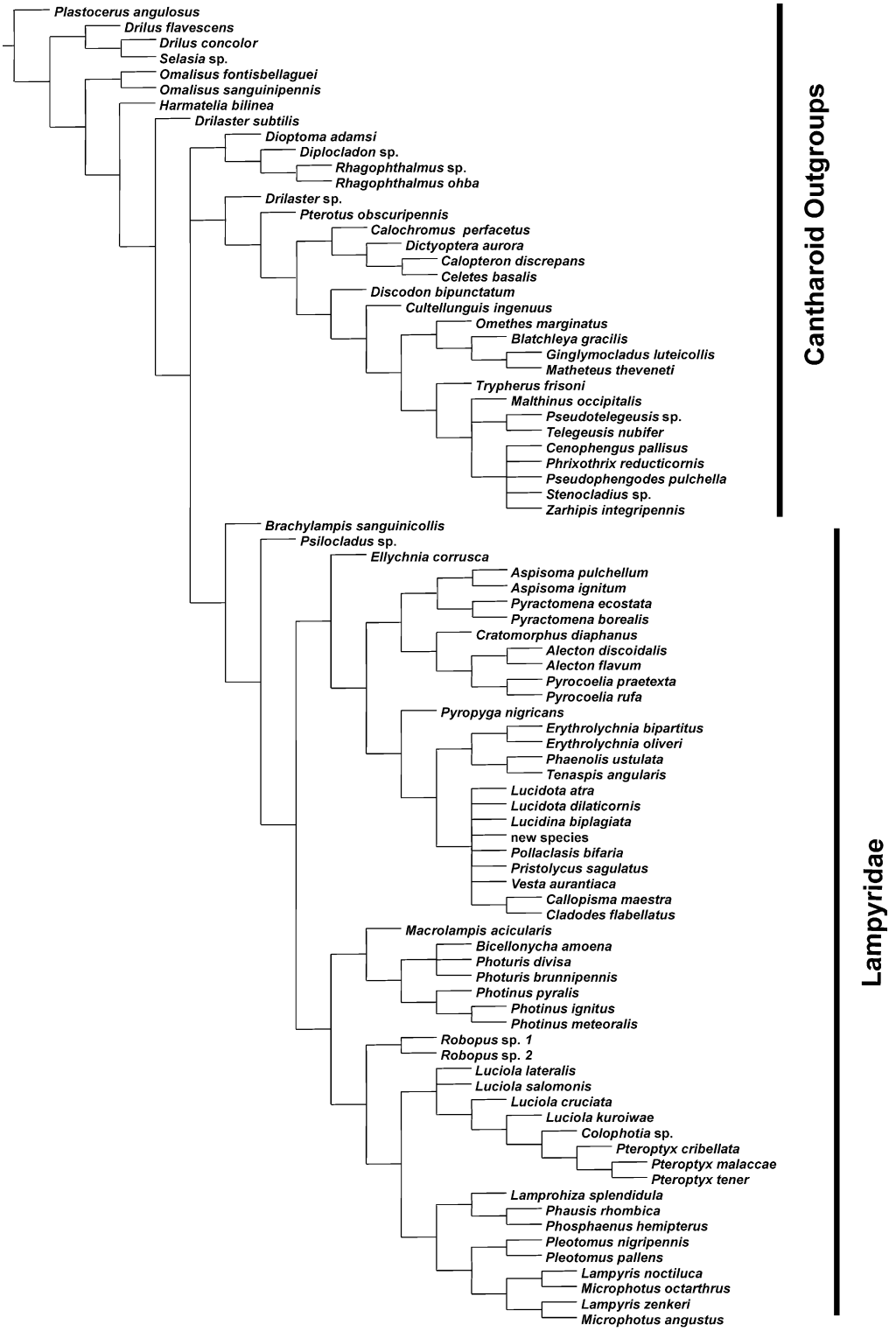

Fig. 1. Strict consensus of 280 most parsimonious trees (848 steps, CI 0.16, RI 0.57). The vertical bars on the far right delimit the cantharoid outgroups and the family Lampyridae.

(1972) comment that, "The fact that ... there are no well established instances of cantharoid beetles luminous as adults but not as larvae, suggests, though it is hardly sufficient to prove, that luminosity first arose in the larval stage." Our analysis confirms proposals by McDermott (1964) and Sivinski (1981) that luminescence first arose in larval cantharoids, was probably maintained through an aposematic function in larvae, and later was co-opted as a sexual signal (perhaps as both a warning and a sexual signal) in the adults of

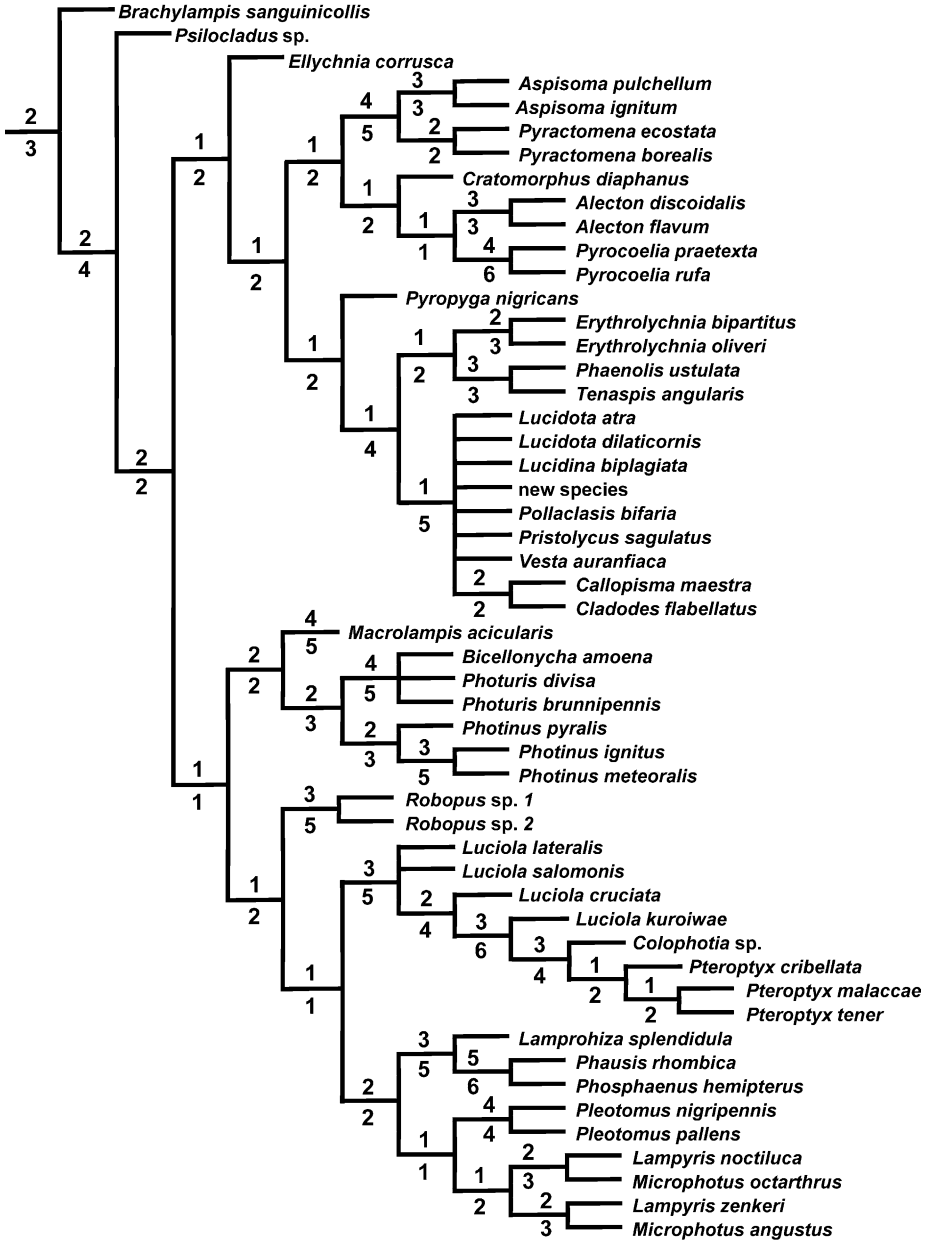

Fig. 2. The lampyrid clade of the strict consensus with Bremer support values (set at a maximum Bremer value of 5) appearing above the nodes, with the numbers below the node indicating the number of synapomorphic characters at that node.

several cantharoid families, while reaching its greatest elaboration and refinement in fireflies.

Lampyrid larvae produce photic signals as aposematic displays to convey to potential predators that they are chemically defended (Belt, 1874; Crowson, 1972; Sivinski, 1981; Tyler, 2001a,b). The chemical substances responsible for this defense were identified by Eisner et al. (1978, 1997) as defensive steroids called lucibufagins, which typically serve as cardiotonic agents. Lucibufagins are structurally related to the bufodienolides of toads and the cardenolides of plants (Budavari et al., 1996; Fieser and Fieser, 1949) and at low concentrations induce nausea and vomiting (Kaiser and Michl, 1958; Kelly and Smith, 1996). Reflex bleeding seems to be the mechanism of defense in adults (Blum and Sannasi, 1974; Kloft et al., 1975) and ingestion of a single *Photinus* can sometimes kill lizards (Knight et al., 1999). Lizards that are naturally sympatric with *Photinus*, such as those in the genera *Anolis*, *Sceloporus*, and *Eumeces*, are known to reject these fireflies prior to ingestion (Lloyd, 1973; Sexton, 1960, 1964; Sydow and Lloyd, 1975). Other predators such as mice and toads can learn to associate luminescence with a distasteful substance (De Cock and Matthysen, 1999; Underwood et al., 1997).

All known lampyrid larvae possess a photic organ of two luminescent spots on the eighth abdominal segment

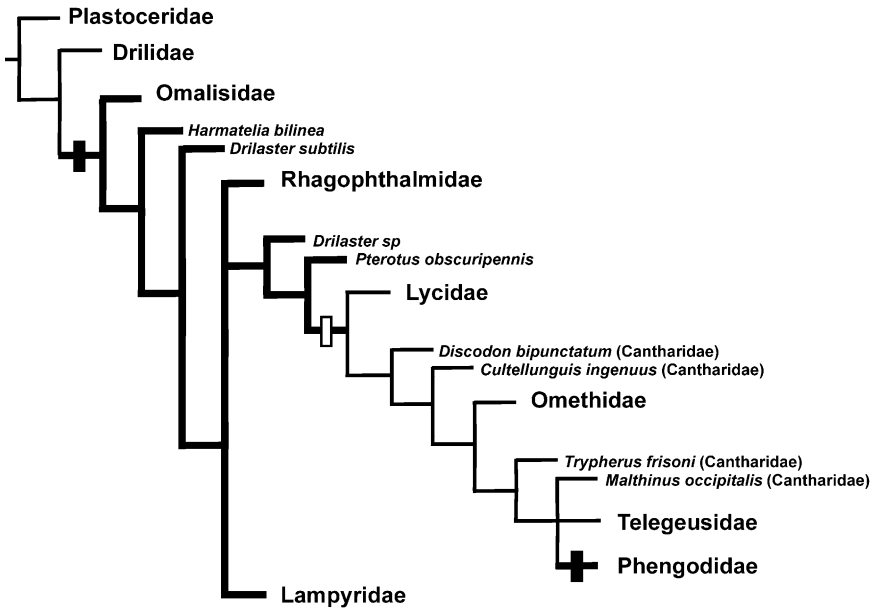

Fig. 3. The evolution of luminescence in cantharoids. A bold branch of the cladogram indicates the known occurrence of luminescence in at least one life stage of each species included in this analysis. Black tick marks indicate origins of bioluminescence, while the white tick mark indicates a loss.

(and sometimes others as well, e.g., segments 2–6 in *Lamprohiza*; Balduf, 1935; Buschman, 1988; Schwalb, 1960). All but one species have the same histological type of photic organ (Buck, 1948; cf. Bugnion, 1929). The larval photic organ appears to be homologous with paired photic organs on the eighth abdominal segment in adults because they are in the same location in the abdomen, have the same shape and same histology, and produce the same type of photic emissions—long continuous glows (Branham and Archangelsky, 2000; Buck, 1948; Hess, 1922; McDermott, 1964). In addition, the emission spectra of the light produced by both adults and larvae of *Photuris* are identical, with a maximum at 552.4 nm (Coblentz, 1912; McElroy and Seliger, 1966).

