



Firefly flashing and jumping spider predation

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Bioluminescent flashing in fireflies, while primarily a sexual signal, is known to deter some predators while attracting others. We tested whether flashing serves an antipredator function against two species of diurnal, visually hunting jumping spiders, *Phidippus princeps* and *Phidippus audax*. To confirm anecdotal reports that fireflies flash during the day in a nonmating context, we documented that adult fireflies (*Photuris* sp.) of both sexes flash when disturbed in daylight. We also confirmed that activity periods of *Phidippus* and fireflies overlap, and that spiders attack fireflies and elicit flashing behaviour. We conducted three experiments to examine the influence of flashing on spider behaviour. (1) We tested whether the sudden onset of a flashing LED startled spiders that had initiated attacks on crickets, and found no evidence that it did so. (2) We used choice tests to determine whether flashing lights attracted or deterred spiders from attacking palatable prey. Spiders more often attacked crickets positioned next to a flashing LED versus an LED that was either off or glowed steadily. (3) Many firefly species are distasteful. Therefore, we tested whether flashing lights facilitate avoidance of unpalatable prey with experience. Spiders were given seven encounters with unpalatable prey (nonluminescent *Ellychnia corrusca* fireflies) associated with either flashing or unlit LEDs. Spiders in the two treatments were equally likely to attack the prey during their first encounter, but spiders exposed to flashing LEDs were significantly less likely to attack unpalatable prey by their seventh trial. Spiders tested with palatable prey showed no decline in attacks after exposure to flashing LEDs. We conclude that, although bioluminescent flash signals may increase attack rates by predatory jumping spiders, they may also facilitate learning about unpalatable prey. Thus, the costs and benefits of flashing may depend on the prevalence of firefly palatability.

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Fireflies (Coleoptera: Lampyridae) are well known for bioluminescent signalling, which is used for species recognition and mate choice (reviewed in Lewis & Cratsley 2008). Firefly signals may also have important consequences outside the arena of sexual communication. Bioluminescence is not always only a sexual signal. Larval fireflies are also bioluminescent, and adult fireflies flash in other contexts besides mating, such as flashing when disturbed during the day (McDermott 1964; De Cock & Matthysen 1999; reviewed in: Sivinski 1981; De Cock 2009). In particular, firefly signals are likely to influence interactions with potential predators. Potential predators of larval and adult fireflies include both vertebrates (birds, mammals, amphibians) and invertebrates (spiders, mantids and other firefly species) (Lloyd 1965, 1973).

Firefly flashing may affect interactions with predators in several ways. First, flashes may act as a cue to eavesdropping predators,

increasing the risk of detection. For example, flashing by the firefly *Photinus* sp. is detected by the predaceous firefly *Photuris* sp. (Woods et al. 2007). Laboratory-reared house mice, *Mus musculus*, that have never encountered fireflies are more likely to select prey next to a flashing light than prey next to a light that is off (Underwood et al. 1997). The little brown bat, *Myotis lucifugus*, preferentially attacks flashing over nonflashing lures (Moosman et al. 2009).

On the other hand, firefly bioluminescence may function to protect against predators (reviewed in: Lloyd 1973; Sivinski 1981; Buschman 1988; De Cock 2009). Bioluminescence serves as an antipredator defence in numerous marine organisms, including dinoflagellates, jellyfish, squid and crustaceans (reviewed in: Widder 2010; Haddock et al. 2010). Recent phylogenetic analyses support the idea that firefly bioluminescence originated in larvae as an antipredator defence, and only later became exapted into an adult courtship signal (Branham & Wenzel 2001; Bocakova et al. 2007; Sagegami-Oba et al. 2007).

Firefly flashing may reduce the risk of predation via at least three mechanisms. First, naïve neophobic or photophobic predators that rarely encounter fireflies may be reluctant to attack flashing prey

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(reviewed in Sivinski 1981). Second, a sudden flash may startle an approaching predator long enough to allow escape, similar to the way io moths, *Automeris io*, startle predators by suddenly exposing the eyespots on their hindwings (Blest 1957). There are anecdotal reports that larval and adult fireflies readily flash when they are physically disturbed either during the day or night (reviewed in: Lloyd 1973; Sivinski 1981; De Cock 2009). Lloyd (1973) summarized casual observations on startle behaviour in response to firefly flashes: horses, raptorial insects, rats, geckos, and a chicken were reported to startle, but toads, frogs, spiders and bats were not. To our knowledge, startle responses of terrestrial, invertebrate predators to sudden flashes of light have not yet been tested.

