Fluorescence in fireflies (Coleoptera: Lampyridae): using sentinel prey to investigate a possible aposematic signal

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Abstract
While fireflies are most renowned for their bioluminescent signals, several species also have been shown to possess UV and blue-induced fluorescence. In a field study, we used a sentinel prey approach to investigate whether this trait might serve as an aposematic function in chemically defended fireflies. We also examined and provide the first detailed description of green and red patterns of blue light-induced fluorescence in the adults and larvae of Ellychnia corrusca (L.) (Coleoptera: Lampyridae). We placed artificial prey models with and without fluorescent markings in a forested area of Belmont, Massachusetts, USA, where they were exposed to natural predators. Clay models were styled to resemble Photinus fireflies, the most common firefly genus in this area, and a close relative of Ellychnia. The field study was conducted from Oct through May in Belmont, Massachusetts; this is the period when adults of the diurnal non-luminescent firefly E. corrusca are overwintering on trees. We observed considerable seasonal variation in attack rates, and marks on the clay models indicated they were attacked by avian, mammalian, and arthropod predators. However, no difference was seen between fluorescent and non-fluorescent models in predator attack rates. This suggests that fireflies’ fluorescent markings do not serve as an aposematic signal, at least for the predator guild active in New England woodlands.

Key Words: predation; Ellychnia; visual signaling; sensory ecology; lampyrine

Resumen
Aunque las luciérnagas son más conocidas por sus señales bioluminiscentes, también se ha demostrado que varias especies poseen fluorescencia inducida por UV y azul. En un estudio de campo, utilizamos un enfoque de presa centinela para investigar si este rasgo podría cumplir una función aposemática en luciérnagas defendidas químicamente. También examinamos y proporcionamos la primera descripción detallada de los patrones verdes y rojos de fluorescencia inducida por la luz azul en los adultos y larvas de Ellychnia corrusca (L.) (Coleoptera: Lampyridae). Colocamos modelos de presas artificiales con y sin marcas fluorescentes en un área de bosque de Belmont, Massachusetts, USA, donde fueron expuestos a depredadores naturales. Los modelos de arcilla fueron diseñados para parecerse a las luciérnagas Photinus, el género de luciérnagas más común en esta área y con una relación cercana a Ellychnia. El estudio de campo se realizó de octubre a mayo en Belmont, Massachusetts, USA; un período en que los adultos de la luciérnaga diurna no luminescente E. corrusca están hibernando en los árboles. Observamos una considerable variación estacional en las tasas de ataque, y las marcas en los modelos de arcilla indicaron que fueron atacados por depredadores aviares, mamíferos y artrópodos. Sin embargo, no se observaron diferencias entre los modelos fluorescentes y no fluorescentes en las tasas de ataque de depredadores. Esto sugiere que las marcas fluorescentes de las luciérnagas no sirven como una señal aposemática, al menos para el gremio de depredadores activos en los bosques de Nueva Inglaterra.

Palabras Clave: predación; Ellychnia; señalización visual; ecología sensorial; lampyrine

Fireflies (Coleoptera: Lampyridae) are charismatic insects most renowned for the spectacular bioluminescent courtship signals used by adults of many species (Lloyd 2002, 2008). Phylogenetic evidence suggests that lampyrid bioluminescence originates in the larval stage (Branham & Wenzel 2003; Martin et al. 2017), where it probably serves as an aposematic signal (Underwood et al. 1997; De Cock & Matthyssen 2003). Recently, bioluminescence produced by flying adult fireflies has been shown also to deter bat predators (Leavell et al. 2018). Such visual aposematism, which most often manifests as conspicuous coloration, is a widespread adaptation used by unpalatable or unprofitable prey to facilitate predator learning (Ruxton et al. 2007). Several firefly taxa are known to be chemically defended (Eisner et al. 1978, 1997; Goetze et al. 1981; Tyler et al. 2008; Smedley et al. 2017) and are repellent to various vertebrate and invertebrate predators in both their adult and larval stages (Lloyd 1973; Leavell et al. 2018). In addition to bioluminescence, some fireflies may deter predators by advertising these chemical defenses with volatile chemicals (Vencl et al. 2016) or auditory and visual signals (Leavell et al. 2018; Moosman et al. 2009).

Previous studies have shown that certain fireflies also are fluorescent, absorbing short wavelength light and reemitting it at longer wavelengths. Metcalf (1943) first described a firefly pigment with UV-induced red fluorescence, which he named lampyrine. In the firefly Photinus marginellus (LeConte) (Coleoptera: Lampyridae), this same author observed lampyrine beneath the pronotum, below the cuticle in the thorax and abdomen, and in the male testes. Examining preserved museum specimens with a UV light, Metcalf noted red fluorescence in 43 different lampyrid taxa, while it was lacking in related beetles he examined in the clades Phengodidae, Cantharidae, and Lycidae. Fluorescence in specific body regions has subsequently been reported in other firefly species (Sannasi 1970; Cicero 2008; Deheyn & Ballantyne 2009; Yiu & Jeng 2018), yet its function remains unexamined. In contrast to bioluminescence, fluorescence may serve as a passive warning signal that is useful in dim or daylight conditions.