Larval photic organs persist throughout the pupal stage in some Phengodidae and Lampyridae (Viviani and Bechara, 1997; Costa et al., 1999; Crowson, 1972), sometimes into the adult in which they produce similar emissions (e.g., *Robopus*; Buck, 1942). In the case of *Pyropyga* (Branham and Archangelsky, unpublished) and *Lucidota atra* (Branham and Archangelsky, 2000) the paired "larval type" photic organs are present in the adults and are functional only for a short time after eclosion. Usually after 24 h following eclosion, and after the cuticle has hardened, the paired photic organs do not appear functional and either disappear completely or remain as twin spots on the abdominal cuticle (McDermott, 1964; M.A.B., personal observation). In the genus *Robopus* larval photic organs are functional in adult males and females, in which they are enlarged and produce sexual signals used in pair formation (Barber, 1941; McDermott, 1964; Farnworth, 1973).

The pattern of flightless females within Lampyridae

Paedomorphic females are found in a few firefly species, the drilid genus *Selasia*, the rhagophthalmid *Diplocladon*, and all known phengodid females, though less so in *Stenocladius* (Branham and Wenzel, 2001; Crowson, 1972; McDermott, 1964). Adult female Lampyridae can possess anything from only a few to an entire suite of larval characters. It is important to note that some authors have incorrectly identified brachypterous, apterous, or physogastric females as "larviform" when in fact they do not display larval morphology. Nonetheless, extreme cases of wing reduction or loss are associated with the possession of the two-spotted, larval-type, photic organs on the ventral surface of the adult females' eighth abdominal segment. All of the cantharoid taxa basal to Lampyridae have flightless females, with the single exception of *Drilaster subtilis* (Branham and Wenzel, 2001). The basal region of the lampyrid clade is composed of species with alate females. The larviform, brachypterous, apterous, or physogastric forms are scattered throughout the clade. It appears that these paedomorphic forms have arisen multiple times within the family. Green (1961) proposed that the phenomenon of brachyptery in *Pyropyga* might be associated with permanent moisture. Lloyd (1999) found varying amounts of female brachyptery in *Pyropyga nigricans* studied from multiple locations across the United States, with some occurrences of brachyptery found in several populations which seemed to be correlated with very restricted habitats around the edge of lakes or marshes. Lloyd suggested that these occurrences were perhaps due

to a "wing polymorphism." One population also showed brachyptery in both males and females (Lloyd, 1999). Losing wings and then regaining them has been argued as extremely improbable, but Andersen (1993), studying a similar adaptive phenomenon, has shown that the ancestor of a group of water striders was flightless or permanently dimorphic, with fully winged species arising repeatedly. We interpret that paedomorphic females occur throughout Cantharoidea, often expressing larval photic organs, but reversal to the typical winged form also occurs. Thus, it seems that larval photic organs can be heterochronically carried over into the adult stage.

Evolution of chemical signals vs visual signals

When long-range attraction of mates is achieved through chemical signals, we refer to these as "pheromones." Use of pheromonal sexual signals is generally restricted to the basal taxa (Fig. 4). This phylogenetic pattern predicts at least three origins of pheromones with at least two losses for the family Lampyridae. Four possible character optimizations exist for the gain and loss of pheromone use within the Lampyridae. These four schemes exist due to two equally parsimonious optimizations within each of the two clades that contain:

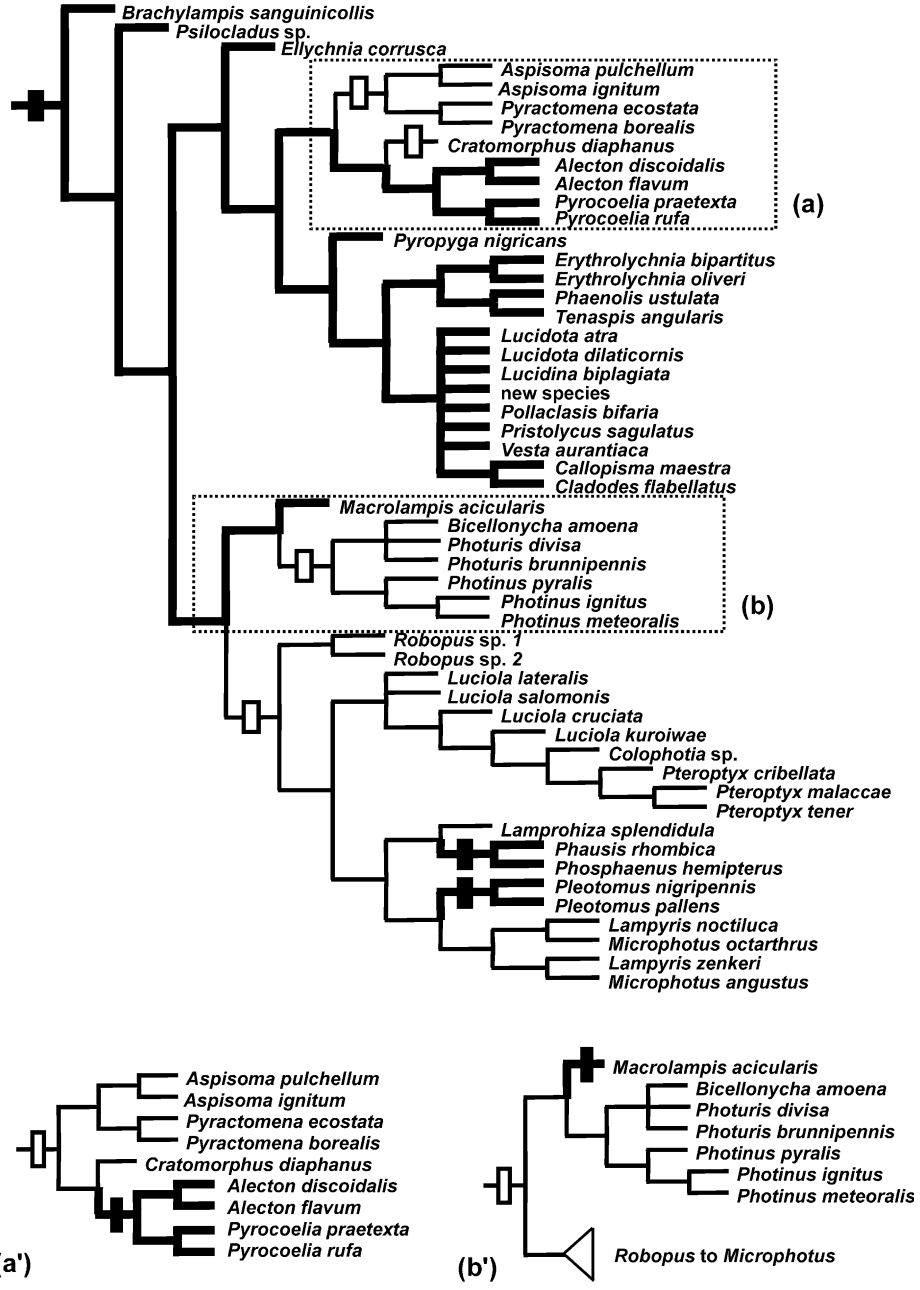

Fig. 4. The evolution of pheromone use in Lampyridae. Four possible optimizations of seven steps each exist for Lampyridae when combining the optimizations (a), (a′), (b), and (b′). The resulting topologies hypothesize from three to five origins and two to four losses of pheromones.

(a) *Aspisoma–Pyrocoelia* and (b) *Macrolampis–Microphotus*. The four possible combinations of optimizations in these clades present three to five origins and then two to four losses (Fig. 4). In all optimization schemes, pheromonal sexual signals were regained in the clade composed of *Phausis* and *Phosphaenus*, as well as in *Pleotomus* (Fig. 4). The basal lampyrid taxa (e.g., *Brachylampis* and *Psilocladus*) that use pheromones are also diurnally active, suggesting that ancestral fireflies were as adults diurnal and employed pheromonal signals for pair formation.

Species that rely on photic signals for sexual communication are generally restricted to the tips of the phylogeny, indicating that the use of photic signals in adults is derived for the family, as is nocturnal behavior. The use of bioluminescent sexual signals has evolved at least four times in the family with at least four losses (Fig. 5). One loss of photic signaling in the adult stage seems to have occurred in the genus *Alecton*, which is diurnal, is brightly colored, and uses pheromones to attract mates. The second loss occurred in the genus *Tenaspis*, in which the males of some species possess a two-spotted photic organ that appears to be nonfunctional on the eighth abdominal segment. The third loss is shown by *Macrolampis*, which contains both luminous and nonluminous species. *Phosphaenus hemipterus* was scored as the fourth loss. In this species, both sexes are diurnal, and while only the female appears to be luminous, the photic emissions do not appear to be used as sexual signals (De Cock, 2000). Therefore the hypothesized ancestor of *P. hemipterus* used photic sexual signals, most likely in conjunction with pheromones, and *P. hemipterus* seems to have subsequently reverted to a diurnal habit and sole use of pheromonal signals for pair formation. Treating pheromonal and photic systems as separate characters, it appears that the combined use of pheromones with photic signals can sometimes serve as a transition between using only pheromones to using only photic signals. Both of these signal modalities (chemical and photic) are present in nine taxa scattered

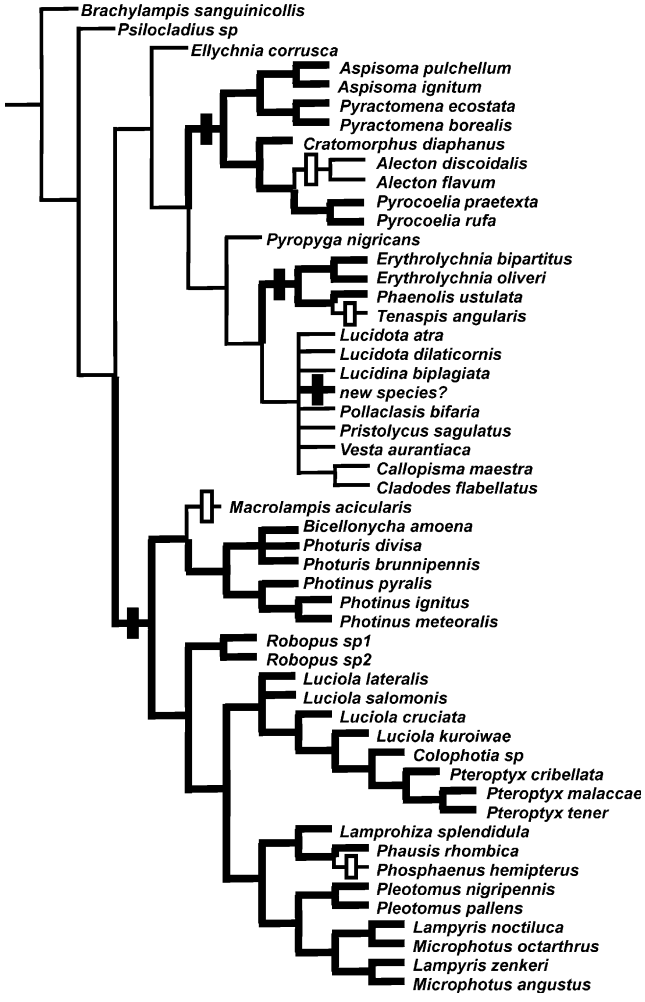

Fig. 5. The evolution of photic signals, produced by either sex, in Lampyridae is represented by a single optimization of four origins and four losses.

