Bioluminescence

Nature's Bright Idea

by Shreeram Akilesh '00

The bright green flash of a firefly is a fascinating sight on lazy summer evenings, but the phenomenon of living creatures producing light, also known as bioluminescence, is far more prevalent than may be expected and deserves our critical investigation. We are all familiar with the fact that green plants harvest light to produce chemical energy. However, the reverse is also true. Creatures ranging from unicellular bacteria to vertebrates use chemical energy to produce light (Wilson & Hastings, 1998). Most light-producing organisms are marine creatures, while only a tiny proportion of land-dwellers possess this capability. The reasons for this skewed distribution are unclear, but evidence suggests that the nature and evolution of bioluminescence as a phenomenon may have something to do with it. This paper will first describe the chemical/physiological phenomenon of bioluminescence and how various organisms use it for diverse purposes. Then it will discuss how these characteristics may have effected the present-day distribution of bioluminescence that strongly favors the marine habitat.

Bioluminescence is not to be confused with fluorescence, though the two phenomena are closely related. In fluorescence, a molecule absorbs a photon with enough energy to elevate an electron to a higher energy level. Following one or more non-radiative or non-visible radiative steps to lower energy level(s), the electron finally drops an energy gap resulting in the release of energy from the system in the form of a visible photon. Consequently, fluorescence is powered by the input of high-energy radiation (Williamson & Cummins, 1983). Bioluminescence, on the other hand, is created by chemical energy. The enzyme luciferase catalyzes the oxidation of an organic molecule, luciferin, to a high-energy form, oxyluciferin, that then relaxes with the subsequent release of enough energy to produce a visible photon[1]. This process is nearly 100% efficient generating little or no heat (Bioluminescence, 2000 February 27). The luciferins are themselves fluorescent molecules and it is here that we see the analogy between bioluminescence and fluorescence—they are very similar processes with different energy inputs. In fluorescence, the original molecule is restored following the fluorescent emission; in bioluminescence, the expended oxy-

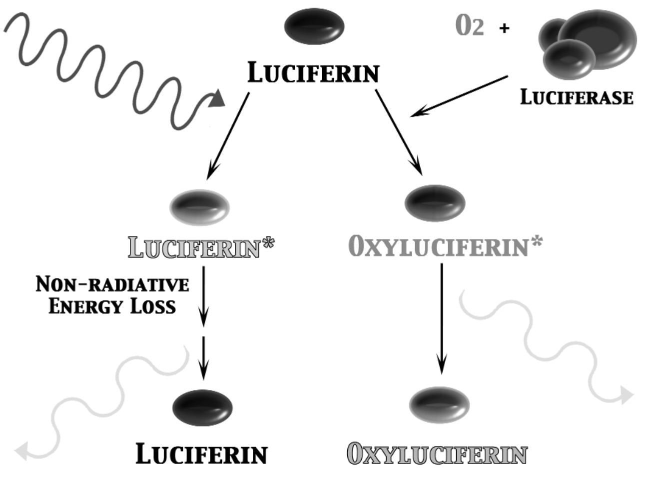


Figure 1. Fluorescence and Bioluminescence. The left side of the flowchart depicts the mechanism of fluorescence: a high energy photon excites ground-state luciferin to an excited state which then loses energy non-radiatively (or as invisible radiation) and then drops to the original ground-state luciferin with the release of a visible photon.[2] The right side of the flowchart shows the mechanism of bioluminescence: luciferase catalyzes the oxidation of luciferin to excited oxyluciferin that then relaxes to produce a visible photon. Ground state oxyluciferin is distinct from the original luciferin. The wavelengths produced by a single luciferin from both these processes may be different.