Third, in firefly species that are chemically defended and thus distasteful, firefly flashing may serve as an aposematic signal (De Cock & Matthysen 1999). Distasteful prey often advertise chemical defences with conspicuous visual signals that deter predators (Ruxton et al. 2004). Many (but not all) fireflies are distasteful to numerous vertebrate and invertebrate predators (reviewed in Lewis & Cratsley 2008). Some firefly genera contain defensive steroidal pyrones known as lucibufagins, which have been shown to deter predation by *Phidippus* jumping spiders (Eisner et al. 1978). Thus, bioluminescent signals may reduce predatory attacks by increasing predator recognition and avoidance of distasteful prey (Guilford 1986; reviewed in De Cock & Matthysen 1999). For example, wild-caught big brown bats, *Eptesicus fuscus*, were less likely to attack flashing compared with nonflashing aerial lures (Moosman et al. 2009). Similarly, when presented with glowing dummy prey resembling glow-worm larvae, wild-caught toads (*Bufo bufo*) showed lower attack rates and longer latencies to attack (De Cock & Matthysen 1999).

Aversion to aposematic signals may be innate or learned (reviewed in Lindström et al. 1999). Visual aposematic signals enhance learned avoidance in avian (reviewed in Rowe & Guilford 2000), aquatic (Aguado & Marin 2007) and invertebrate predators (Prudic et al. 2006). Bioluminescent signals in particular may help predators learn and retain information that signalling prey are distasteful (Guilford & Cuthill 1989). In terrestrial ecosystems, the role of learning has been tested only in vertebrate predators. House mice, *M. musculus*, learn to avoid unpalatable food more rapidly when it is positioned next to a glowing light (Underwood et al. 1997). Toads (*B. bufo*) exposed to distasteful, glowing firefly larvae (*Lampyrus noctiluca*), become more reluctant to attack glowing (but not nonglowing) dummy prey (De Cock & Matthysen 2003).

Diurnal and nocturnal spiders are known predators of fireflies (reviewed in Lloyd 1973). Here we investigate the effect of bioluminescent flashes on the behaviour of jumping spiders in the genus *Phidippus* (Araneae: Salticidae), specifically *Phidippus princeps* and *Phidippus audax*. These North American spiders have a broad geographical range that overlaps that of many firefly species (Lloyd 1966; Edwards 2004). Jumping spiders have excellent vision and are able to see colour, motion and image detail (reviewed in Richman & Jackson 1992). Instead of using a prey-capture web, jumping spiders stalk and tackle prey. Members of the genus *Phidippus* prey upon a wide variety of insects (Freed 1984; Edwards & Jackson 1993). *Phidippus audax* have been used in bioassays for firefly chemical defences, as they readily attack these insects (Eisner et al. 1978, 1997). However, the relationship of firefly flashing to spider behaviour was not recorded during these studies. Furthermore, spiders are capable of learning to avoid aversive stimuli (reviewed in Jakob et al. 2011). In particular, *P. princeps* has been shown to reduce its attacks on toxic prey after repeated experiences (Skow & Jakob 2006).

Fireflies begin sexual signalling at dusk, whereas jumping spiders are generally diurnal. However, firefly flashing might be important to jumping spiders both during daylight and at dusk. We

had noticed that fireflies resting on vegetation during the day flashed when disturbed, such as when we brushed against their plants while walking. Thus, during daylight, a spider might sometimes see a flashing firefly that had been disturbed, or might itself induce flashing by touching or attacking a firefly. We conducted daytime trials to more rigorously quantify flashing in response to gentle disturbance. We also observed interactions between fireflies and spiders at dusk under natural light in order to examine whether their activity periods overlapped, and whether interactions resulted in flashing. We tested three hypotheses about the role of bioluminescent flash behaviour in predator–prey interactions between fireflies and jumping spiders: (1) jumping spiders will be startled by sudden light flashes and stop their attack, (2) flashing light affects the prey choice of spiders, and (3) after multiple encounters, spiders are less likely to attack unpalatable prey associated with a flashing versus an unlit light.

METHODS

Study Organisms

The spiders used in these experiments were female *P. princeps* and *P. audax*, collected in western Massachusetts (Quabbin Reservoir, Hampshire County, U.S.A.) in the late summer and autumn of 2009 and 2010. These two species co-occur in the same fields and we routinely capture them in the same microhabitats (see Skow & Jakob 2006 for habitat description). We used *P. princeps* in all experiments except for dusk activity trials where *P. audax* were used. Dusk activity trials were conducted in late spring of 2011 before *P. princeps* could be collected in large numbers. We housed spiders individually in plastic cages enriched with leaves of plastic plants and wooden dowels painted green (Carducci & Jakob 2000) and fed them *Acheta domesticus* house crickets weekly. Crickets were also used as a palatable prey item in the experiments described below. Before each experiment, spiders were starved for 5–9 days to ensure they were hungry.