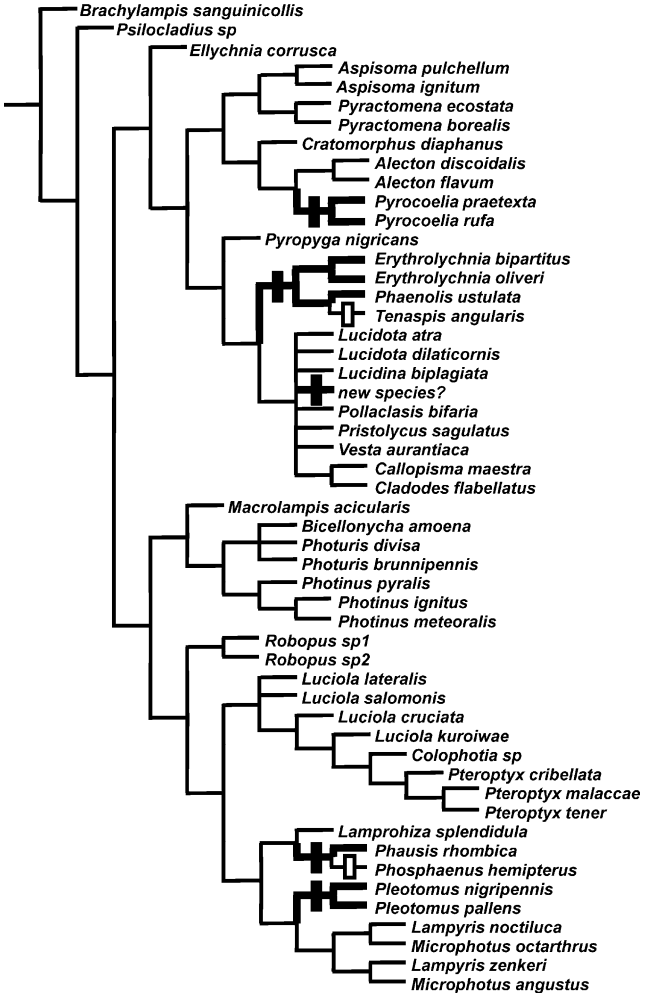

Fig. 6. The evolution of signaling systems that use a combination of both pheromonal and photic signals. Five possible origins and two losses are hypothesized.

across the family (in the order appearing in Fig. 6, *Pyrocoelia*, *Phaenolis*, *Erythrolychnia*, a new species, *Phausis*, and *Pleotomus*).

Evolution of photic signal systems

The context as well as the manner in which photic signals are produced play an important role in the type of signal system required to elicit courtship and pair formation. The photic signal systems of fireflies can be complex, with various aspects of some systems being either retained or eliminated in the communication systems of other species. Whereas two general types of photic signal systems were noticed by early firefly workers (Blair, 1915, 1924a,b; Gorham, 1880; Mast, 1912; McDermott, 1914), it was Lloyd (1971) who defined these two signal system types and posed a hypothetical evolutionary scheme for their evolution.

Lloyd (1971) used Signal System I to represent firefly species in which one sex, generally the female, is stationary and broadcasts a species-specific signal, to which the other sex is attracted. While not explicitly stated, Signal System I seems to be applied only to sedentary females producing species-specific *photic* emissions rather than *pheromonal* signaling alone. In contrast, Signal System II is represented in species in which one sex, usually the flying male, broadcasts a species-specific signal that is answered by a species-specific response produced by the opposite sex. The primary signaler is then attracted to the response. In addition, critically timed parameters are employed by some taxa using Signal System II. Lloyd (1971) was also aware of signal systems that used elements of both Signal Systems I and II, termed "Compound Signal Systems," such as those found in the synchronously flashing aggregations of several *Pteroptyx* species. Lloyd (1971) proposed a potential transitional system, with *Phausis reticulata* as an example, and placed it in a position that seemed intermediate between Signal System I and Signal System II. In this signal system, glowing females attract glowing males, and nonglowing females also will initiate glowing in response to glowing males. After studying the behavior of *Luciola obsoleta* (Olivier), Lloyd (1972b) realized that such a simple classification as Systems I and II was not adequate for the larger scenario of sexual signaling and proposed the use of the term "protocol" to replace "signal system" (Lloyd, 1978). As the old classification of signal systems (I + II) was unsatisfactory, Lloyd (1983) recommended that workers focus on the "key factors" of firefly mating protocols that "emerge from modern studies of sexual selection and ecology." Lloyd (1997) additionally hypothesized that Signal Systems I and II could have arisen multiple times. He pointed out various components that are shared between the four signal systems, or dropped from some, thereby highlighting the complexity of trying to define communication protocols. Of course, Lloyd's original definitions include multiple components, and such composite characters may disintegrate under a cladistic analysis of the components themselves. Signal Systems I and II are not supported by our analysis as forming an evolutionary series, System I did not give rise to System II through an intermediate stage, such as in *Phausis reticulata*, and both appear to have evolved more than once.

Signal system components

In order to address signal system evolution, components should be examined, rather than the complete or overall communication system, because some components may covary while others do not. Among basal taxa, the primary signaler is a sedentary female producing pheromonal signals (Fig. 7). Subsequently, sedentary signaling is used by species that produce both pheromonal and photic signals. The combined use of pheromones and photic signals in courtship appears to have evolved at least five times: once in *Pyrocoelia*, once in the common ancestor of *Erythrolychnia* and *Phaenolis*, once in a new species from Puerto Rico that cannot be placed with any currently described genus, once in *Phausis*, and once in *Pleotomus* (Figs. 6 and 7). Additionally, there seems to be a correlation between females functioning as the primary signaler when the primary signaler is sedentary (Fig. 7). In contrast, primary signalers that produce photic signals while in flight appear to be derived and strongly correlated with signaling males. This syndrome seems to have evolved at least four times in the family: once in the *Aspisoma–Pyractomena* clade, once in *Cratomorphus*, once in the *Bicellonycha–Photuris–Photinus* clade, and once in *Robopus* (Fig. 7).

Sedentary, synchronously flashing aggregations of fireflies

Luciola cruciata (Lloyd, 1979; Ohba, 1983, 1984) possesses a photic signaling system that seems to be intermediate between asynchronous flashing found in most flashing fireflies and the true synchrony found in *Pteroptyx cribellata*, *P. tener*, and *P. malaccae.* The males of *L. cruciata* emit synchronous flashes only while in flight. Sedentary females respond to these flashing males. Upon receiving a response, males break from the group synchrony and engage in a "male–female flash interaction," which ultimately leads to copulation (Ohba, 1984). Yajima (1978) determined that the female response to male signals did not involve critical timing parameters on the part of the male. *L. cruciata* shows little sedentary signaling during courtship, though when it occurs, it is after the male has landed near the female. Aggregations in this species occur only among females during oviposition. Females emit unique flashes at these aggregations and tend to attract other females to an oviposition site (Kuribayashi, 1980; Yuma, 1981). The lineage of *Luciola cruciata* is basal to *Pteroptyx* and may

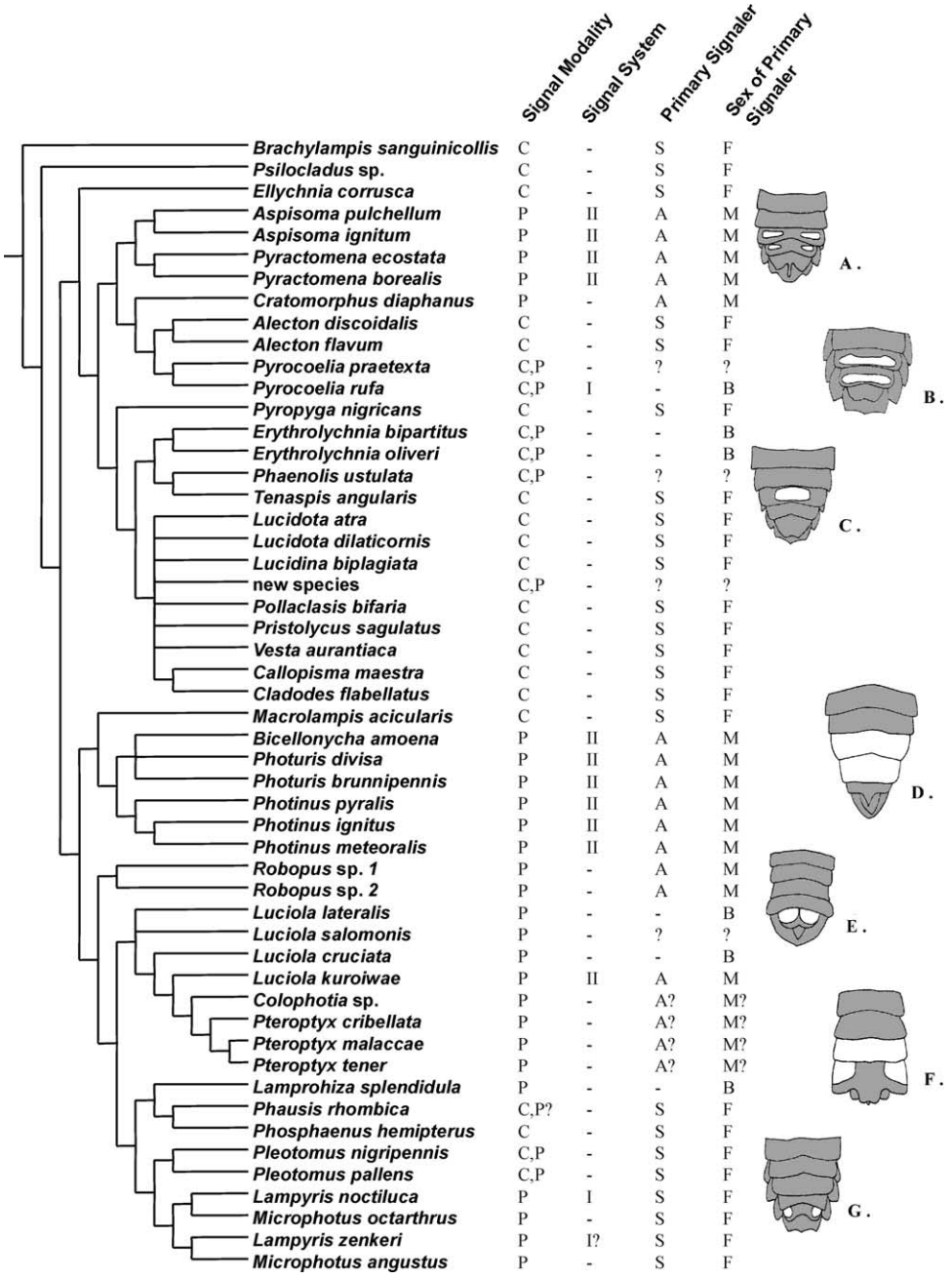

Fig. 7. The signal system modalities used, evolution of sexual signal systems, sedentary or active primary signalers, and the sex of the primary signaler. C, chemical signals; P, photic signals; I, Signal System I; II, Signal System II; S, sedentary primary signaler; A, active primary signaler; F, primary signaler is female; M, primary signaler is male. Representative adult male photic organ morphologies: (A) *Cratomorphus diaphanous*; (B) *Pyrocoelia rufa*; (C) *Erythrolychnia oliveri*; (D) *Bicellonycha amoena*; (E) *Robopus* sp. #2; (F) *Pteroptyx tener*; (G) *Pleotomus pallens*.

serve as an important model for how sedentary signaling, synchrony, and the aggregation of signaling individuals evolved as components in the sexual signal system of three *Pteroptyx* species, *P. cribellata*, *P. tener*, and *P. malaccae*.

Evolution of photic organs

No adult photic organs exist in (*Brachylampis* and *Psilocladius*) the basal lineages of Lampyridae. For lampyrids that use both pheromones and photic signals, only restricted regions of their fifth, sixth, and seventh ventrites (true abdominal segments VI, VII, and VIII) are used for photic emission in either males and females. Species that rely solely on photic signals for pair formation use most of the ventral surface of the fifth and sixth ventrite. The genus *Robopus*, however, retains larval-type photic organs into the adult stage—the two-spot condition on the seventh ventrite. These adults produce photic signals and are not known to use pheromones.

Luminescence in adult Lampyridae seems to have arisen in adult males and females at about the same time. Paedomorphic females have paired photic spots that seem identical to larval photic organs both in structure and in types of emission, i.e., intermittent glows (M.A. Branham, unpublished data). Males and females in many genera bear only the twin-spotted photic organ on the seventh ventrite that is either nonfunctional or capable of only a very faint glow (the bright glow of *Robopus* being an exception). This configuration is found throughout lampyrid phylogeny with the exception of the basal lineages, i.e., *Brachylampis*, *Psilocladus*, *Ellychnia*, etc., and is present in close to half of the taxa sampled for our analysis. This arrangement seems to have arisen eight times in the family Lampyridae, which is substantially more than the number of origins hypothesized for any other photic organ arrangement (Fig. 8). It seems possible that the family's frequent carryover of larval features into adults may play a role in the numerous independent origins of this morphology.

