

Correspondences

Bioluminescent aposematism in millipedes

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Bioluminescence — the ability of organisms to emit light — has evolved about 40–50 times independently across the tree of life [1]. Many different functions for bioluminescence have been proposed, for example, mate recognition, prey attraction, camouflage, and warning coloration. Millipedes in the genus *Motyxia* produce a greenish-blue light at a wavelength of 495 nm that can be seen in darkness [2]. These detritivores defend themselves with cyanide, which they generate internally and discharge through lateral ozopores [3]. *Motyxia* are an ideal model system to investigate the ecological role of bioluminescence because they are blind, thus limiting their visual signalling to other organisms, for example predators. While the biochemical mechanisms underlying *Motyxia* bioluminescence have been studied in detail [2,4], its adaptive significance remained unknown [5,6]. We here show that bioluminescence has a single evolutionary origin in millipedes and it serves as an aposematic warning signal to deter nocturnal mammalian predators. Among the numerous examples of bioluminescence, this is the first field experiment in any organism to demonstrate that bioluminescence functions as a warning signal.

Aposematic colors warn potential predators of distasteful or unpleasant qualities, such as spines, venoms, or chemical explosions [7]. When disturbed, blind millipedes in the order Polydesmida generate a hydrogen cyanide toxin [3]. Many diurnal millipedes in this group display aposematic coloration in yellow, orange, or red [8]. In contrast, the members of the genus *Motyxia* (formerly *Luminodesmus*) are nocturnal and do not display conspicuous color in daylight. Instead,

Motyxia species are bioluminescent, producing a greenish-blue light that gradually intensifies when the millipede is disturbed (Figure 1).

Currently, eight of the 12,000 described millipede species are known to be bioluminescent [5,6]. Here, we show that these species comprise a single clade and are geographically restricted to three counties in California (for the phylogeny of *Motyxia* species and close relatives, see Supplemental information). Light from *Motyxia* originates in the exoskeleton and involves a photoprotein that contains a chromophore with porphyrin as its functional group [2,4]. The basic photogenic mechanism is more similar to that of the GFP-jellyfish, *Aequorea victoria*, than that of the more closely related firefly *Photinus pyralis* [4]. Nevertheless, the structure of the luminescent molecules remains unknown and their homologies to molecules of other animals are uncertain [2,4].

Several hypotheses for the function of bioluminescence in *Motyxia* have been suggested. One hypothesis states that luminescence serves as an aposematic signal, warning would-be predators of its noxiousness [6]. It has also been suggested that the bioluminescence serves no function at all [5], or even that it inadvertently attracts predators. Until now, these hypotheses have not been tested experimentally.

We used live and clay model millipedes in field trials (where *Motyxia*

naturally occur in California) to test the prediction that luminescent individuals are attacked less often than non-luminescent individuals. We collected 164 living millipedes of the species *Motyxia sequoiae* from Giant Sequoia National Monument in California, and painted the surface of half (82 individuals) with paint to conceal their bioluminescence. Using polymer clay, we also constructed 300 clay millipedes from a bronze cast of *M. sequoiae*. Clay models were covered with the same paint, and in half, a chemiluminescent pigment was mixed into the paint to generate luminescence. To capture the naturally patchy distribution of indigenous predators, we distributed living and model millipedes along separate transects, with a random distribution of luminescent and non-luminescent millipedes spaced five meters apart. Experiments were run over night, after which predation marks among luminescent and non-luminescent groups were tallied and statistically analyzed to evaluate the null hypothesis of parity between treatments.

Significantly more of the non-luminescent millipedes (both clay and live) were attacked than luminescent millipedes (Figure 1C–E). Nearly half (48.6%) of the non-luminescent clay millipedes were attacked, while only 22.4% of the luminescent models were attacked. Experiments conducted with live millipedes exhibited a similar attack pattern. Nearly one-fifth (17.9%) of the