For firefly disturbance and dusk activity trials, we collected adult male and female *Photuris* sp. in early summer 2010 and 2011 from three locations in Massachusetts (Greenfield, Franklin County and Quabbin Reservoir and University of Massachusetts Amherst, Hampshire County). We housed fireflies in clear plastic cups with ventilated lids and gave them a wet piece of filter paper and a piece of apple to maintain humidity in the cage and a piece of vegetation from plastic plants to rest on.

To examine avoidance of unpalatable prey with experience, we used the diurnal firefly, *Ellychnia corrusca*, which our preliminary observations showed to be unpalatable to *P. princeps*. Overwintering adult fireflies were collected in January 2010 on oak trees, *Quercus rubra* (Cadwell Forest, Pelham, Hampshire County, MA). Fireflies were housed communally in a plastic container outfitted similarly to those for *Photuris* sp.

Firefly Disturbance and Flash Behaviour

Previous reports indicated that adult fireflies flash when disturbed by contact with potential predators (reviewed in: Lloyd 1973; Fu 2006). To confirm these reports, we recorded the response of female and male *Photuris* sp. to physical disturbance. During daylight trials in the laboratory, we placed individual fireflies in a clear plastic, open-topped vial (3 cm diameter × 5.7 cm long). We observed undisturbed fireflies for 3 min and scored whether they flashed. We then gently tapped each firefly with a paint brush and again scored whether they flashed. We compared flashing before and after disturbance using McNemar's exact test, appropriate for paired samples (Daniel 1978).

Dusk Activity Trials

To examine potential interactions between jumping spiders and fireflies during the fireflies' active hours, we placed fireflies (*Photuris* sp.) and spiders (*P. audax*) together at dusk and recorded their interactions. Trials were conducted in June 2011, between 1930 and 2200 hours outside on a mowed lawn in Amherst, Massachusetts. We placed spiders individually in 9 cm petri dishes and allowed them to acclimate for at least 1 h. Once fireflies began to flash spontaneously, typically around 2030–2100 hours (21 lx), two fireflies were placed into each dish. For 1 h, we recorded any touches or attacks made by the spiders and the flashing behaviour of the fireflies before and after interaction with the spider.

Tests of the Startle Hypothesis for Flashing

We tested whether *P. princeps* were startled by light flashes that occurred during a predatory attack. We simulated firefly flashes with green light-emitting diodes, LEDs (570 nm, 50 mcd; Radio-Shack, Model 276-009), which closely matches the wavelength of *Photuris* sp. fireflies (Biggley et al. 1967), many species of which are found locally. Firefly flash rates vary widely between species and individuals, as well as within individuals across temperatures. The flash rate that we chose was within the biological range of local fireflies. LEDs were connected to a 555 timer chip, and a variable resistor was used to adjust the LED flash rate to 2 MHz with a flash duration of 150 ms for this and subsequent experiments.

The testing arena consisted of a covered 9 cm diameter petri dish with a LED inserted through the centre of the bottom. For each trial, we glued a single cricket (0.7–1 cm in length) to card stock (see Jakob et al. 2007) and placed it next to the LED; secured crickets were still able to make small movements of the head and body. This and all subsequent experiments were conducted under full spectrum bulbs (GE Reveal, 120 V, 65 W flood) at 1292 lx, approximating daylight conditions.

We introduced spiders individually into the arena using a modified 20 cc syringe with its tip cut off. The sides of the syringe were opaque to eliminate visual cues, and spiders remained in the syringe for 5 min to acclimate before we gently introduced them into the arena through a small hole in the lid.

During each trial in the control treatment, spiders were placed in the arena with a cricket positioned next to an unlit LED. For the flashing treatment, we placed spiders in the arena with a cricket positioned next to an LED that we activated to flash using a wireless remote when the spider oriented to and began to approach the cricket. The light remained on as long as the spider was oriented to the cricket. In both treatments, we recorded each spider's behaviour for 15 min or until it began to consume the cricket. We defined startle responses as a pause, with the spider suddenly stopping its forward motion towards the prey; a retreat, moving its body rapidly backwards; or a defensive posture, tucking its posterior legs under its body and raising the anterior two legs. All trials were scored in real time and were also videorecorded; these recordings were reviewed to refine and confirm scoring. Syringes and arenas were cleaned with isopropyl alcohol and allowed to dry between trials to remove possible chemical cues from previous spiders. We compared the frequency of spider startle responses in control and flashing treatments using a chi-square contingency test.