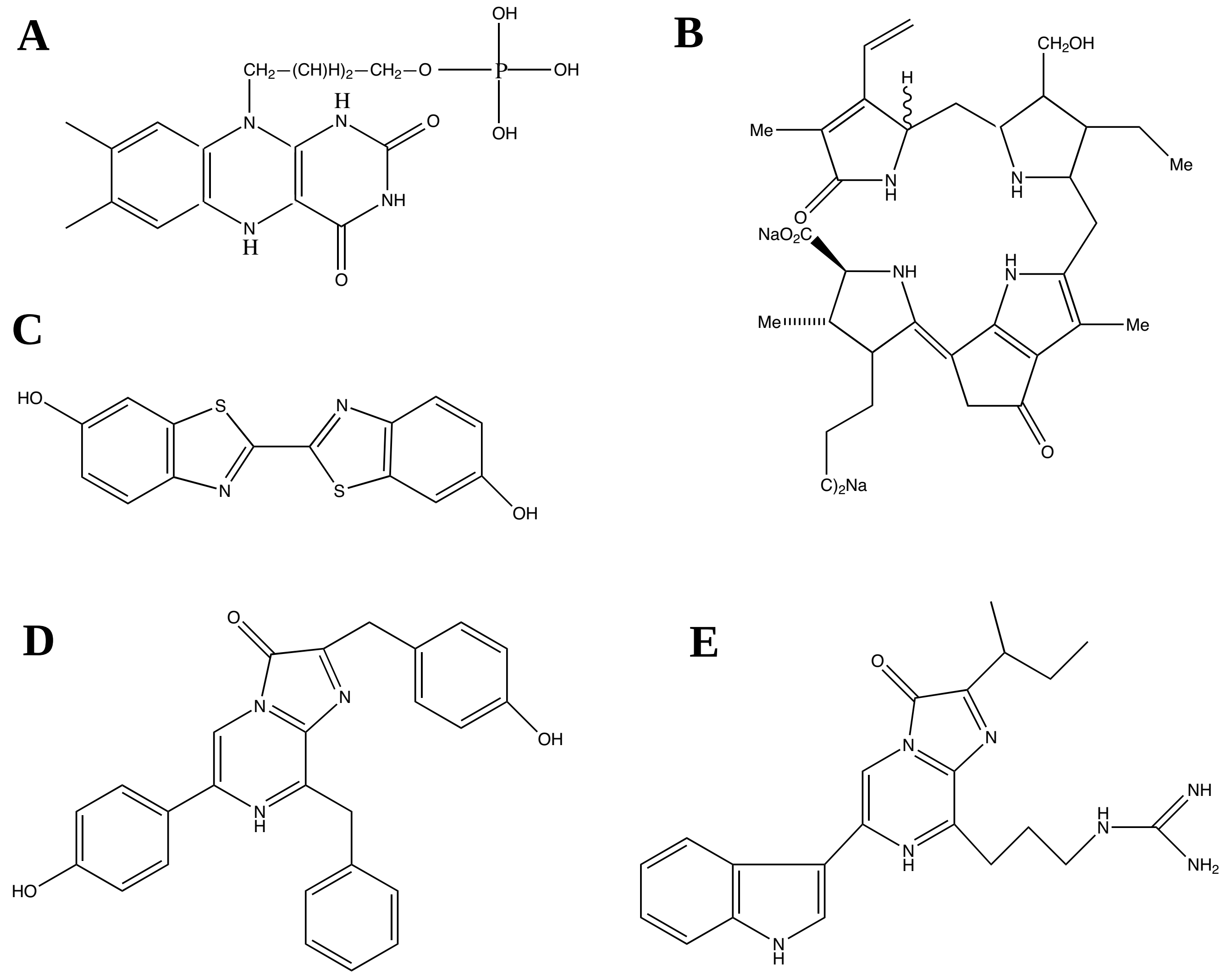


Figure 2. Chemical structures of different luciferins. (BL Web: Chemistry details, 2000). Luciferins from different biological sources are chemically unrelated. They tend to be polycyclic aromatic compounds that are inherently fluorescent since their molecular orbitals have multiple energy levels. **A**: Bacterial luciferin, a derivative of riboflavin; **B**: Dinoflagellate luciferin, structurally related to chlorophyll; **C**: Firefly luciferin, requires ATP for bioluminescence; **D**: Coelenterazine, an extremely common luciferin found in several species; **E**: Vargulin, found in some shrimp species.

luciferin is distinct from the original luciferin and must be replaced either by biochemical synthesis or from the diet (BL Web: Chemistry, 2000). Figure 1 depicts the two phenomena and their similarities and differences.

