Shedding light on millipede evolution

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Motyxia bistipita emits a blue green light. Millipedes help maintain soil health by digesting decomposing leaves and depositing a rich manure that renders soil high in nitrogen. It is estimated that they annually contribute more than two tons of compost, about two small automobiles worth, to an acre of the forest. They can be thought of as little garbage men of the forest floor. Credit: Paul Marek, Entomology Department, Virginia Tech

As an National Science Foundation (NSF)-funded entomologist, Virginia Tech's Paul Marek has to spend much of his time in the field, hunting for rare and scientifically significant species. He's provided NSF with an inside look at a literal bug hunt, and the fascinating world of bioluminescence.

It is midnight on a winter night in 2013, and I am in the middle of a dark oak forest near San Luis Obispo, Calif. I am here, alone, with my millipede-finding ultraviolet flashlight and wanderlust to explore. My goal is to find millipedes that are bioluminescent—meaning they produce light through a chemical reaction.

As an entomologist who is fascinated by millipedes, I chose this site because back in 1967, two immature, inch-long bioluminescent millipedes were first discovered here. But between 1967 and 2013, this species had not been seen and its biology had remained a mystery.

In hot pursuit of a millipede

Looking for these 62-legged millipedes is an adventure. Their habitats are in giant sequoia forests, coastal live oak groves, and mountain skunk cabbage meadows in the High Sierra of California. Collecting them requires conducting fieldwork from dusk to dawn.

These millipedes not only glow in the dark but also fluoresce—meaning that when they are illuminated with ultraviolet (UV) light, they shine a brilliant green hue. So I search the dark forest with just the purple light of a UV torch, which makes fluorescent creatures glow.

Over by a small bubbling stream, I find one member of this enigmatic species, then two more (which were mating) and then three more. Their green fluorescence shines brightly under the UV light. I switch the light off and the millipede disappears; I turn it back on and it reappears as a glowing beacon of lime green.

I excitedly, but gently, scoop up the millipedes to avoid disturbing them, which could cause them to ooze cyanide as a defense mechanism. I must place each of them into a small plastic cup with a bit of soil and leaves that provide moisture.

The millipedes that I seek hold clues about the evolution of bioluminescence and how this trait, which functions as a deterrent to predators, is rare evolutionarily and geographically.

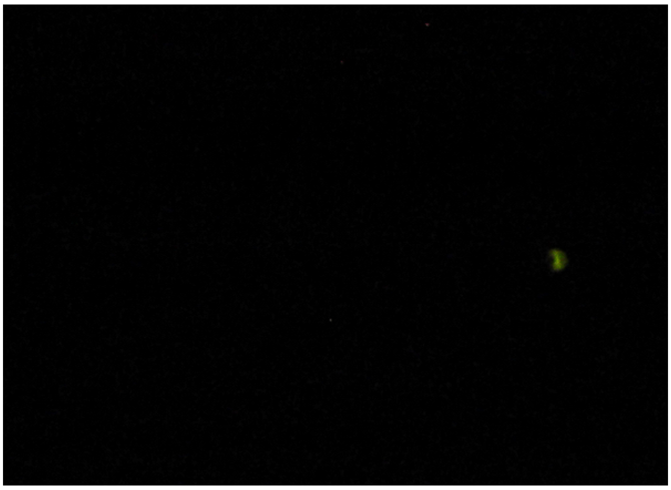
My research team, which is funded by NSF, explores the evolution of bioluminescence in a genus known as Motyxia, the only millipedes in North America that are known to be bioluminescent.

In a previous article for NSF, I explained that Motyxia's "glow means no!" to predators. That is, the green glow of nocturnal Motyxia—which are exclusively found in the Sierra Nevada Mountains of California—wards off nocturnal predators. Motyxia's bright coloring warns predators that when these millipedes feel threatened, they ooze toxins, including hydrogen cyanide, an extremely poisonous gas.

However my recent research indicates that millipedes may not have always used bioluminescence as a defense mechanism. Rather, bioluminescence may have originated in a millipede species named Xystocheir bistipita for an entirely different function and slowly evolved into its current defensive function for Motyxia.

All in the family

Xystocheir bistipita, which was not known to be bioluminescent, had not been seen in about 50 years until I recently rediscovered this species in the oak forest near San Luis Obispo. When I rediscovered it, I surprisingly found that this species does, in fact, emit a faint glow.



A railroad worm from Madera Canyon, Ariz., exhibits 8 pairs of bioluminescent lamps on its abdomen and a lamp behind its brain. The role of bioluminescence in this animal is uncertain, but it may be a warning to deter nocturnal predators or a social signal to notify cannibalistic siblings to avoid eating their kin. Credit: Paul Marek, Entomology Department, Virginia Tech

In order to provide a context to the evolution of bioluminescence, my team and I sequenced the DNA of Xystocheir bistipita, and positioned it on an evolutionary tree with other species of Motyxia and their closest relatives. These and other analyses showed that Xystocheir bistipita should now be classified in the genus Motyxia along with other glowing millipedes. So in honor of its family ties, I gave Xystocheir bistipita a new name: Motyxia bistipita.

Brightening up

Our analyses also showed that the faint bioluminescence of the low-lying cousins of Motyxia bistipita—which I'll refer to as simply M. bistipita—represents an older trait than the brighter bioluminescence of their mountain relatives. In addition, millipede species that live at higher elevations exhibit the brightest bioluminescence.

Furthermore, millipedes with brighter bioluminescence have larger cyanide glands, suggesting that millipede toxicity may be linked to the intensification of their bioluminescence.