Effect of Flashing on Spiders' Prey Choice

To test whether flashing altered the likelihood of *P. princeps* attack, we conducted tests in which spiders were allowed to choose between two palatable crickets, each positioned next to an LED. These LEDs were either unlit, flashing (2 MHz flash rate, flash

duration 0.15 s) or glowing (continuously on). The testing arena was a covered 14 cm diameter petri dish with two LEDs inserted through the bottom of the arena at opposite sides, 1 cm from the edge. Size-matched crickets (0.7–1 cm length) were used in each trial, with one secured cricket positioned next to each LED. We tested two different pairwise comparisons: (1) flashing versus unlit LEDs and (2) flashing versus steadily glowing LEDs. For each trial, we randomly assigned the flashing LED to be on either the left or right side of the arena. After a 5 min rest period in the syringe, individual spiders were introduced through a hole in the centre of the arena as described for the startle trial. Each spider was observed for 15 min to determine which cricket it attacked first. Spiders that did not attack either cricket within 15 min were retested after 24 h. Spiders that never attacked were dropped from the experiment after three trials. For each comparison, we tested whether spiders discriminated between the two prey treatments with exact binomial tests.

Effect of Flashing on Avoidance of Unpalatable Prey over Multiple Encounters

To test whether flashing increases spiders' avoidance of unpalatable prey over multiple encounters, we compared *P. princeps* attack rates before and after seven trials with unpalatable *E. corrusca* fireflies. The adults of this species are nonluminescent (Williams 1917; Rooney & Lewis 2000) so we coupled them with LEDs that were either flashing or unlit. We chose seven trials because Skow & Jakob (2006) found that eight trials were sufficient for spiders to learn an association between contextual cues and distasteful prey, and inspection of the raw data suggested that seven trials would also be sufficient. Spiders were placed in one of 10 testing arenas (9 cm petri dishes with an LED inserted through the centre of the bottom) that were visually isolated from each other. Spiders were allowed to adjust to the arena for 30 min, after which LEDs in 5 randomly chosen arenas began flashing (2 MHz for 150 ms), while the other LEDs remained off. At the same time, a single *E. corrusca* adult was placed into each arena. Each spider was given seven consecutive 10 min trials, with 30 min rest periods between each trial; we observed each spider's behaviour during the first and last trials and recorded whether the spider attacked the firefly. During rest periods we removed fireflies and turned off all LEDs so that spiders were alone in the arena, and we added new fireflies at the start of each trial. To compare spider attack rates between the flashing and nonflashing treatments, we used chi-square tests of two 2×2 contingency tables (one for the first trial and another for the last trial).

As a further control to test whether spiders became blinded or otherwise disturbed across trials by the flashing light and changed their predatory behaviour as a result, we performed a second set of trials with different *P. princeps* spiders. We used the same protocol as above, except that we offered all spiders palatable *Drosophila hydei* as prey on the first and seventh trials. Spiders were each tested twice, once in the flashing and once in the nonflashing treatment, with at least 1 day between tests. Treatment order was randomly assigned for each spider. Spiders that failed to attack prey in both the first and seventh trial in a particular treatment were retested up to three times. Two spiders still failed to attack prey and were omitted from the analysis, leaving a sample size of 20 individuals. Because the same spiders were tested in both treatments we used a McNemar's test for significance of changes, appropriate for a paired design (Daniel 1978).

RESULTS

Firefly Disturbance and Flash Behaviour

Under daylight conditions in the laboratory, *Photuris* sp. adult fireflies exhibited no flashing when undisturbed, but they were

significantly more likely to flash when gently tapped (McNemar's exact test: $\chi^2_1 = 9.09$, $N = 14$, $P = 0.003$). Three fireflies did not flash in either the resting or disturbance trials.

Dusk Activity Trials

During the dusk trials, all 13 spiders contacted a firefly: 11 spiders touched a firefly at least once, and nine attacked at least once. We observed a total of 40 touches and 25 attacks. Twelve attacks (48%) and eight touches (20%) were immediately followed by the firefly flashing. Two spiders oriented to and then approached a flashing firefly. During one interaction, a spider oriented towards and approached a firefly 11 times, during which the firefly flashed continuously. These fireflies appeared to be distasteful to the spiders, because all spiders released them after the attack.