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Here we examined the possibility that firefly fluorescence may serve as an aposematic signal that deters predators. Under certain light environments, externally visible fluorescence can contribute substantially to body coloration (Marshall & Johnsen 2017). For example, under twilight or nocturnal incident illumination, Taboada et al. (2017) showed that fluorescence in South American tree frogs generated 20 to 30% of their total emerging light (fluoresced plus reflected photons). Across firefly taxa, courtship aggregations are found in diverse light environments: certain bioluminescent fireflies court at dusk, whereas others are fully nocturnal, and species with non-luminescent adults are active during the d (Lloyd 2008). In addition, under various light conditions fireflies are likely to be encountered by insectivorous birds and mammals while resting on the ground or in vegetation. Here we experimentally investigate whether externally visible fluorescence altered predation rates using artificial firefly models with or without fluorescent markings as sentinel prey. Additionally, we measured seasonal variation in predation rates on the model prey (Lovei & Ferrante 2016; Roslin et al. 2017; Ferrante et al. 2017). Finally, we provide a detailed description of blue-induced fluorescence in adults and larvae of Ellychnia corrusca (L.) (Coleoptera, Lampyridae), an unusual diurnal non-luminescent firefly that overwinters on tree trunks during its adult stage.

SEASONAL VARIATION IN PREDATION RATES

Because E. corrusca adults overwinter in exposed locations on tree trunks, we also used our sentinel prey experiment to investigate whether predation rates varied seasonally. Sentinel prey techniques are useful for measuring spatial and temporal variation in predation (Lovei & Ferrante 2016; Ferrante et al. 2017; Roslin et al. 2017). To investigate how seasonal changes in daylength and temperature might influence the total number of predator attacks, we conducted separate linear regressions using average photoperiod (h) and air temperature during each of the 11 assays periods (data obtained from Wunderground.com; analyses conducted in R using packages “Car” and “MASS”).

FLUORESCENCE IN ELLYCHNIA FIREFLIES

Ellychnia corrusca fireflies have been shown to contain toxic lucibufagins that deter potential predators (Smedley et al. 2017). This species has shown distinctive coloration on its pronotum (head shield): a central black area is bordered with red, and surrounded on both sides by pale, parentheses-shaped areas. To investigate patterns of fluorescence, we photographed E. corrusca adults that we collected between Apr and Jun in Lincoln (42.424629°N, 71.307482°W) and Arlington, Massachusetts, USA (42.411750°N, 71.168096°W). Adult Ellychnia were freeze-killed for 5 min, then we immediately photographed their dorsal and ventral aspects. We also photographed a few E. corrusca larvae that we reared from eggs laid in the laboratory. Larvae were kept in darkness in small Petri dishes, and periodically fed with small pieces of earthworms.

Table 1. Total number and percentage of attacks on fluorescent and non-fluorescent artificial prey recorded from Oct 2017 to Feb 2018.

| Treatment | Attacked | Not attacked | % Attacked |
|-----------|----------|--------------|------------|
| Fluorescent | 52 | 340 | 46.4 |
| Non-Fluorescent | 60 | 332 | 53.6 |
Photographs were taken under illumination with a Nightsea Blue-Star light (Nightsea, Lexington, Massachusetts, USA) emitting 440 to 460 nm, with a longpass yellow filter (500 nm cutoff). Photographs were taken with either a Fujifilm Finepix HS10 camera (Fujifilm, Edison, New Jersey, USA), or under a Nikon dissecting microscope fitted with a Spot Insight camera (Nikon, Melville, New Jersey).

**Results**

Across the entire study, about 50% of our models showed signs of predator attack (Table 1). However, we found no significant difference between the fluorescent and the non-fluorescent treatments in the percentage of model prey that were attacked (Fisher’s exact test; 1 df; \( P = 0.47; \) odds ratio = 0.846).

We noted marked seasonal variation in the total number of attacks (Fig. 1). Attack rates on model prey increased through the fall until mid- to late-Nov, then declined during winter mo. While attack rates on non-fluorescent models showed an increase during Feb and Mar, no such increase was observed for fluorescent models (Fig. 1). This seasonal variation in attack rates was not associated with changes in either temperature (Fig. 2a; least squares minimization linear regression \( F_{(1,9)} = 0.04, P = 0.84 \)) or daylength (Fig. 2b; \( F_{(1,9)} = 0.71, P = 0.42 \)).

Approximately 20% of attacked models showed 2 parallel grooves about 3 mm long that could be indicative of small rodents (P. Marek, personal communication). We also observed deeper gashes ranging from 4 to 7 mm long in 30% of attacked models that may have resulted from bird attacks (L. Long, personal communication). Approximately 10% of attacked models showed shallow scrape marks conceivably caused by arthropod mandibles, possibly ants or spiders. Approximately 35% of attacked models showed shallow gashes about 3 mm long that were more difficult to attribute to a particular predator; these could represent marks made by the claws or beak of a bird, or claw marks of a small mammal. Finally, 5% of our models had chunks missing, were dismembered, or were removed from the tree; in these cases, no likely attacker could be determined. The trail camera revealed a white-breasted nuthatch (*Sitta carolinensis* (Latham)) (Sittidae: Sitta) and a grey squirrel (*Sciurus carolinensis* (Gmelin)) (Sciuridae: Sciurus) foraging on trees near our models.