Photic organs on the seventh ventrite are functional in both adult sexes of taxa such as *Robopus*, *Pleotomus*, and *Microphotus*, but these organs appear not to be functional beyond teneral adults in taxa such as *Vesta*, *Pollaclasis*, *Tenaspis*, and *Lucidota atra.* Even though the two-spot condition is functional in males of both *Pleotomus* and *Microphotus*, it appears to have no role in courtship, though they glow quite visibly when distressed. It may also be noted that lampyrids are diurnal in taxa in which the two-spot photic organ morphology is nonfunctional. The only exception appears to be

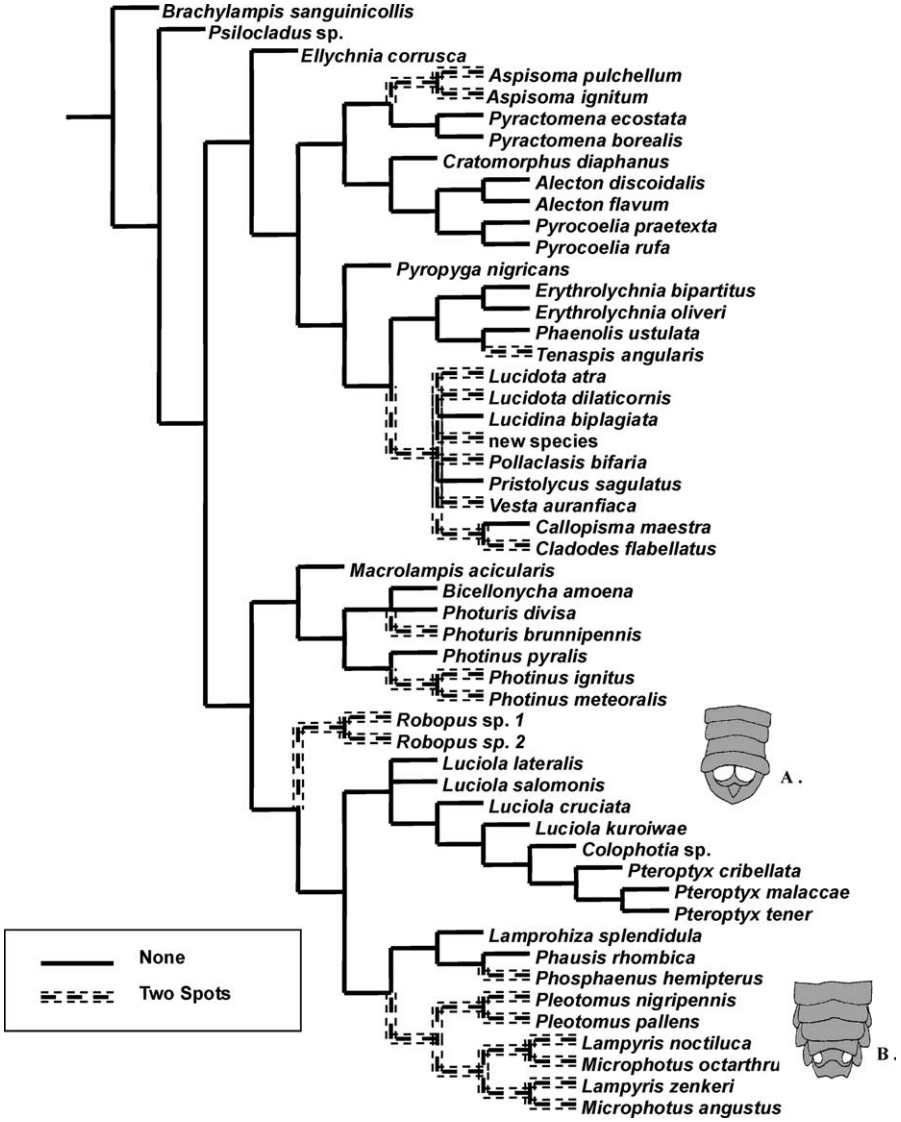

Fig. 8. Adult male photic organ evolution on abdominal ventrite seven (true abdominal segment VIII). Only one photic organ morphology, the two-spot condition, occurs in this abdominal segment in male fireflies. The occurrence of the two-spot condition (whether it appears to be functional or not) is indicated by the dashed branches of the cladogram. This photic organ morphology is widespread throughout the family and has evolved at least eight times. Representative adult male photic organ morphologies include (A) *Robopus* sp. #2; (B) *Pleotomus pallens*.

Phosphaenus hemipterus, which is diurnal and of which the female glows (in daylight) from a two-spotted photic organ when molested or attacked by predators (De Cock, 2000).

Only three types of photic organ configuration are known to occur on the male's sixth ventrite (two luminous spots, a luminous center strip, and the entire ventral surface of the sixth ventrite luminous), and all have evolved repeatedly. The two-spot morphology arose twice, once in *Cratomorphus* and once in *Pteroptyx*. The center strip morphology seems to have arisen three times: once each in the genera *Pyrocoelia*, *Luciola*, and *Lamprohiza*. The photic organ that covers the entire ventral surface of the ventrite appears to have arisen three times: once in the *Aspisoma–Pyractomena* clade, once in the *Bicellonycha–Photuris–Photinus* clade, and once in the *Luciola–Cholphotia–Pteroptyx* clade (Fig. 9).

The photic organs on male ventrite five show four configurations. The one-spot condition appears in *Erythrolychnia* and the two-spot condition appears in *Cratomorphus*. The center strip condition appears to have arisen twice, once in *Pyrocoelia* and once in *Lamprohiza*. The largest of all photic organs appear on ventrite five, of which the entire surface is luminous. This has three origins: once in the *Aspisoma–Pyractomena* clade, once in the *Bicellonycha–Photuris–Photinus* clade, and once in

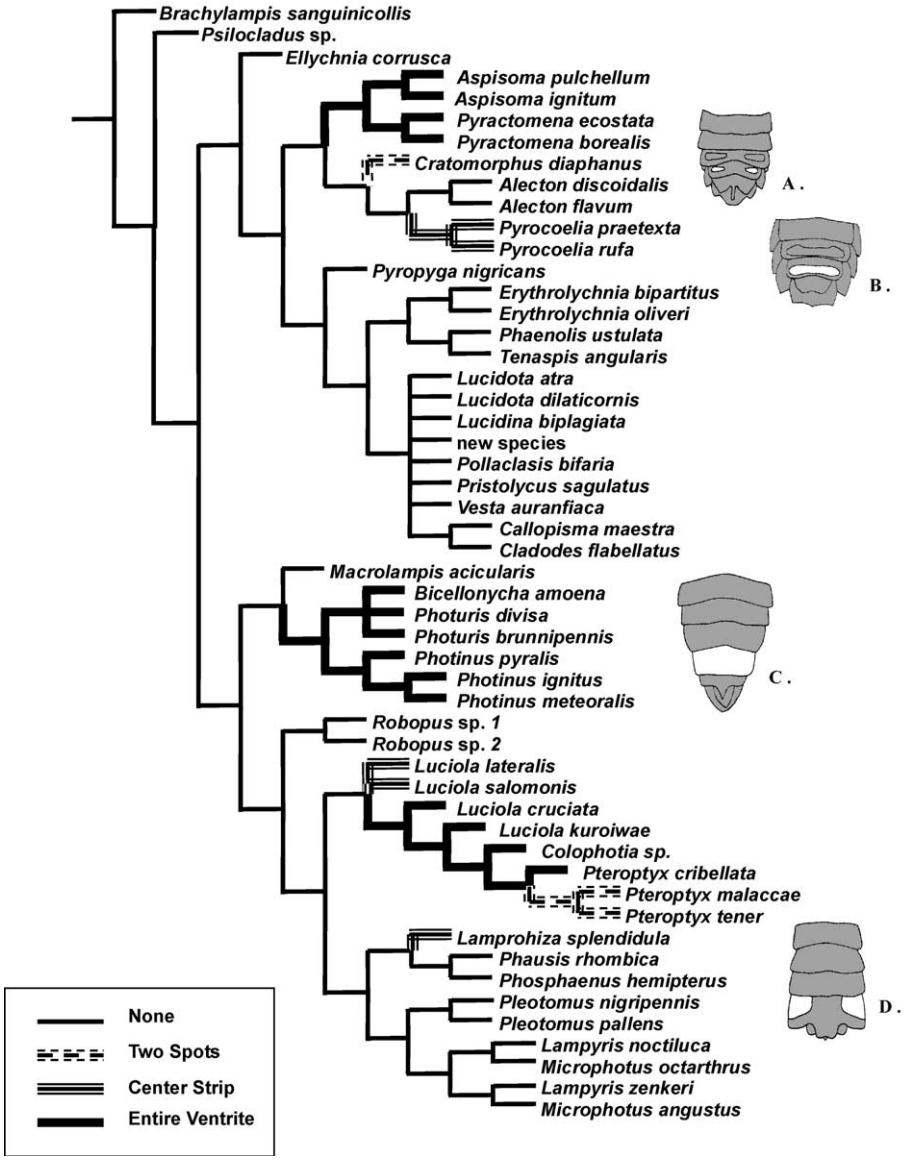

Fig. 9. Male photic organ evolution on abdominal ventrite six (true abdominal segment VII). Only three types of photic organ morphology appear in this abdominal segment, the two-spot, the center strip, and the entire ventrite photic organ morphology. Whenever these morphologies are present, they are functional. The two-spot photic condition appears to have evolved twice, while both the center strip and the entire ventrite photic organ morphology appear to have each evolved at least three times in the seventh abdominal segment of Lampyridae. Representative adult male photic organ morphologies: (A) *Cratomorphus diaphanous*; (B) *Pyrocoelia rufa*; (C) *Bicellonycha amoena*; (D) *Pteroptyx tener*.

the *Luciola–Colophotia–Pteroptyx* clade (Fig. 10). In addition, the presence of large photic organs covering the entire ventrite also is associated with species that produce flashed signals (M.A.B., personal observation) This is true of males in the three clades mentioned above, indicating that flashed signals evolved at least three times in the family Lampyridae (Fig. 10). Physiological modification of the male photic organs to support rapid flashing has already been demonstrated relative to the larval ground plan. In *Photuris pennsylvanica*, larval innervation of photic organs is direct and light emission is triggered slowly, whereas innervation in the derived, adult photic organs (anterior to the eighth abdominal segment) is indirect and about 100 times faster (Oertel et al., 1975). The larval glow lasts about 50 times as long as that of the adult, meaning that adults turn off faster as well. This is consistent with the concept that adult male photic organs are shaped by sexual selection, just as is known in acoustic signaling systems (Branham and Greenfield, 1996).

Sexual selection and phylogeny

This study confirms the common assumption that sexually selected characters may evolve convergently, but it refutes the suspicion that they are poor choices for interpreting phylogenetic history. Convergence is seen in the repeated evolution of large photic organs (Figs. 9