The spectrum of the radiation observed in bioluminescence is very broad and ranges from violet to red, but blue/blue-green is by far the most common. The reason can be attributed to bioluminescence being largely prevalent in the oceans where the seawater is especially transparent to blue light (How do animals make light?, 2000). Light of other colors does not travel far in water and consequently, evolution has selected for blue bioluminescence. The primary determinant of color in bioluminescence depends heavily on the structure of the luciferin itself (Wilson & Hastings, 1998). Different luciferins will produce different colors upon activation as a result of their chemical structure. Examples of the chemical structures of several luciferins are shown in Figure 2.

The color can also be influenced by the three-dimensional structure and amino acid sequence of the luciferase protein. Figure 3 depicts the 3D structure of bacterial and firefly luciferase obtained from the Protein Data Bank (http://www.rcsb.org/pdb) and analyzed with RasMol. It is readily apparent that the two enzymes have very different structures that in turn affect their respective luciferins in different ways. The presence of accessory proteins or other chromophores can affect the spectrum of radiated light by influencing either the microenvironment in which the luciferin is situated or by intercepting and re-radiating the energy released by the luciferin (Wilson & Hastings, 1998).

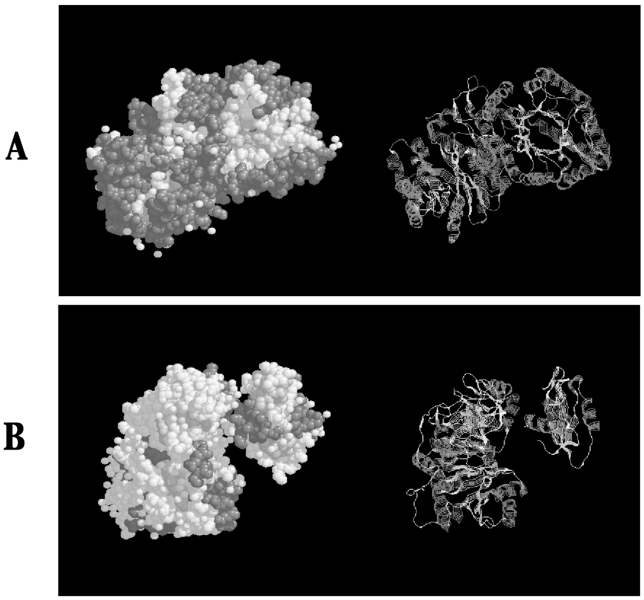
The chemical basis of bioluminescence is conceptually straightforward, but the physiological control of the phenomenon is far more complicated. This would include where in the cell the light producing chemicals are stored; how they are regenerated; how they are controlled or induced; and in the cases of rapid flashes, how the light signal can be quickly turned on and off. In truth, the range of biological diversity in the physiological control of bioluminescence is too staggering for this article to elaborate in detail. The simplest characterization would be that there are a wide variety of mechanisms for subcellular localization, signal induction, and chemical regeneration. In bioluminescent bacteria, the photochemicals are present throughout the cytoplasm, and they glow continuously without flashing. Fireflies have a highly structured specialized light-producing organ (the lantern) that is probably regulated by the flow of oxygen to it. Dinoflagellates (unicellular plants, explained in more detail below) have special light producing organelles (scintillons) that are regulated by shifts in pH. Lastly, it is thought that some bioluminescent worms control light flashes by controlling calcium entry into cells (Wilson & Hastings, 1998). Thus, while the basic chemical principle of bioluminescence is preserved across diverse species, the physiological implementation of bioluminescence is extremely variegated.

Diverse organisms have developed bioluminescence as a means to protect themselves. One such organism is the dinoflagellate which can bioluminesce and produce beautiful displays during red tide blooms. Dinoflagellates use their bioluminescence as a defense mechanism. Dinoflagellates follow a circadian rhythm of bioluminescence—they only flash during the night or during their ‘dark’ cycle. At night, when they are mechanically disturbed, for example by a wave or the motion of a nearby fish, they trigger a light flash. The logic behind this event is that the light flash both distracts the primary predator of the dinoflagellate and can also make the primary predator visible to a larger secondary predator. Consequently, the primary predator quickly evacuates for fear of being eaten (Latz, 2000).