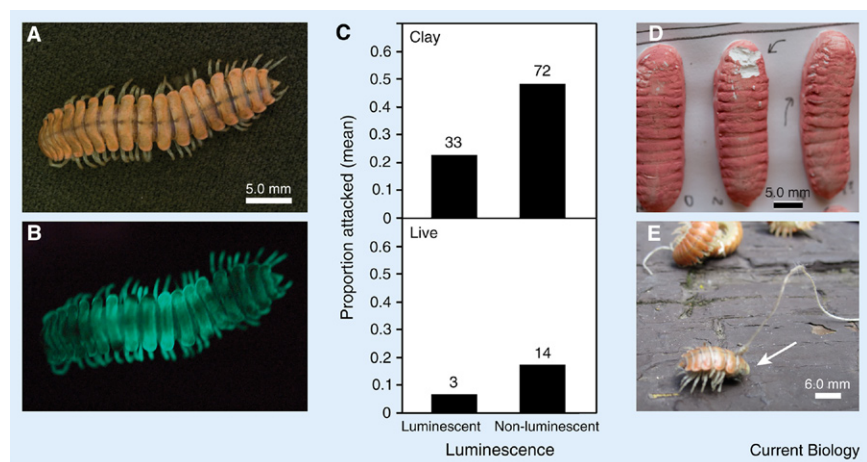


Figure 1. Millipede luminescence and predation. (A) *Motyxia sequoiae* photographed in natural light, and (B) entirely with light from bioluminescence; (C) Mean proportion of millipedes attacked versus luminescence. Number of individuals attacked above bars; (D) Rodent incisor marks in clay millipede; (E) Live millipede (arrow) with anterior segments 1–14 missing after predator attack.

non-luminescent live millipedes were attacked as opposed to only 4.0% of the luminescent individuals. The asymmetrical attack ratios between luminescent and non-luminescent groups differed significantly from the null hypothesis of a one-to-one ratio for clay millipedes (G-test, $G = 14.839$, 1 df, $P < 0.001$; exact binomial test $P < 0.001$) and for live millipedes ($G = 7.723$, 1 df, $P = 0.005$; exact binomial test $P = 0.013$). Rodents were found to inflict most of the predation marks, as indicated by oppositional incisor impressions on the clay surface (Figure 1D). Most nocturnal rodents can detect millipede luminescence by way of scotopic (night) vision, as a result of a reflective tapetum and a high density of rod photoreceptor cells in the retina [9]. While surveying the experiment site, small neotomine rodents, likely the southern grasshopper mouse (*Onychomys torridus*), were observed to be active nocturnally.

Our results demonstrate strong predatory avoidance of luminescent coloration, results which are inconsistent with the expectation that a luminescent object would attract nocturnal predators and initiate exploratory sampling. We found that non-luminescent millipedes were attacked more than twice as often. Thus, our experimental evidence indicates luminescence serves an aposematic function of deterring predators. Aposematism involves two main qualities: noxiousness and a signal of noxiousness. By using actual live millipedes in conjunction with clay models, our experiment indicates that these two main qualities (noxious chemical defense and luminescent signal), even when treated separately, function to deter predator attacks.

With our clay model experiment, we demonstrate that luminescence in and of itself, and no other cue such as odor or taste, repels predators. The live millipede data show that a luminescent millipede does not repel attack as well if the light is concealed. (Luminescent millipedes were attacked four times less often than their non-luminescent counterparts.) This finding suggests that visual luminescent cues are sufficient to deter predation.

However, non-visual cues, such as olfactory and gustatory (e.g., from chemical defenses in live millipedes), though not directly compared against

luminescence, also contribute to *Motyxia* aposematism. How much more protection is conferred by the combinatory effect of visual plus non-visual cues is uncertain, and how predators come to recognize *Motyxia* bioluminescence as an indication of their cyanogenic-based noxiousness remains unclear.

As with aposematic signals generally, the response to the signal may reflect avoidance learning, dietary conservatism (including neophobia), or unlearned avoidance that evolved in predator populations [10]. Future work will need to investigate how predators develop an avoidance of *Motyxia*.

A simple follow-up would be to repeat the experiment in a similar habitat outside the geographical range of bioluminescent millipedes. If allopatric predator populations similarly avoided bioluminescent millipedes, it might suggest that avoidance is rooted in neophobia, or a prior evolutionary association with bioluminescent millipedes that led to unlearned avoidance. If predator populations in allopatry do not avoid bioluminescent millipedes, then it would suggest there has been coevolution between predators and millipedes, which would provide a basis for future investigations into whether learning is involved. Bioluminescence in *Motyxia* provisionally represents an adaptive innovation, which is particularly impressive in terms of the small geographic and evolutionary scale on which it appears to have occurred.

Supplemental Information

Supplemental Information includes experimental procedures and a supplemental figure and can be found with this article online at doi:10.1016/j.cub.2011.08.012.

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