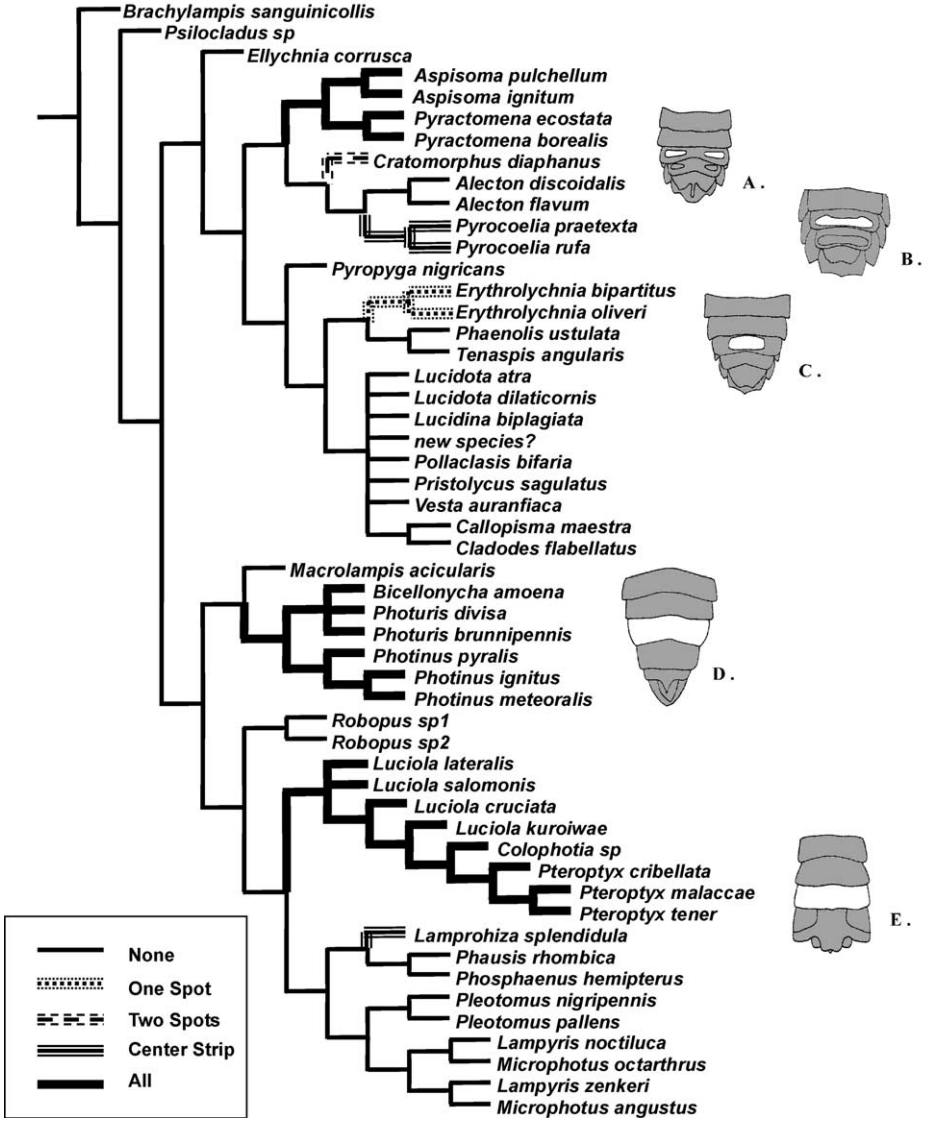

Fig. 10. Male photic organ evolution on abdominal ventrite five (true abdominal segment VI). Whenever these morphologies are present, they are functional. The one-spot, two-spot evolved once and the center strip photic organ morphology evolved twice in the family Lampyridae. The photic organ morphology, in which the photic organ covers all of the surface area of the ventrite, appears to have evolved at least three times in the sixth abdominal segment of Lampyridae. Representative adult male photic organ morphologies: (A) *Cratomorphus diaphanous*; (B) *Pyrocoelia rufa*; (C) *Erythrolychnia oliveri*; (D) *Bicellonycha amoena*; (E) *Pteroptyx tener*.

and 10), and of flashed signals (Signal System II; Fig. 7), and indeed the co-occurrence of these traits. Our demonstration of convergence is decisive because the characters of the photic organs were included in the analysis. Arguments for "total evidence" (Kluge and Wolf, 1993) usually rely on the concept of greater explanatory power. Our study examines directly the characters of concern (in the spirit of Wenzel, 1997), providing ample opportunity for homology of photic organs to be indicated rather than the convergence we found. Objections to including characters of interest usually rely on accusations of circularity, but no such complaint can be made here because we conclude *against* the proposal of a single origin. Thus, convergence is not merely a logical proposal (because male displays are under intense sexual selection to be visible from afar and easily recognized), but also empirically supported.

It is simpler to interpret the sexually selected characters than was formerly thought. Examining character suites and their levels of homoplasy indicates that sexually selected characters are comparable to other characters (Table 1). Both antennae (chemical communication) and photic organs (luminescent display) show CI of approximately 0.4 and RI of approximately 0.5, whereas general morphological characters show CI of 0.3 and RI of 0.6. In fact the lowest average CI and RI (0.1 and 0.4, respectively) are found among characters of wing venation, which are not associated with any special process we know of.

The homoplasy associated with sexually selected characters is often due to elaboration that can be distinguished during character coding, such as origin of photic organs on ventrite five, which is desirable. This analysis separates components of the signal systems as independent statements of homology, and separate derivations of similar endpoints can be seen in the final cladogram (Figs. 7–10). Rather than confusing the issue, the sexually selected characters provide structure in the cladogram because of state changes (see especially the *Aspisoma–Pyrocoelia* clade or the *Robopus–Microphotus* clade in Figs. 8–10) throughout the tree.

Table 1
A comparison of levels of homoplasy between various suites of characters

Character suite	Character No.	CI	RI
Antennae	2–18	0.44	0.53
Photic organs	60–63	0.38	0.52
Wing veins	64–73	0.12	0.40
General morphology	0, 1, 19–59	0.30	0.62

Note. The Antennae and Photic organ character suites are associated with sexual signaling and, therefore, may be evolving via sexual selection. The Wing vein characters represents a suite that is more convergent (hence, lower CI) than either characters associated with sexual signaling or general morphological characters.

Conclusions

The origin of bioluminescence occurred early in the evolutionary history of cantharoids, preceding the origin of the family Lampyridae. Cladistic analysis indicates that luminescence first appeared in larvae, probably as an aposematic warning display, and is still found in many cantharoid larvae today. It appears as though luminescence in adults is a carryover from larvae. Luminescence in the adult appears to function as an aposematic warning display, which has been co-opted in many species to serve also as a sexual signal used in courtship.

The evolution of firefly signals is accompanied by a change from diurnal to nocturnal mating behavior. The overall trend is the use of pheromones in basal species, then pheromones used in conjunction with photic signals, then the sole use of photic signals (Branham and Wenzel, 2000). Signal systems that employ only luminescence usually involve flashed signals, perhaps using critically timed parameters, rather than glows. Not only do some components of the signal systems appear to be convergent, but so does photic organ morphology. In addition, several character associations have evolved repeatedly across the family. First, primary signalers that are sedentary during signaling are almost always females. Female primary signalers that belong to taxa appearing basal in the family, such as *Brachylampis* and *Psilocladus*, employ only pheromonal signals; those in more derived taxa, such as *Lampyris* and *Microphotus*, employ only photic signals. Second, species that possess large, complex photic organs have adult males who are primary signalers. This relationship may reflect intense sexual selection.

By comparing individual components, we can recognize and better interpret adaptive correlations despite convergence or loss. We demonstrate that convergently evolving traits can be investigated phylogenetically and in fact are best illuminated by cladistic analysis.

Acknowledgments

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Appendix A. List of taxa used in the analysis

Sources of material

The material studied for this article was borrowed from the following institutions: California Academy of Sciences, San Francisco (CASC); Field Museum of Natural History, Chicago (FMNH); Florida State Collection of Arthropods, Gainesville (FSCA); collection of one of the authors (MABC); Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (MCZC); Ohio State University, Columbus (OSUC); Snow Entomological Museum, University of Kansas, Lawrence (SEMC); Museum of Zoology, University of Michigan, Ann Arbor (UMMZ); and National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM).

List of species studied

The higher classification used here is based on Lawrence and Newton (1995) and, in the case of Rhagophthalmidae, Wittmer and Ohba (1994).

Plastoceridae
- *Plastocerus* (= *Ceroplatus*) *angulosus* (Germar) [FMNH]

Omalisidae
- *Omalisus* (= *Omalysus*, *Homalisus*) *fontisbellaguei* (Fourar) 1785 [FMNH]
- *O. sanguinipennis* Cast. 1840 [FMNH]

Drilidae
- *Drilus concolor* Ahr. 1812 [FMNH]
- *D. flavescens* G.A. Olivier 1790 [USNM]
- *Selasia* sp. [FMNH]

Omethidae
- Matheteinae
  - *Matheteus theveneti* LeConte 1874 [CASC]
  - *Ginglymocladus luteicollis* Van Dyke 1918 [CASC]
- Omethinae
  - *Omethes marginatus* LeConte 1861 [CASC]
  - *Blatchleya gracilis* Blatchley 1910 [OSUC]

Phengodidae
- Phengodinae
  - *Cenophengus pallidus* Schaeffer 1904 [FSCA]
  - *Phrixothrix reducticornis* Wittmer 1963 [UMMZ]
  - *Pseudophengodes pulchella* Guer 1843 [USNM]
  - *Zarhipis integripennis* LeConte 1874 [MABC], [UMMZ]
- Rhagophthalminae
  - *Dioptoma adamsi* Pascoe 1860 [USNM]
  - *Diplocladon* sp. [CASC]

Rhagophthalmidae
- *Rhagophthalmus ohbai* Wittmer and Ohba 1994 [MABC]
- *Rhagophthalmus* sp. [SEMC], [CASC]

Telegusidae
- *Pseudotelegeusis* sp. [SEMC]
- *Telegeusis nubifer* Martin 1931 [SEMC]

Lycidae
- Calochrominae
  - *Calochromus perfacetus* (Say) 1825 [OSUC]
- Lycinae
  - *Calopteron discrepans* (Newman) 1838 [OSUC]
  - *Celetes basalis* LeConte 1851 [OSUC]
- Erotinae
  - *Dictyoptera aurora* (Herbst) 1789 [OSUC]

Cantharidae
- Cantharinae
  - *Cultellunguis ingenuus* LeConte 1881 [OSUC]
- Silinae
  - *Discodon bipunctatum* Schaeffer 1908 [OSUC]
- Malthininae
  - *Malthinus occipitalis* LeConte 1851 [OSUC]
- Chauliognathinae
  - *Trypherus frisoni* Fender 1960 [OSUC]

Lampyridae
- Pterotinae
  - *Pterotus obscuripennis* LeConte 1859 [UMMZ]
- Cyphonocerinae
  - *Pollaclasis bifaria* (Say) 1835 [MCZC], [FSCA]
- Ototretinae
  - *Brachylampis sanguinicollis* Van Dyke 1939 [CASC]
  - *Drilaster subtilis* (E. Olivier) 1908 [CASC]
  - *Driliaster* sp. [MABC]
  - *Harmatelia bilinea* Walker 1858 [CASC]
  - *Stenocladius* sp. [MABC]
- Amydetinae
  - *Cladodes flabellatus* Solier 1849 [CASC]
  - *Psilocladus* sp. [MABC]
  - *Vesta aurantiaca* E. Olivier 1886 [USNM]
- Lampyrinae
  - *Alecton discoidalis* Laporte 1833 [MCZC]
  - *A. flavum* Leng and Mutchler 1922 [MCZC]
  - *Aspisoma ignitum* (Linnaeus) 1767 [OSUC]
  - *A. pulchellum* (Gorham) 1880 [FSCA]
  - *Callopisma maestra* Mutchler 1923 [CASC]
  - *Cratomorphus diaphanus* (Germar) 1824 [USNM]
  - *Ellychnia corrusca* (Linnaeus) 1767 [MABC]
  - *Erythrolychnia bipartitus* (E. Olivier) 1912 [FSCA]
  - *E. olivieri* Leng and Mutchler 1922 [FSCA]
  - *Lamprohiza splendidula* (Linnaeus) 1767 [CASC]
  - *Lampyris noctiluca* Linnaeus 1767 [CASC], [FSCA]
  - *L. zenkeri* Germar 1817 [FMNH]
  - *Lucidina biplagiata* (Motschulsky) 1866 [MABC]
  - *Lucidota atra* (G.A. Olivier) 1790 [MABC]
  - *L. dilaticornis* (Motschulsky) 1854 [FMNH]
  - *Macrolampis acicularis* (E. Olivier) 1907 [CASC]
  - *Microphotus angustus* LeConte 1874 [FMNH]
  - *M. octarthrus* Fall 1912 [MABC]
    - New species [MABC]
  - *Phaenolis ustulata* Gorham 1880 [FSCA]
  - *Phausis rhombica* Fender 1962 [MABC]

Photinus ignitus Fall 1927 [OSUC]
P. meteoralis (Gorham) 1881 [CASC]
P. pyralis (Linnaeus) 1767 [MABC]
Phosphaenus hemipterus (Fourcroy) 1785 [CASC]
Pleotomus nigripennis LeConte 1885
P. pallens LeConte 1866 [MABC]
Pristolycus sagulatus Gorham 1883 [CASC]
Pyractomena ecostata (LeConte) 1878 [FSCA], [UMMZ??]
P. borealis (Randall) 1828 [MABC]
Pyrocoelia praetexta E. Olivier 1911 [MABC]
P. rufa E. Olivier 1886 [MABC]
Pyropyga nigricans (Say) 1823 [MABC], [CASC]
Robopus sp. #1[MABC]
Robopus sp. #2[MABC]
Tenaspis angularis (Gorham) 1880 [CASC], [MCZC]

Luciolinae
Colophotia sp. [MABC]
Luciola cruciata Motschulsky 1854 [MABC]
L. kuroiwae Matsumura 1918 [MABC]
L. lateralis Motschulsky 1860 [CASC]
L. salomonis (E. Olivier) 1911 [CASC]
Pteroptyx cribellata (E. Olivier) 1891 [MABC], [UMMZ]
P. malaccae (Gorham) 1880 [MABC]
P. tener E. Olivier 1907 [MABC]

Photurinae
Bicellonycha amoena Gorham 1880 [FSCA]
Photuris brunnipennis Jacq.-Duv. 1856 [OSUC]
P. divisa LeConte 1852 [MABC]

Appendix B. Characters and character states

Multistate characters treated as ordered are specified below. Values for CI and RI for each character in the analysis as they appear on the consensus tree are indicated after the last character state (CI, RI). The character-taxon matrix is presented in Appendix C. The morphological terminology of Lawrence and Britton (1991) and Snodgrass (1993) was used. Wing venation scheme follows that used in Kukalova-Peck and Lawrence (1993).