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**Fig. 1.** Seasonal changes in the number of recorded attacks on artificial prey having fluorescent (short-dashed line) and non-fluorescent (long-dashed line) markings between Oct 2017 and Feb 2018. For each trial, 36 observations were made for each treatment; in the fifth and sixth trials there were only 34 observations due to sampling difficulties. Total number of models attacked is also shown.

**Fig. 2.** Relationship between climatic variables and the total number of attacks recorded on artificial prey (both treatments combined) during each wk-long assay conducted from Oct 2017 through May 2018 (\( n = 11 \) wk): (a) photoperiod (d length in h), (b) average temperature per wk.

**Fig. 3.** Dorsal and ventral aspects of the head area of an *Ellychnia corrusca* adult illuminated with (a, b) Nightsea BlueStar light emitting 440 to 460 nm, and photographed with a longpass yellow filter (500 nm cutoff), and (c, d) the same individual under white light illumination.
the crepuscular firefly *Cicero* (2008), Deheyn and Ballantyne (2009), as well as Yiu and Jeng (2018) have identified various body regions, visible, UV-induced fluorescent markings on various body regions, as well as Yiu and Jeng (2018). Also fluorescence has been observed in diurnal and nocturnal species that differ remarkably in range, behavior, and life history. In the crepuscular firefly *P. marginellus*, Metcalf (1943) identified lampyrine, a fluorescent pigment present in fat body and responsible for the bright red coloration on the pronotum and other body regions. Lamyprine is likely to be responsible for the red fluorescence we observed underlying areas of clear cuticle on the ventral meso- and meta-thorax in the diurnally active *E. corrusca*. Though widely observed in preserved specimens across the Lampyridae, Metcalf (1943) noted that lampyprine was absent from 2 species, *Tenaspsis angularis* (Gorham) (Coleoptera: Lampyridae) and *Photuris* (Coleoptera: Lampyridae) sp., indicating interspecific variation that bears investigating.

The green fluorescence that we observed on the pronotum of *E. corrusca* has not been previously described, and may arise in certain areas of unattended, unpigmented cuticle. Fluorescence has been found in the light producing organs of the firefly *Bourgeoisia hypocrita* (Olivier) (Coleoptera: Lampyridae), as well as in dorsal and ventral markings (Deheyn & Ballantyne 2009). Yiu and Jeng (2018) recently described whole-body blue-green fluorescence in the neotenic, flightless females of a newly described nocturnal lampyrid from Hong Kong, *Oculogyrophus chenghoiyanae* Yiu & Jeng (Coleoptera: Lampyridae). Wiesenborn (2011) reported representatives of several insect orders that show blue fluorescence (emission maximum at 420 nm) associated with resilin, a structural protein providing elasticity. The green fluorescence we observed in *E. corrusca* is not likely from resilin, based on its distinct emission characteristics.

When we used sentinel prey to investigate the possible aposematic function for firefly fluorescence, we found no difference in predator attack rates on artificial prey models that had been painted with red and green fluorescent inks. This suggests that fireflies’ fluorescent markings do not serve as an aposematic signal, at least for the predator guild active in New England woodlands. However, while our fluorescent models resembled generic firefly patterning to us, it is possible that they did not adequately match their brightness or spectral emission as perceived by natural predators. While measuring spectral properties was beyond the scope of this study, future work should quantify emission characteristics. Without measuring the exact excitation we cannot know how closely our models resembled actual fireflies. Additionally, it is possible that fluorescence may be perceived only as an effective warning in concert with other signals; for instance, Leavell et al. (2018) demonstrated that bioluminescence acts synergistically with some unknown acoustic signal to deter bat predation on fireflies in flight.

Fluorescence has been observed in fireflies that differ remarkably in their range, behavior, and life history, and several alternative explanations for firefly fluorescence are possible (Yiu & Jeng 2018). First, it is possible that firefly fluorescence has no adaptive value and is merely correlated with other features of body pigmentation. Other possible adaptive functions of fluorescence in fireflies that remain to be tested include sexual signaling or species recognition in dim light environments. Although courtship in many lampyrads takes place at night, others court during daytime or at dusk.

In summary, we found little evidence that fireflies gain any advantage in predation deterrence from conspicuous fluorescent markings. Our data show no substantial difference in predator attacks between models with or without fluorescence. These data do not support the hypothesis that firefly fluorescence serves as an aposematic signal. However, because of the prevalence of fluorescence in lampyrads and its variation across different species and genera, future studies should explore other possible functions for such a trait, including intraspecific and sexual communication. UV and blue-induced fluorescence has the potential to contribute substantively to body coloration in crepuscular and other dim light environments, and remains an exciting area for future investigations.

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