Tests of the Startle Hypothesis for Flashing

Phidippus princeps jumping spiders showed no startle behaviours in any trial. Whether the LED remained unlit ($N = 9$) or flashed ($N = 12$), every spider attacked and captured the cricket prey (chi-square contingency test: $\chi^2_1 = 0$, $P = 1$).

Effect of Flashing on Spiders' Prey Choice

Spiders were significantly more likely to attack cricket prey positioned next to a flashing LED than cricket prey positioned next to an unlit LED (exact binomial test: $N = 29$, $P = 0.03$). Spiders also attacked cricket prey positioned next to a flashing LED significantly more often than cricket prey positioned next to a steadily glowing LED (binomial test: $N = 25$, $P = 0.007$).

Effect of Flashing on Avoidance of Unpalatable Prey over Multiple Encounters

During the first trial, spiders were equally likely to attack unpalatable, nonbioluminescent *E. corrusca* fireflies that were presented with either a flashing or an unlit LED (Fisher's exact test, $N = 30$, $P = 0.49$; Fig. 1). By the seventh trial, however, attack rates

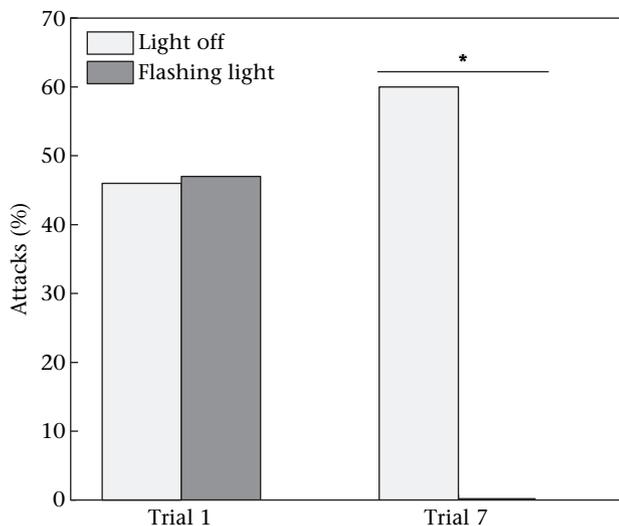


Figure 1. Percentage of attacks by *Phidippus princeps* spiders on unpalatable prey (*Ellychnia corrusca* nonluminescent fireflies) that were presented next to LEDs that were either flashing (light grey) or unlit (dark grey). Seven consecutive 10 min trials were interspersed with 30 min rest periods. Results shown for the first and last trials ($N = 30$ spiders in each). Asterisk indicates $P < 0.05$ by contingency test.

differed significantly between spiders tested with flashing and unlit lights (Fisher's exact test: $N = 30$, $P < 0.007$). Spiders that had been offered prey accompanied by an unlit LED maintained similar attack rates (53% versus 47% during their first trial; Fig. 1). In contrast, when prey were presented with a flashing LED, spider attack rate was reduced from 60% to 0% by the seventh trial. In all instances, *E. corrusca* fireflies attacked by spiders were rejected after contact and released. The fireflies appeared unharmed by the attack.

In contrast, when tested with palatable *D. hydei*, spiders in the two light conditions did not differ in their behaviour. In the non-flashing treatment, five spiders attacked prey in the first trial but not the last, six attacked in the last trial but not the first, and nine attacked in both trials. In the flashing treatment, three spiders attacked prey in the first trial but not the last, five attacked prey in the last trial but not the first, and 12 attacked in both trials. We found no difference between the behaviour of spiders in the flashing and nonflashing conditions in either the first trial (McNemar's exact test: $\chi^2_1 = 0$, $P = 1$) or the last trial ($\chi^2_1 = 0.25$, $P = 0.625$).

DISCUSSION

Jumping spiders may interact with fireflies both during the day and at dusk. Under daylight conditions in the laboratory, we found that adult fireflies (*Photuris* sp.) flashed readily when disturbed, but they never flashed when undisturbed. Thus, diurnally active jumping spiders may elicit flashes from resting fireflies they encounter while hunting or may be attracted to a flashing firefly that has been disturbed in some other way. Furthermore, we found that *P. audax* remains active during the dusk activity period of the fireflies and interacts with fireflies during this period. When 13 spiders were allowed to interact with fireflies during the fireflies' active period (between 2030 and 2100 hours), all 13 attacked or touched a firefly. Fireflies flashed in response to 48% of the attacks and 20% of the touches.