0. *Head position*: 0—exposed; 1—partially exposed; 2—covered (CI 0.18, RI 0.72).
1. *Head shape*: 0—deflexed between eyes; 1—partially deflexed; 2—not deflexed (CI 0.8, RI 0.52).
2. *Antennal insertions* (ordered): 0—widely separated; 1—moderately approximate; 2—approximate (CI 0.8, RI 0.61).
3. *Antennal sockets*: 0—prominent; 1—flush (CI 0.5, RI 0.33).
4. *Number segments (antennomeres) in male antennae* (ordered): 0—8; 1—10; 2—11; 3—12; 4—13 (CI 0.44, RI 0.50).
5. *Antennal segment 3 (flagellomere 1)* (ordered): 0—short, 1—same as segment 4; 2—long (CI 0.7, RI 0.52).
6. *Antennal features (general)*: 0—filiform; 1—serrate; 2—flabellate; 3—pectinate; 4—bipectinate (CI 0.25, RI 0.63).
7. *Distal antennal flagellomeres* (ordered): 0—longer than wide; 1—about as long as wide; 2—much wider than long (CI 0.14, RI 0.29).
8. *Basal antennal flagellomere(s)*: 0—not symmetrical with apical flagellomeres; 1—symmetrical with apical flagellomeres (CI 0.14, RI 0.53).
9. *Distal margins of flagellomeres*: 0—straight; 1—concave (CI 0.11, RI 0.46).
10. *Distal margin of antennal flagellomeres*: 0—approximating proximal margin in width; 2—wider than proximal margin (CI 0.7, RI 0.38).
11. *Antennal flagellomere 2* (ordered): 0—not compressed; 1—slightly compressed; 2—greatly compressed (CI 0.8, RI 0.60).
12. *Lateral margins of the distal antennal flagellomeres*: 0—parallel; 1—nonparallel (CI 0.5, RI 0.52).
13. *Antennal lobes produced from* (ordered): 0—basal region of flagellomere; 1—medial region of flagellomere; 2—apical region of flagellomere (CI 0.25, RI 0.62).
14. *Number of elongated antennal lobes per segment*: 0—one lobe; 1—two lobes (CI 0.25, RI 0.62).
15. *Antennal lobes*: 0—compressed; 1—not compressed (CI 0.20, RI 0.33).
16. *Length of antennal lobes* (ordered): 0—less than length of flagellomere; 1—approximating length of flagellomere; 2—greater than length of flagellomere (CI 0.50, RI 0.66).
17. *Antennal lobe/flagellomere juncture*: 0—broad; 1—narrow (CI 0.16, RI 0.44).
18. *Antennal lobes*: 0—not bearing a sensory depression at apex; 1—bearing a sensory depression at apex (CI 1.0, RI 1.0).
19. *Mandibles* (ordered): 0—prominent; 1—normal sized; 2—reduced; 3—very reduced (CI 0.14, RI 0.68).
20. *Mandible tooth*: 0—absent; 1—present (CI 1.0, RI 1.0).
21. *Mandible width*: 0—stout; 1—slender (CI 0.12, RI 0.68).
22. *Mandible shape*: 0—apices acute (inside angle $< 90°$); 1—apices nonacute (inside angle near 180°) (CI 0.5, RI 0.56).
23. *Mandible type*: 0—normal type (arcuate, regularly narrowing to tips); 1—specialized type (tips slender and glabrous with discontinuous curvature) (CI 0.25, RI 0.84).
24. *Hypomera*: 0—not extending to anterior edge of pronotal shield; 1—narrowly extending to anterior edge of pronotal shield; 2—broadly extending to anterior edge of pronotal shield; 3—lacking (CI 0.30, RI 0.82).
25. *Hypomera space around head (side view)*: 0—head (eyes) not able to retract between hypomera;

1—head (eyes) partially enclosed (up to half width of eyes); 2—head (eyes) retractable (less than half eye width exposed) (CI 0.11, RI 0.65).
26. *Maxillary palpi*: 0—filiform; 1—clavate compressed; 2—clavate; 3—modified (CI 0.12, RI 0.57).
27. *Maxillary palp apical segment*: 0—filiform; 1—securiform; 2—elongate; 3—greatly elongate and flattened; 4—conical (CI 0.4, RI 0.5).
28. *Labial palpi*: 0—filiform; 1—clavate compressed; 2—clavate; 3—modified (CI 0.16, RI 0.44).
29. *Labial palp apical segment*: 0—filiform; 1—securiform; 2—elongate; 3—greatly elongate and flattened (CI 0.20, RI 0.52).
30. *Eyes*: 0—oval; 1—emarginate (CI 1.0, RI 1.0).
31. *Eyes posteroventrally* (ordered): 0—separated; 1—approximate; 2—contiguous (CI 0.25, RI 0.66).
32. *Pronotum border*: 0—smooth; 1—margined; 2—explanate (CI 0.22, RI 0.46).
33. *Hind angles of pronotum*: 0—truncate (juncture between lateral and hind margin 90°); 1—acute (juncture $< 90°$); 2—laterally expanded (juncture $> 90°$); 3—notched (juncture $< 90°$ due to deep notch in hind margin) (CI 0.13, RI 0.58).
34. *Overall surface area of hypomeron* (ordered): 0—absent; 1—small; 2—large/broad (CI 0.22, RI 0.58).
35. *Scutellum shape*: 0—distinct; 1—poorly developed (CI 0.25, RI 0.40).
36. *Scutellum*: 0—membranous, 1—sclerotized (CI 1.0, RI 1.0).
37. *Prosternum* (ordered): 0—small; 1—medium; 2—large (CI 0.22, RI 0.53).
38. *Mesosternum (anterior margin)*: 0—straight; 1—emarginate (CI 0.50, RI 0.75).
39. *Mesal margins of metepisterna*: 0—sigmoid; 1—straight or nearly so (CI 0.33, RI 0.83).
40. *Anterior coxae* (ordered): 0—contiguous; 1—nearly contiguous; 2—separate at base (CI 0.33, RI 0.42).
41. *Anterior coxal shape*: 0—conical; 1—subconical; 2—triangular; 3—broad; 4—bulbous (CI 0.19, RI 0.54).
42. *Middle coxae* (ordered): 0—contiguous; 1—nearly contiguous; 2—separate (CI 0.22, RI 0.63).
43. *Hind coxae* (ordered): 0—contiguous; 1—nearly contiguous; 2—separate (CI 0.09, RI 0.33).
44. *Hind coxae/femoral plates* (ordered): 0—plates obsolete; 1—less than length of coxae; 2—entire length of coxae (CI 0.16, RI 0.74).
45. *Trochanter attachment to femora*: 0—oblique; 1—very oblique; 2—interstitial (CI 0.22, RI 0.82).
46. *Middle trochantins*: 0—setiferous; 1—glabrous (CI 0.10, RI 0.47).
47. *Femora*: 0—slender; 1—normal; 2—flattened; 3—swollen (CI 0.22, RI 0.56).
48. *Tibiae*: 0—slender; 1—normal; 2—flattened; 3—swollen (CI 0.14, RI 0.50).
49. *Tibial spurs* (ordered): 0—absent; 1—small; 2—well developed (CI 0.06, RI 0.42).
50. *Hind tarsal segment 1*: 0—normal; 1—elongate (CI 0.22, RI 0.53).
51. *Tarsal segment 3*: 0—simple; 1—lobed beneath (CI 0.25, RI 0.66).
52. *Tarsal segment 4*: 0—simple; 1—lobed beneath (CI 0.25, RI 0.0).
53. *Claws*: 0—simple; 1—cleft (CI 0.50, RI 0.66).
54. *Male elytra* (ordered): 0—fully covering abdomen; 1—somewhat reduced; 2—greatly reduced (CI 0.28, RI 0.54).
55. *Elytral surface*: 0—slight punctures with no costae; 1—slight punctures with longitudinal costae; 2—deep window-shaped punctures with longitudinal costae; 3—coarse punctures with no costae; 4—slightly coarse punctures with longitudinal costae (CI 0.26, RI 0.54).
56. *Elytral epipleural fold* (ordered): 0—absent; 1—narrow; 2—broad at base (CI 0.22, RI 0.84).
57. *Abdominal ventrite number (including pygidium)* (ordered): 0—6 visible; 1—7 visible; 2—8 visible (CI 0.20, RI 0.72).
58. *Male ninth abdominal tergite*: 0—not emarginate behind; 1—emarginate behind (CI 0.11, RI 0.75).
59. *Setae on claws*: 0—absent; 1—present (CI 0.50, RI 0.85).
60. *Abdominal segment 6, shape of photic organ(s)*: 0—two spots; 1—one spot; 2—all; 3—center strip; 4—none (CI 0.57, RI 0.85).
61. *Abdominal segment 7, shape of photic organ(s)*: 0—two spots; 2—strip; 3—all; 4—none (CI 0.37, RI 0.73).
62. *Abdominal segment 8, photic organ(s)*: 0—absent; 1—present (CI 0.08, RI 0.47).
63. *Paired photic organs on segments 1–7*: 0—absent; 1—present (CI 0.50, RI 0.0).
64. *Wing vein r3*: 0—absent; 1—present (CI 0.08, RI 0.26).
65. *Wing vein r4* (ordered): 0—absent; 1—partial; 2—complete (CI 0.09, RI 0.42).
66. *Wing radial cell*: 0—open; 1—closed; 2—not present (CI 0.13, RI 0.23).
67. *Wing vein MP3*: 0—contacting MP1+2; 1—not contacting MP1+2 (CI 0.07, RI 0.27).
68. *Wing first cubitoanal cell*: 0—absent; 1—present (CI 0.16, RI 0.54).
69. *Wing second cubitoanal cell*: 0—absent; 1—present (CI 0.05, RI 0.55).
70. *Wing CuA1(cross vein)*: 0—absent; 1—partial; 2—complete (CI 0.11, RI 0.30).
71. *Wing CuA1 vein intersecting MP vein*: 0—above fork (MP3a and MP3b); 1—at fork 2–below fork; 3—other (no fork present) (CI 0.16, RI 0.21).
72. *Wing CuA2(cross vein)*: 0—absent; 1—partial; 2—complete (CI 0.23, RI 0.67).
73. *Wing AA3+4 vein*: 0—absent; 1—present (CI 0.08, RI 0.57).