Another example of a bioluminescent defense mechanism is the case of the shrimp species *Systellaspis debilis* (Herring, 2000). This particular species squirts a cloud of symbiotic bioluminescent bacteria at an attacker similar to a smokescreen. In the perpetual darkness of the deep ocean, the bright bioluminescent flash blinds and disorients the attacker, giving the shrimp enough time to escape. Bioluminescence is also used for camouflage at mid-ocean depths where light is still available. In fact close to 90% of the animals in the 200-1000 m depth range (the mesopelagic zone) are bioluminescent (Bioluminescence, 2000 February 23). During the day, some animals produce a faint blue bioluminescence on their underbellies that blends with the blue colors of the surface water and masks the silhouette of the animal from predators lurking below. This decreases the chances of the animal being seen by the predator and confers a survival advantage (Why do animals make light? Defense, 2000). See figure 4 for an illustration of this strategy. The few cases of terrestrial bioluminescence (besides the case of fireflies, which is discussed later) include bioluminescent millipedes, centipedes and worms. In these cases, their bioluminescence may serve as a defense mechanism warning potential predators that they do not taste pleasant (Bioluminescence, 2000 February 27).

In addition to defense, organisms also use bioluminescence to procure food. An extremely unusual example of this is the 2 foot long cookie-cutter shark, *Isistius brasiliensis*. To understand how the small cookie-cutter shark is able to prey on much larger predators, we must examine its use of bioluminescence (Cookie-cutter shark, 2000).

Figure 3: Luciferase Structures
A Bacterial Luciferase **B** Firefly Luciferase



During the day, the shark swims in shallow waters and like other species that use bioluminescence for camouflage, the underbelly of the shark is covered with light producing organs. However, a patch of skin near the throat of the shark does not have these bioluminescent organs. Consequently, when viewed from below, the shark displays the silhouette of a small fish instead of a larger shark (see Figure 4). This attracts large predators. As they move in for the kill, the shark quickly twists, suctions itself to the larger fish, and clamps into its flesh with its teeth. The twisting motion of the shark allows a cookie-shaped chunk of flesh to be bitten off from the larger predator as it lunges forward.

Another interesting adaptation of bioluminescence as an aid to feeding is the case of deep sea fishes such as angler fish and black dragonfish. The angler fish has a bioluminescent bait at the end of an appendage on its head. The bait attracts unsuspecting prey close enough to the angler fish to be eaten (Why do animals make light? Finding food., 2000). The black dragonfish has an even more fascinating adaptation. This fish actually has two different bioluminescent organs—one that produces a blue-green light and another, just under the eye, that produces a long wavelength red light. Since most deep sea animals cannot see the red light produced by the dragonfish, the creature essentially has an infrared sight with which it can see its prey without alerting them. While the red light does not travel very far, it is nonetheless a vital adaptation for the dragonfish (BL Web: Malacosteid Fish and Red Luminescence, 2000).

The last major use of bioluminescence is in attracting and signaling potential mates. On land, fireflies[3] attract members of the opposite gender of the same species by varying their light output. The light production can be a continuous glow, a certain frequency of flashes, or a sequence of repeated flashes that can alternate in frequency. Signaling by light allows fireflies to choose a compatible mate and also allows them to discern the health of the mate by the intensity or frequency of the signals (high intensity/frequency/duration indicates better health, and therefore a stronger evolutionary advantage) (Branham, 2000). Female deep-sea angler fish also use this technique to attract males. The male, who is tiny compared to the female, attaches himself to the female with his mouth. The bloodstream of the male then fuses with the female's so that he becomes a sperm-producing parasite of the female (Why do animals make light? Finding a mate, 2000). In this way, various animals have evolved a startling array of adaptations to utilize the basic phenomenon of bioluminescence to defend themselves, feed, or mate.

Understanding the nature and use of bioluminescence allows us to address why bioluminescence is far more prevalent in the oceans than on land. This article mentioned previously that the only common feature among the diverse forms of bioluminescence was in the basic nature of the reaction itself—the oxidation of a luciferin by a luciferase followed by the relaxation of the excited oxyluciferin with the emission of a visible photon. However, in nature, this same chemical 'truth' has been reinvented at least 30 different times (Wilson & Hastings, 1998). From an evolutionary perspective, these different systems are completely unrelated, as the diverse structures of the various luciferins used (Figure 2) and the varied three-dimensional conformations of the luciferases (Figure 3) indicate. The different luciferins are chemically unrelated and the luciferases themselves do not show any similarity in their amino acid sequence[4]. This indicates that there was a biological need to produce light, and that need was so great that nature reinvented the bioluminescence "wheel" 30 times over.