We then examined three hypotheses concerning the role of bioluminescent flashing in mediating behavioural interactions between fireflies and a jumping spider predator, *P. princeps*. We found that spiders did not startle due to the sudden onset of a flashing light, nor did they stop their predatory attack. We also found that flashing lights influenced the spiders' prey choice. Spiders preferred prey secured next to a flashing light compared with prey secured next to a light that was always off or on. Spiders stopped attacking unpalatable prey associated with a flashing light after seven trials, but they continued to attack unpalatable prey associated with an unlit light.

We tested whether bioluminescent signals elicit a startle response from spiders, as has been found in other species. For example, the sudden flashing of dinoflagellates reduces predation by copepod predators (Esaías & Curl 1972). However, we found no evidence that flashing serves to startle *P. princeps* jumping spiders, as sudden activation of a flashing light did not deter any spiders from successful predatory attacks on palatable prey. However, because multiple authors have observed that firefly flashing elicits startle responses from other potential predators (rats, geckos and chickens; reviewed in Lloyd 1973), expanding these tests to additional predators would be useful.

Our results also showed that jumping spiders were significantly more likely to attack palatable prey associated with flashing LEDs compared with prey associated with either unlit or steadily glowing LEDs. In vertebrates, responses to flashing lights vary. House mice (Underwood et al. 1997) and little brown bats (Moosman et al. 2009) are attracted to artificial prey associated with flashing lights. In contrast, wild-caught *B. bufo* toads (De Cock & Matthysen 1999) and big brown bats are both reluctant to attack glowing lures. We are aware of only one study of firefly flashing and invertebrate predators: predatory *Photuris* fireflies are attracted to flashing LEDs (Woods et al. 2007). Thus, one possible cost of bioluminescent

firefly flashes may be an increased risk of attack by some vertebrate and invertebrate predators.

Although bioluminescent flashing may attract *P. princeps* spiders, it may also facilitate learning about unpalatable prey, which has not yet been demonstrated in a terrestrial invertebrate predator. In our experiment, spiders that captured adult *E. corrusca* fireflies quickly dropped them, apparently unharmed; these and many other fireflies secrete defensive chemicals through reflex bleeding when they are disturbed (reviewed in Lewis & Cratsley 2008). During our dusk trials, spiders all touched or attacked fireflies but then dropped them unharmed. Forty-eight per cent of attacks were followed by flashing, providing the spiders an opportunity to associate the flash with the distasteful firefly.

In the presence of a flashing LED, spiders ceased attacking unpalatable prey within seven trials, while spiders exposed to an unlit LED continued to attack. At least three different mechanisms may have caused the decline in attacks in the presence of a flashing light. First, the flashing light may have harmed the spiders' vision, making them less likely to see prey in later trials. This is unlikely given that, in a separate test, we found that spiders that had been exposed to a flashing light easily captured *D. hydei*. Second, the flashing light may have acted as a contextual cue (i.e. a background cue that enhances retention of a particular association). This interpretation is supported by previous work showing that *P. princeps* attends to visual background cues when learning to avoid distasteful prey (Skow & Jakob 2006). Finally, the flashing light may have increased the spiders' arousal and thus enhanced their attention to taste cues.

These results, together with previous work on mice (Underwood et al. 1997) and toads (De Cock & Matthysen 2003), suggest that bioluminescence can serve as an aposematic signal helping both vertebrate and invertebrate predators learn to identify and subsequently avoid unpalatable prey. The importance of learning in nature depends on how often spiders encounter distasteful and palatable fireflies. Chemical defences have been studied in very few firefly taxa, but they appear to vary both in their composition and effectiveness against predators (reviewed in Lewis & Cratsley 2008). Steroidal pyrones called lucibufagins have been found in adults of two nocturnal North American fireflies (*Photinus ignitus* and *Photinus marginellus*) and a diurnal firefly (*Lucidota atra*; Gronquist et al. 2006), and these compounds have been shown to deter predation by *P. audax* jumping spiders (Eisner et al. 1978). It is possible that lucibufagins may also be responsible for *E. corrusca*'s unpalatability to *P. princeps* spiders. However, in *Photuris* fireflies the newly emerged adults entirely lack lucibufagins and are readily consumed by *P. audax* jumping spiders; *Photuris* fireflies only acquire protection against their own predators after they have eaten fireflies in the genus *Photinus* (Eisner et al. 1997). Thus, jumping spiders and other naïve predators are likely to encounter a mixture of toxic and nontoxic firefly prey, which may slow the rate at which they learn to avoid flashing prey.