These discoveries provide the bases for a possible explanation of the evolutionary origins of bioluminescence in millipedes. Over time, bioluminescence gradually escalated from the faintly glowing species of Motyxia that live at low elevations to the brighter and more highly toxic species of Motyxia that live at high elevations.

This evolution might have occurred because millipedes that live at high elevations share their habitat with many more predators than do lower-elevation millipedes. Therefore, they need a brighter glow to advertise to predators the greater toxicity of their cyanide weapon. The more glow, the more emphatic the "No!"

Our analyses of the chemical reaction used by M. bistipita to generate its faint glow suggests that this species might not have originally acquired bioluminescence as a defense mechanism. Rather, it might have acquired its faint glow to help adapt to the dry heat of its habitat—before other Motyxia acquired bioluminescence.

Evidence for this idea includes the particular chemical reaction that M. bistipita uses to create light. This reaction neutralizes potentially harmful chemical byproducts, such as peroxides, which the millipede's body produces when it metabolizes oxygen in hot weather. Bioluminescence by M. bistipita thereby protects the millipede from what would otherwise potentially threaten it in a hot habitat.

According to my explanation for the origins of bioluminescence in millipedes, as their evolution progressed and Motyxia colonized higher elevations that support more predators, this millipede repurposed and intensified its glow as a way to warn predators of its greater toxicity.

These findings show that even seemingly complex, intricate traits we see today may have evolved through many small steps, and from an original function unrelated to its present day role. These findings also provide insights into how the spectacular biodiversity of our planet evolved.

Different functions in different organisms

In addition providing the adaptive and defensive functions discussed here, bioluminescence also serves more than 10 other functions for various organisms.

For example, it fills other types of defensive roles, from creating smoke screens and fireworks that startle predators and give prey precious time to escape, to lighting up body parts that detach to distract predators from the organism's vulnerable structures. It can also play offensive roles, serving as a lure for predators, confusing prey and providing illumination.

Railroad worms of the genus Phrixothrix have a red bioluminescent lamp in their head and eight pairs of green lamps lining the sides of their abdomens. Because this light is invisible to its prey (since most insects are unable to see in red light), it has been hypothesized that the carnivorous Phrixothrix uses its bioluminescence as its own "private" illumination source—although it is not yet known whether Phrixothrix can see its own red light.

Many remaining mysteries

Bioluminescent organisms produce light through chemical reactions involving hundreds of various unrelated enzymes and other light-producing proteins that evolved independently in various groups of organisms.

But so far, researchers understand the biochemical reaction that produces light in only a handful of species. We know the most about how light is produced by fireflies, specifically the big dipper firefly, Photinus pyralis.

In addition, new bioluminescent creatures are still frequently being discovered—particularly on land and sea in tropical areas, and most often in coral reefs, which are among the most diverse ecosystems on Earth.

With so much still to discover about bioluminescence, various aspects of this phenomena form the bases of vibrant and active fields that are currently being researched by biochemists, entomologists, marine biologists, and engineers.

Societal benefits

Research into bioluminescence has already produced many practical applications. Those include:

- Helping to revolutionize our understanding of cancer by literally shining a light on what researchers could not see any other way. Scientists can make particular types of cells in laboratory animals bioluminescent—including cancer cells. Using high-tech imaging tools, researchers can track the movement of these bioluminescent cells by following the light they emit. This technique enables researchers to view proliferating and metastasizing bioluminescent cancer cells in real time, and thereby gain important insights about how cancer grows.
- With NSF funding, Jennifer Prescher of the University of California-Irvine is creating a new way to view specific biological events,

including those that involve cancer growth. Her work involves bioluminescent tools that produce light only when cells come into direct contact with one another.
- Those tools could, for example, be applied to tumor cells and cancer-fighting immune system cells. When the different types of cells are separate, they would generate minimal light or none at all. But when one type of cell makes contact with the other, they would light up. That luminescence could help researchers test potential cancer therapies, as it would signal that cancer-fighting cells were able to successfully reach cancer cells, and could be useful for cancer therapies.
- Revolutionizing how we study cells. NSF-funded biologist Osamu Shimomura's search for the source of the green glow of the jellyfish Aequorea victoria led him to discover a protein called green fluorescent protein (GFP). GFP is now widely used in biological and biomedical research as a fluorescent tag.
- Researchers can label a particular type of biomolecule of interest, such as a protein, antibody, or amino acid, by chemically attaching GFP to it. They may then track the labelled biomolecules by following the green fluorescence attached to them.
- Such tagging helps researchers track specific biological activities, such as the production of insulin and the movement of HIV proteins. In 2008, Shimomura, along with Martin Chalfie and Roger Tsien, received the Nobel Prize in Chemistry for the discovery and development of GFP.
- Helping us find cellular activity. Enzymes responsible for bioluminescence in fireflies are used by researchers to detect ATP (adenosine triphosphate), which is an essential substance for living cells. Because ATP is ubiquitous in cells, its presence is an indicator of biological activity and can be used to determine the cleanliness of drinking water, rate of fermentation, and microbial activity of soils.
- Providing new ways to find important ions. Many types of physiological processes trigger changes in concentrations of intracellular calcium ions, an essential component of biological processes. One way to track intracellular calcium concentrations in is to insert a type of photoprotein known as aequorininto cells. Like GFP, aequorin is derived from the jellyfish Aequorea victoria. Aequorin reacts with calcium, emitting light when it does so, which signals the element's concentration.

The next time you notice glowing animals, remember there is still much to learn about why and how their wondrous lights evolved, and how research about them may benefit society in many and varied ways.

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