There is a theory to explain bacterial bioluminescence as having developed to facilitate DNA repair (Marchant, 2000). This hypothesis does not explain the bioluminescence of higher organisms such as fish; and it certainly does not explain the marine dominance of the phenomenon, especially since terrestrial animals are naturally exposed to more UV radiation than their deep-sea counterparts. Another theory posits that the common chemical basis of bioluminescence, the oxidation of luciferin, developed as a natural protection mechanism against toxic oxygen radicals when organisms were exposed to an atmosphere that was gradually increasing in oxygen content (Rees et al., 1998). If this is the case, then the theory does not explain the marine prevalence of bioluminescence when terrestrial organisms are constantly exposed to a higher oxygen content than aquatic organisms.

Bioluminescence is therefore an excellent example of convergent evolution—when different organisms develop the same physical/functional feature through separate evolutionary routes. This is analogous to the reason whales and sharks have fins, even though the former are mammals and are unrelated to sharks, which are fish. The prevalence of marine bioluminescence in contrast to

Figure 4. Bioluminescence for camouflage or feeding. A: Without bioluminescence, a fish swimming near the surface has a distinct, identifiable silhouette when viewed from below. **B**: Some fish have bioluminescent underbellies that masks their silhouette when viewed from below. **C**: The cookie-cutter shark has a bioluminescent underbelly except for the patch under the throat. This fools large predators swimming below who think that it is a small fish based on the small silhouette it

terrestrial bioluminescence therefore argues that there was a greater evolutionary need for this adaptation in the oceans than there was on land.

We can infer then, that ocean dwelling creatures may have had a greater need for bioluminescent adaptations than their land-dwelling counterparts. Indeed, from the examples given above, marine creatures at all levels of the evolutionary scale have produced remarkable forms of bioluminescence to give themselves an evolutionary edge. Additionally, they have fine-tuned their bioluminescence to be produced and detected in the blue/blue-green wavelength range that is most easily transmitted in seawater (with notable exceptions such as the black dragonfish). The terrestrial examples of bioluminescence fail to display this level of complexity; from an evolutionary standpoint, bioluminescence is less essential for day-to-day survival on land where light is abundant and life is mostly restricted to the earth's surface.

Therefore we see that the incredible array of bioluminescent mechanisms present in nature argues that if bioluminescence conferred a survival advantage, organisms would have evolved to exploit it. As the marine habitat is vastly different from the terrestrial one, the survival pressures on marine organisms are vastly different from those faced by terrestrial creatures. Organisms that always live in the dark face a natural selection pressure to develop light-producing organs that enable them to survive, while those living in better lit waters still find bioluminescence useful for camouflage, feeding (cookie-cutter shark), or predator deterrence (dinoflagellates). In response to the unique pressures they face, marine organisms evolved to exploit bioluminescence in a dazzling multitude of applications. The other theories proposing bioluminescence as an oxygen-defense mechanism or a DNA repair facilitator are paradoxical since terrestrial organisms should be exposed to both more UV radiation and oxygen, yet only a tiny fraction of terrestrial animals are bioluminescent. Consequently, it should be of no surprise that the unique marine habitat and the particular suitability of bioluminescence in a normally dark environment has resulted in the overwhelming abundance of the phenomenon in the ocean in myriad forms and its relative absence on land. ■

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1 The terms luciferase and luciferin are generic and apply to the respective components of any specific bioluminescent system. In fact, there are multiple forms of both luciferase and luciferin representing a remarkable case of convergent evolution that will be examined later.

2 This is of course an oversimplification. In reality, there can be a series of energy level drops, any of which can produce a visible photon. The visible photon need not be produced during the drop to the ground state.

3 Actually, fireflies are not really 'flies' but beetles in the family Lampyridae.

4 Proteins are made up of one or more chains of amino acids. There are at least 20 naturally occurring amino acids that can be arranged in unlimited combinations, and in multiple chains to form proteins. Comparing the amino acid sequences of proteins allows us to determine if they descended from a common ancestor – since if they did, a large portion of their sequences would match.

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