In summary, in the context of jumping spider predation, bioluminescent flashing may increase predation risk for nontoxic fireflies, especially those not in sympatry with toxic fireflies: spiders were attracted to and not startled by flashing lights. However, bioluminescent flashing can reduce predator attacks on toxic fireflies by improving the ability of spiders to learn about them. Thus, the usefulness of bioluminescence as an aposematic signal should depend on the relative frequency of nontoxic and toxic prey in the local firefly fauna, as well as on the composition of local predator guilds.

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References

- Agudo, F. & Marin, A. 2007. Warning coloration associated with nematocyst-based defenses in aeolidioid nudibranchs. *Journal of Molluscan Studies*, **73**, 23–28.
- Biggley, W. H., Lloyd, J. E. & Seliger, H. H. 1967. The spectral distribution of firefly light II. *Journal of General Physiology*, **50**, 1681–1692.
- Blest, A. D. 1957. The function of eyespot patterns in the Lepidoptera. *Behaviour*, **11**, 209–256.
- Bocakova, M., Bocak, L., Hunt, T., Teravainen, M. & Vogler, A. P. 2007. Molecular phylogenetics of Elateriformia (Coleoptera): evolution of bioluminescence and neoteny. *Cladistics*, **23**, 477–496.
- Branham, M. A. & Wenzel, J. W. 2001. The evolution of bioluminescence in cantharoids (Coleoptera: Elateroidea). *Florida Entomologist*, **84**, 565–586.
- Buschman, L. L. 1988. Light organs of immature fireflies (Coleoptera: Lampyridae) as eye-spot/false-head displays. *Coleopterists Society*, **42**, 94–97.
- Carducci, J. & Jakob, E. 2000. Rearing environment affects behaviour of jumping spiders. *Animal Behaviour*, **59**, 39–46.
- Daniel, W. 1978. *Applied Nonparametric Statistics*. Boston: Houghton Mifflin.
- De Cock, R. 2009. Biology and behaviour of European lampyrids. In: *Bioluminescence in Focus: a Collection of Illuminating Essays* (Ed. by V. B. Meyer-Rochow), pp. 163–200. Kerala, India: Research Signpost.
- De Cock, R. & Matthysen, E. 1999. Aposematism and bioluminescence: experimental evidence from glow-worm larvae (Coleoptera: Lampyridae). *Evolutionary Ecology*, **13**, 619–640.
- De Cock, R. & Matthysen, E. 2003. Glow-worm larvae bioluminescence (Coleoptera: Lampyridae) operates as an aposematic signal upon toads (*Bufo bufo*). *Behavioral Ecology*, **14**, 103–108.
- Edwards, G. B. 2004. Revision of the jumping spiders of the genus *Phidippus* (Araneae: Salticidae). *Occasional Papers of the Florida State Collection of Arthropods*, **2**, viii–156.
- Edwards, G. B. & Jackson, R. R. 1993. Use of prey-specific predatory behaviour by North American jumping spiders (Araneae, Salticidae) of the genus *Phidippus*. *Journal of Zoology*, **229**, 709–716.
- Eisner, T., Wiemer, D. F., Haynes, L. W. & Meinwald, J. 1978. Lucibufagins: defensive steroids from the fireflies *Photinus ignitus* and *P. marginellus*. *Proceedings of the National Academy of Sciences, U.S.A.*, **75**, 905–908.
- Eisner, T., Goetz, M. A., Hill, D. E., Smedley, S. R. & Meinwald, J. 1997. Firefly “femmes fatales” acquire defensive steroids (lucibufagins) from their firefly prey. *Proceedings of the National Academy of Sciences, U.S.A.*, **94**, 9723–9728.
- Esaias, W. E. & Curl, H. C., Jr. 1972. Effects of dinoflagellate bioluminescence on copepod ingestion rates. *Limnology and Oceanography*, **17**, 901–906.
- Freed, A. N. 1984. Foraging behaviour in the jumping spider *Phidippus audax*: bases for selectivity. *Journal of Zoology*, **203**, 49–61.
- Fu, X. 2006. Reflex-bleeding in the firefly *Pyrocoelia pectoralis* (Coleoptera: Lampyridae): morphological basis and possible function. *Coleopterists Bulletin*, **60**, 207–215.
- Gronquist, M., Schroeder, F. C., Ghiradella, H., Hill, D., McCoy, E. M., Meinwald, J. & Eisner, T. 2006. Shunning the night to elude the hunter: diurnal fireflies and the “femmes fatales”. *Chemoecology*, **16**, 39–43.
- Guilford, T. 1986. How do ‘warning colours’ work? Conspicuousness may reduce recognition errors in experienced predators. *Animal Behaviour*, **34**, 286–288.
- Guilford, T. & Cuthill, I. 1989. Aposematism and bioluminescence. *Animal Behaviour*, **37**, 339–341.
- Haddock, S. H. D., Moline, M. A. & Case, J. F. 2010. Bioluminescence in the sea. *Annual Review of Marine Science*, **2**, 443–493.
- Jakob, E. M., Skow, C. D., Haberman, M. P. & Plourde, A. 2007. Jumping spiders associate food with color in a T maze. *Journal of Arachnology*, **35**, 487–492.
- Jakob, E. M., Skow, C. & Long, S. 2011. Plasticity, learning and cognition. In: *Spider Behaviour: Flexibility and Versatility* (Ed. by M. E. Herberstein), pp. 307–333. Cambridge: Cambridge University Press.
- Lewis, S. M. & Cratsley, C. K. 2008. Flash signal evolution, mate choice, and predation in fireflies. *Annual Review of Entomology*, **53**, 293–321.
- Lindström, L. A., Alatalo, R. V. & Mappes, J. 1999. Reactions of hand-reared and wild-caught predators toward warningly colored, gregarious, and conspicuous prey. *Behavioral Ecology*, **10**, 317–322.
- Lloyd, J. E. 1965. Aggressive mimicry in *Photuris*: firefly femmes fatales. *Science*, **149**, 653–654.
- Lloyd, J. E. 1966. Studies on the flash communication system in *Photinus* fireflies. *University of Michigan Miscellaneous Publications*, **130**, 1–95.
- Lloyd, J. E. 1973. Firefly parasites and predators. *Coleopterists Bulletin*, **27**, 91–106.
- McDermott, F. E. 1964. The taxonomy of the Lampyridae. *Transactions of the American Entomological Society*, **90**, 1–72.
- Moosman, J. P. R., Cratsley, C. K., Lehto, S. D. & Thomas, H. H. 2009. Do courtship flashes of fireflies (Coleoptera: Lampyridae) serve as aposematic signals to insectivorous bats? *Animal Behaviour*, **78**, 1019–1025.
- Prudic, K. L., Skemp, A. K. & Papaj, D. R. 2006. Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behavioral Ecology*, **18**, 41–46.
- Richman, D. B. & Jackson, R. R. 1992. A review of the ethology of jumping spiders (Araneae, Salticidae). *Plant Pathology*, **9**, 33–37.