Appendix C. Morphological character matrix

Character Number (10)	1	2	3	4	5	6	7
Character Number	0123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123						
Plastocerus angulosus	02002130101012002100?000100000001220120114202111111000031 1-143000211112221
Drilus flavescens	10002230001112002000001010000000222011103102111100110001201430010 0-100-00
Drilus concolor	1200221201111------000001000000022201111011021121000100022?14300100-000-00
Selasia sp.	11012221000001002100?000100100001220111101102101100010002201430010 0-000-00
Omalisus fontisbellaguei	1210200010101------1?0101001010012201211011211220101003 11-043001111112031
Omalisus sanguinipennis	1210200010101------1?0101021210012201211011211220101003 11-043001210102000
Harmatelia bilinea	11002030101002002100001010010100122011102000201210101001120 0---10211112221
Drilaster subtilis	1201200010010------1001010210100222010110100210221101001120043?01210112221
Dioptoma adamsi	1211321200110------0010011011111222010110200211220101000120 0---11211112321
Diplocladon sp.	1201321100011201101001001104001122101011000001022210100112004300021102201
Rhagophthalmus sp.	1011321000010201001001001104001222201011000021022210100122004300020111222 0
Rhagophthalmus ohba	1011321000011201001001001004001122201011101121022101100122004300121111222 0
Drilaster sp.	1201201010121------0011001210100222010110111211202101001120043001110112221
Pterotus obscuripennis	1200223010000100200001001100000021201 0?1011121122210100112004300121010220 1
Calochromus perfacetus	2201211010111------0011010010100211110010321021222011001020043000001100-01
Dictyoptera aurora	2220201000111------0011010212100212010011321020222011002020043001100102101
Calopteron discrepans	2220201010121------1010010111100211110012321020221011002020043001210102001
Celetes basalis	1220201011121------1010010212100212110012322020220011002020043000111100-00
Discodon bipunctatum	1201210010110------0011000010100212010100100011111010010 2-043001210102001
Cultellunguis ingenuus	0200210010100------0011010010100001010100002010112101001 02-043001210100-00
Omethes marginatus	0200200010101------0110010010100231010100200210112111000 01-043001110012200
Blatchleya gracilis	1101200000101------0101010000000231010110000010000111100001?043001110102201
Ginglymocladus luteicollis	1100203000000200110110110110100131010100200210202111001 01-043001110112221
Matheteus theveneti	2100203000010100100010100001010023211010020001000021110011004300121011222 1
Trypherus frisoni	0111200010100------0010030010100131010100101010111101010 02-043000010100-00
Malthinus occipitalis	0201200010101------1010010000000131010100001010111101013 02-043000100100-00
Pseudotelegeusis sp.	0200211010101101100001002002000013010 0?000000101011?? 01002004300003-000-00
Telegeusis nubifer	0101200010101------0?1002033300013010010000001010210101002004300000-000-00
Cenophengus pallisus	0221304001101010210001002001010000001110000001010010102002004300111000 0-00
Phrixothrix reducticornis	0200314001000010210000101000000010201010200101011210002002004300023110230 0
Pseudophengodes pulchella	0200205010000011210001001000000013101 0?000000102000010100200431001001 00-00
Stenocladius sp.	1211203010000000210100101100000000101011002111000010000012004300013010 0-00
Zarhipis integripennis	0200304001001011210001001000000021101010020001011010101112043001210112221
Brachylampis sanguinicollis	2200210010110------0011012210000222010110200201221101000220043001110102021
Psilocladus sp.	2211204010000011210101101121000021201011020020022010100112004300121011202 1
Ellychnia corrusca	2211211010110------1001001111100212010110200200221101001221043001210111021
Pyractomena ecostata	2211210010110------2011102111100202010110200201220001001221122001210112221
Pyractomena borealis	2111210010110------2011102111100202010110200201221001001221122001210112221
Aspisoma pulchellum	2121210010010------2011101211100212010110200100221001001220122101210102221
Aspisoma ignitum	2211210010110------2010102111100212011110200200221001001220122101210102021
Cratomorphus diaphanus	2021211010110------20111021 0?10121201011021120022110100122100000121011222 1
Pyrocoelia praetexta	2121201011121------2011102111100212010110401200220101001221031001211112221
Pyrocoelia rufa	2021211111121------2011101111100212010110401200220101001221031001211112221
Alecton discoidalis	2121211210101------2010102111100212010110101200221101001220043001210112221
Alecton flavum	2121211210111------2010102111100212010110201200221101001220043001210112221
Pyropyga nigricans	2201211010120------1010002111100212010110200200221100001221043001210112221
Phaenolis ustulata	2221204010120210200001110211010020201111010120022010100121104300120010202 1
Tenaspis angularis	2211201011121------1011102210100212010110100200221001001211043101210112021
Erythrolychnia bipartitus	2021211010121------1010102212100202010110200200221101001211013001210012220
Erythrolychnia oliveri	2021201010121------1010102112100202010110200200221101001211013001211112220
Lucidota atra	2121201011121------1010002210100212010110200100220101001221043101210112221
Lucidota dilaticornis	2121201011121------0010001210100212010110201200221101001220043101201112221
Lucidina biplagiata	2211201011121------0010001212100212010110400200220101001221043001100112221
New species	2221211011121------0010001112100212010110200200220101000220043101210112221
Pollaclasis bifaria	2111214011101010210001001201010021201011020121021010100122104310021011222 1
Pristolycus sagulatus	2211201011121------2010012112100221010110301210220101004101043001011112221
Vesta aurantiaca	2221?01011121------0010001110100222010110200200221101001221043101111112021
Callopisma maestra	2220201010120------0011001212100212010110200200220101001221043001210100-01
Cladodes flabellatus	2221202011021200210001100221210021201011020120022010100122104310121011 0-21
Macrolampis acicularis	2011210010110------0001001112100202010110201200221101001220043001210112021
Photinus pyralis	2111210010020------0001001210100202010110100200222101001220022001210101011
Photinus ignitus	2101220010010------1001001110100212010110100200221101001221022101210112121
Photinus meteoralis	2201210010020------1001001211100212011110101200222101001221022101210112121
Bicellonycha amoena	2021200010100------0001001110100222010110200210222101101220022001110101321
Photuris divisa	1020200010100------0000000112100212010110200210222101100220022001210110-20
Photuris brunnipennis	2021200010100------0001000010100212010110200210222101101220022101210102201
Robopus sp1	2121210010020------1011001110100202010110200200220101001220043101210110-11
Robopus sp2	2121210010020------0011001110100202010110200200220101001220043101210111221
Luciola lateralis	2011210010111------1011011010101222010110200201211101001200021-01210112220
Luciola salomonis	2021220010111------1011011210101212010110200200211101000200021-01210112221
Luciola cruciata	1220210010111------0001011112101222010110200210220101001200022-01211112221
Luciola kuroiwae	1021220110101------1011011112101202010110200 20?220101001200022-01211002200
Colophotia sp.	2111200010000------1000011112100232010110200211110101001201022-00211000-00
Pteroptyx cribellata	1001220010100------0010011110100232010110200200220101001201022-01211002100
Pteroptyx malaccae	1010210010111------0010011110100232010110200210220101000201020-01210000-00

Appendix C. (*continued*)

Character Number (10)	1	2	3	4	5	6	7
Character Number	0123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123						
Pteroptyx tener	1011210010101------0010011110100232010110200210110101001201020-01210002200						
Lamprohiza splendidula	2021210010101------2011001010101212010110200201222101001211031001100102201						
Phausis rhombica	2110210110010------1010102210100212010110400201220101001221043 00-100101001						
Phosphaenus hemipterus	2020220110100------101000221??00202010?020120122000102022104310----------						
Pleotomus higripennis	2021402010100210200101110211010121201111020020022110100121104310121011 2221						
Pleotomus pallens	2021402010100210200101110221010121201011010120022100100122104310121011 2221						
Lampyris zenkeri	2021200010110------3011101110102212011110200100221101001221043101000110-21						
Microphotus angustus	2021110210100------301?101210102212011110200100220101001221043101001102020						
Lampyris noctiluca	2021210110110------301?101212101202010110101100221101001221043101210112221						
Microphotus octarthrus	2021020210100------301?101210002202010110101100220101011221043100210112221						

References

Andersen, N.M., 1993. The evolution of wing polymorphism in water striders (Gerridae): a phylogenetic approach. Oikos 67, 433–443.

Andersson, M.B., 1994. Sexual Selection. Princeton University Press, Princeton, NJ.

Archangelsky, M., Branham, M.A., 2001. Description of the last instar and pupa of *Pyropyga nigricans* (Coleoptera: Lampyridae: Photinini), and comparison with larvae of other Photinini genera. Can. Entomol. 133, 1–10.

Balduf, W.V., 1935. The Bionomics of Entomophagous Coleoptera. Swift, St. Louis.

Ballentyne, L.A., 1992. Revisional studies on flashing fireflies (Coleoptera: Lampyridae). University of Queensland, St. Lucia, Brisbane. (Ph.D. Thesis).

Barber, H.S., 1941. Species of fireflies in Jamaica (Coleoptera, Lampyridae). Proc. Rochester Acad. Sci. 8, 1–13.

Barber, H.S., 1951. North American fireflies of the genus *Photuris*. Smithsonian Inst. Collect. 117, 1–58.

Belt, T., 1874. The Naturalist in Nicaragua, third ed. University of Chicago Press, Chicago (1985 printing).

Beutel, R.G., 1995. Phylogenetic analysis of (Elateriformia Coleoptera): polyphaga based on larval characters. J. Zool. Syst. Evol. Res. 33, 145–171.

Blair, K.G., 1915. Luminous insects. Nature 96, 411–415.

Blair, K.G., 1924a. Some notes on luminosity in insects. Entomol. Monthly Mag. 60, 173–178.

Blair, K.G., 1924b. Remarks on the luminosity of the Lampyridae. Trans. R. Entomol. Soc. London, vii.

Blum, M.S., Sannasi, A., 1974. Reflex bleeding in the lampyrid *Photinus pyralis*: defensive function. J. Insect Physiol. 20, 451–460.

Branham, M.A., Greenfield, M.D., 1996. Flashing males win mate success. Nature 381, 745–746.

Branham, M.A., Archangelsky, M., 2000. Description of the last larval instar and pupa of *Lucidota atra* (G.A. Olivier 1790) (Coleoptera: Lampyridae), with a discussion of abdominal segment homology across life stages. Proc. Wash. Entomol. Soc. 102, 869–877.

Branham, M.A., Wenzel, J.W., 2000. The evolution of sexual communication in fireflies. XIth International Symposium on Bioluminescence and Chemiluminescence. Luminescence 15, 202 (Abstract).

Branham, M.A., Wenzel, J.W., 2001. The evolution of bioluminescence in cantharoids (Coleoptera: Elateriodea). Fla. Entomol. 84, 565–586.

Buck, J.B., 1937. Studies on the firefly. I. The effects of light and other agents on flashing in *Photinus pyralis* with special reference to periodicity and diurnal rhythm. Physiol. Zool. 10, 45–58.

Buck, J.B., 1942. Problems in the distribution and light organ structure of Jamaican lampyrid fireflies. Yearbook Am. Philos. Soc., 124–129.

Buck, J.B., 1948. The anatomy and physiology of the light organ in fireflies. Ann. N. Y. Acad. Sci. 49, 397–482.

Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P.E. (Eds.), 1996. The Merck Index. Merck, Rahway, NJ.

Bugnion, E., 1929. Le ver-luisant provencal et la luciole nicoise. Mem. Assoc. Nat. Nice Alpes-Maritimes (Suppl. Riviera Sci.).

Buschman, L.L., 1988. Light organs of immature fireflies (Coleoptera: Lampyridae) as eye-spot/false-head displays. Coleopt. Bull. 42, 94–97.

Coblentz, W.W., 1912. A physical study of the firefly. Bull. Carnegie Inst. 164, 3–47.

Costa, C., Vanin, S.A., Casari, S.A., Viviani, V.R., 1999. Larvae of neotropical Coleoptera. XXVII. *Phrixothrix hirtus*: immatures, neotenic female, adult male and bionomic data (Phengodinane, Phengodidae, Coleoptera). Iheringia Ser. Zool. Porto Alegre 86, 9–28.

Cowles, R.B., 1959. Zulu Journal. University of California Press, Berkeley, CA.

Crowson, R.A., 1972. A review of the classification of Cantharoidea (Coleoptera), with the definition of two new families, Cneoglossidae and Omethidae. Rev. Univ. Madrid 21, 35–77.

De Cock, R., 2000. Rare, or simply overlooked? Practical notes for survey and monitoring of the small glow-worm *Phosphaenus hemipterus* (Coleoptera: Lampyridae). Belg. J. Zool. 130, 93–101.

De Cock, R., Matthysen, E., 1999. Aposematism and bioluminescence: experimental evidence from glow-worm larvae (Coleoptera: Lampyridae). Evol. Ecol. 13, 619–639.

Desutter-Grandcolas, L., 1997. Acoustic communication in crickets (Orthoptera: Grylloidea): a model of regressive evolution revisited using phylogeny. In: Grandcolas, P. (Ed.), Mémoires, Vol. 173, The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. Mus. Natl. Hist. Nat., Paris, pp. 183–202.

Eberhard, W.G., 1985. Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge, MA.

Eisner, T., Wiemer, D.F., Haynes, L.W., Meinwald, J., 1978. Lucibufagins: defensive steroids from the fireflies. Proc. Natl. Acad. Sci. USA 75, 905–908.