- Rooney, J. A. & Lewis, S. M.** 2000. Notes on the life history and mating behavior of *Ellychnia corrusca* (Coleoptera: Lampyridae). *Florida Entomologist*, **83**, 324–334.
- Rowe, C. & Guilford, T.** 2000. Aposematism: to be red or dead. *Trends in Ecology & Evolution*, **15**, 261–262.
- Ruxton, G. D., Sherratt, T. N. & Speed, M. P.** 2004. *Avoiding Attack: the Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*. Oxford: Oxford University Press.
- Sagegami-Oba, R., Takahashi, N. & Oba, Y.** 2007. The evolutionary process of bioluminescence and aposematism in cantharoid beetles (Coleoptera: Elateroidea) inferred by the analysis of 18S ribosomal DNA. *Gene*, **400**, 104–113.
- Sivinski, J.** 1981. The nature and possible functions of luminescence in coleoptera larvae. *Coleopterists Bulletin*, **35**, 167–179.
- Skow, C. & Jakob, E.** 2006. Jumping spiders attend to context during learned avoidance of aposematic prey. *Behavioral Ecology*, **17**, 34–40.
- Underwood, T. J., Tallamy, D. W. & Pesek, J. D.** 1997. Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). *Journal of Insect Behavior*, **10**, 365–370.
- Widder, E. A.** 2010. Bioluminescence in the ocean: origins of biochemical, chemical, and ecological diversity. *Science*, **328**, 704–708.
- Williams, F. X.** 1917. Notes on the life-history of some North American Lampyridae. *Journal of the New York Entomological Society*, **25**, 11–33.
- Woods, W. A., Hendrickson, H., Mason, J. & Lewis, S. M.** 2007. Energy and predation costs of firefly courtship signals. *American Naturalist*, **170**, 702–708.