Eisner, T., Goetz, M.A., Hill, D.E., Smedley, S.R., Meinwald, J., 1997. Firefly "femmes fatales" acquire defensive steroid (lucibufagins) from their firefly prey. Proc. Natl. Acad. Sci. USA 94, 9723–9728.

Farnworth, E.G., 1973. Flashing behavior, ecology and systematics of Jamaican lampyrid fireflies. University of Florida, Gainesville. (Ph.D. Thesis).

Fieser, L.F., Fieser, M., 1949. Natural Products Related to Phenanthrene. Reinhold, New York.

Gorham, H.S., 1880. On the structure of the Lampyridae, with reference to their phosphorescence. Trans. R. Entomol. Soc. London, 63–67.

Goloboff, P., 1993. Nona, Version 1.8 (32 bit). Published by the author, Instituto Miguel Lillo, Miguel Lillo 205, 400 Sierra Madre de Tucuman, Argentina.

Green, J.W., 1961. Revision of the species of *Pyropyga* (Lampyridae). Coleopt. Bull. 15, 65–74.

Harvey, E.N., 1957. A history of luminescence. Am. Philos. Soc. Philadelphia 44, 669.

Hess, W.N., 1922. Origin and development of the light organs of *Photuris pennsylvanica* De Geer. J. Morphol. 36, 245–277.

Hormiga, G., Scharff, N., Coddington, J.A., 2000. The phylogenetic basis of sexual size dimorphism in orb-weaving spiders (Araneae, Orbiculariae). Syst. Biol. 49, 435–462.

Kaiser, E., Michl, H., 1958. Die Biochemie der Tierischen Gifte. Deuticke, Vienna.

Kelly, R.A., Smith, T.W., 1996. Pharmacological treatment of heart failure. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 809–838.

Kloft, W.J., Lloyd, J.E., Bhatkar, A.P., 1975. Nachweis von Reflex-Bluten bei Lampyriden (Coloeptera) mit Hilfe der Tracer-Methode. Experientia 31, 410.

Kluge, A.G., Wolf, A.J., 1993. Cladistics: what's in a word? Cladistics 9, 183–199.

Knight, M., Glor, R., Smedley, S.R., Gonzalez, A., Alder, K., Eisner, T., 1999. Firefly toxicosis in lizards. Chem. Ecol. 25, 1981–1986.

Kukalova-Peck, J., Lawrence, J.F., 1993. Evolution of the hind wing in Coleoptera. Can. Entomol. 125, 181–258.

Kuribayashi, S., 1980. Genjibotaru *Luciola cruciata*. Nature Book, Tokyo (In Japanese with English abstract).

Lawrence, J.F., 1988. Rhinorhipidae, a new beetle family from Australia, with comments on the phylogeny of the Elateriformia. Invert. Taxonomy 2, 1–53.

Lawrence, J.F., Britton, E.B., 1991. Coleoptera. In: CSIRO (Ed.), The Insects of Australia, second ed., vol. 2. Cornell University Press, Ithaca, NY, pp. 543–683.

Lawrence, J.F., Newton, A.F., 1995. Families and subfamilies of Coleoptera (with selected genera, notes references and data on family-group names). In: Pakaluk, J., Slipinski, S.A. (Eds.), Biology, Phylogeny, and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson. Muz. Inst. Zool. PAN, Warsaw, Poland, pp. 849–861.

Lloyd, J.E., 1964. Notes on flash communication in the firefly *Pyractomena dispersa* (Coleoptera: Lampyridae). Ann. Entomol. Soc. Am. 57, 260–261.

Lloyd, J.E., 1966. Studies on the flash communication system in *Photinus* fireflies. Misc. Pub. Mus. Zool. Univ. Mich. 130, 1–95.

Lloyd, J.E., 1968. Illumination, another function of firefly flashes? Entomol. News 79, 265–268.

Lloyd, J.E., 1971. Bioluminescent communication in insects. Annu. Rev. Entomol. 16, 97–122.

Lloyd, J.E., 1972a. Chemical communication in fireflies. Environ. Entomol. 1, 265–266.

Lloyd, J.E., 1972b. Mating behavior of a New Guinea *Luciola* firefly: a new communicative protocol (Coleoptera: Lampyridae). Coleopt. Bull. 26, 155–163.

Lloyd, J.E., 1973. Firefly parasites and predators. Coleopt. Bull. 27, 91–106.

Lloyd, J.E., 1978. Insect bioluminescence. In: Herring, P. (Ed.), Bioluminescence in Action. Academic Press, New York, pp. 241–272.

Lloyd, J.E., 1979. Sexual selection in luminescent beetles. In: Blum, M.S., Blum, N.A. (Eds.), Sexual Selection and Reproductive Competition in Insects. Academic Press, New York, pp. 293–342.

Lloyd, J.E., 1983. Bioluminescence and communication in insects. Annu. Rev. Entomol. 28, 131–160.

Lloyd, J.E., 1990. Firefly semiosystematics and predation: a history. Fla. Entomol. 73, 52–66.

Lloyd, J.E., 1997. Firefly mating ecology, selection, and evolution. In: Choe, J.C., Crespi, B.J. (Eds.), The Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge, UK, pp. 184–192.

Lloyd, J.E., 1999. On research and entomological education. III. Firefly brachyptery and wing "polymorphism" at Pitkin Marsh and watery retreats near summer camps (Coleoptera: Lampyridae, *Pyropyga*). Fla. Entomol. 82, 165–179.

Mast, S.O., 1912. Behavior of fire-flies (*Photinus pyralis*)? with special reference to the problem of orientation. J. Anim. Behav. 2, 256–272.

McDermott, F.A., 1911. Some further observations on the light emission of American Lampyridae: the photogenic function as a mating adaptation in the Photinini. Can. Entomol. 43, 399–406.

McDermott, F.A., 1912. A note on *Photinus castus*. Can. Entomol. 44, 312.

McDermott, F.A., 1914. The ecologic relations of the photogenic function among insects. Z. Wiss. Insekt. 10, 303–307.

McDermott, F.A., 1958. The Fireflies of Delaware. Soc. Nat. Hist., Delaware, Wilmington.

McDermott, F.A., 1964. The taxonomy of Lampyridae (Coleoptera). Trans. Am. Entomol. Soc. 90, 1–72.

McDermott, F.A., 1966. Lampyridae. In: Steel, W.O. (Ed.), Coleopterorum Catalogus Supplementa, second ed. The Hague, Junk, pp. 1–149.

McElroy, W.D., Seliger, H.H., 1966. Firefly bioluminescence. In: Johnson, F.H., Haneda, Y. (Eds.), Bioluminescence in Progress. Princeton University Press, Princeton, NJ, pp. 427–458.

Nixon, K.C., 2000. Winclada version 0.9.99m8.1 (BETA). Published by the author, Cornell University, Ithaca, NY.

Nixon, K.C., Carpenter, J.M., 1993. On outgroups. Cladistics 9, 413–426.

Nylin, S., Wedell, N., 1994. Sexual size dimorphism and comparative methods. In: Eggleton, P., Vane-Wright, R.I. (Eds.), Linnean Society Symposium Series, Phylogenetics and Ecology, vol. 17. Academic Press, London, pp. 253–280.

Oertel, D., Linberg, K.A., Case, J.F., 1975. Ultrastructure of the larval firefly light organ as related to control of light emission. Cell Tissue Res. 164, 27–44.

Ohba, N., 1980. Mating behavior of a Japanese *Hotaria* firefly (Coleoptera: Lampyridae). Sci. Rep. Yokosuka City Mus. 27, 13–18.

Ohba, N., 1983. Studies on the communication system of Japanese fireflies. Sci. Rep. Yokosuka City Mus. 30, 1–62.

Ohba, N., 1984. Synchronous flashing in the Japanese firefly, *Luciola cruciata* (Coleoptera: Lampyridae). Sci. Rep. Yokosuka City Mus. 32, 23–32.

Ohba, N., 1986. Calling signal of female *Hotaria parvula* (Coleoptera: Lampyridae). Sci. Rep. Yokosuka City Mus. 34, 17–23.

Ohba, N., Goto, Y., 1992a. Morphology and behavior of *Pyrocoelia discicollis* and *P. fumosa* Coleoptera: Lampyridae. Sci. Rep. Yokosuka City Mus. 40, 1–5.

Ohba, N., Goto, Y., 1992b. Flying and flashing activity of *Curtos okinawana* and *Luciola kuroiwae* (Coleoptera: Lampyridae). Sci. Rep. Yokosuka City Mus. 40, 7–10.

Ohba, N., Goto, Y., 1993. Geographical variation on the morphology and behavior of *Curtos costipennis* and *C. okinawana* (Coleoptera: Lampyridae) in the Southwestern Islands. Sci. Rep. Yokosuka City Mus. 41, 1–14.

Osten-Sacken, B., 1861. Die amerikanischen Leuchtkafer. Stettiner Entomol. Ztg. 22, 54–56.

Papi, F., 1969. Light emission, sex attraction and male flash dialogues in a firefly, *Luciola lusitanica* (Charp.). Monit. Zool. Ital. (N.S.) 3, 135–184.

Schwalb, H.H., 1960. Beiträge zur Biologie der Einheimischen Lampyriden, *Lampyris noctiluca* Geoff. und *Phausis splendidula* Lec., und experimentelle Analyse ihrer Beutfang-und Sexualverhaltens. Zool. Jb. Syst. 88, 399–550.

Sexton, O.J., 1960. Experimental studies of artificial Batesian mimics. Behaviour 15, 244–252.

Sexton, O.J., 1964. Differential predation by the lizard *Anolis carolinensis* upon unicoloured and polycoloured insects after an interval of no contact. Anim. Behav. 12, 101–110.

Sivinski, J., 1981. The nature and possible functions of luminescence in Coleoptera larvae. Coleopt. Bull. 35, 167–179.

Snodgrass, R.E., 1993. Principles of Insect Morphology. Cornell University Press, Ithaca, NY.

Susuki, H., 1997. Molecular phylogenetic studies of Japanese fireflies and their mating systems (Coleoptera: Cantharoidea). Tokyo Metro. Bull. Nat. Hist. 3, 1–53.

Susuki, H., Sato, Y., Fujiyama, S., Ohba, N., 1996. Biochemical systematics of Japanese fireflies of the subfamily Luciolinae and their flash communication systems. Biochem. Genet. 34, 191–200.

Sydow, S.L., Lloyd, J.E., 1975. Distasteful fireflies sometimes emetic, but not lethal. Fla. Entomol. 58, 312.

Tyler, J., 2001a. Are glow-worms *Lampyris noctiluca* (Linnaeus) (Lampyridae) distasteful? Coleopterist 9, 148.

Tyler, J., 2001b. A previously undescribed defence mechanism in the larval glow-worm *Lampyris noctiluca* (Linnaeus) (Lampyridae)? Coleopterist 10, 38.

Underwood, T.J., Tallamy, D.W., Pesek, J.D., 1997. Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). Insect Behav. 10, 365–370.

Viviani, V.R., Bechara, I.J.H., 1997. Bioluminescence and biological aspects of Brazilian railroad-worms (Coleoptera: Phengodidae). Ann. Entomol. Soc. Am. 90, 389–398.

Wenzel, J.W., 1997. When is a phylogenetic test good enough? In: Grandcolas, P. (Ed.), Mémoires, Vol. 173, The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. Mus. Natl. Hist. Nat., Paris, pp. 31–45.

West-Eberhard, M.J., 1979. Sexual selection, social competition and evolution. Proc. Am. Philos. Soc. 123, 222–234.

West-Eberhard, M.J., 1983. Sexual selection, social competition and speciation. Q. Rev. Biol. 58, 155–183.

West-Eberhard, M.J., 1984. Sexual selection, competitive communication and species-specific signals in insects. In: Lewis, T. (Ed.), Insect Communication. Academic Press, Orlando, FL, pp. 283–324.

Wittmer, W., Ohba, N., 1994. Neue Rhagophthalmidae (Coleoptera) aus China und benachbarten Ländern. Jpn. Entomol. 62, 341–355.

Yajima, M., 1978. Diurnal activity and luminous signals of fireflies—the case of *Luciola cruciata*. Insectarium 15, 12–19 (in Japanese).

Yuma, M., 1981. Gregarious ovipositions on *Luciola cruciata* Motschulsky (Coleoptera: Lampyridae). Physiol. Ecol. Jpn. 18, 93–112.