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The function and consequences of fluorescence in tetrapods

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Fluorescence, the optical phenomenon whereby short-wavelength light is absorbed and emitted at longer wavelengths, has been widely described in aquatic habitats, in both invertebrates and fish. Recent years have seen a stream of articles reporting fluorescence, ranging from frogs, platypus, to even fully terrestrial organisms such as flying squirrels, often explicitly or implicitly linking the presence of fluorescence with sexual selection and communication. However, many of these studies fail to consider the physiological requirements of evolutionary stable signaling systems, the environmental dependence of perception, or the possible adaptive role of fluorescent coloration in a noncommunicative context. More importantly, the idea that fluorescence may simply constitute an indirect by-product of selection on other traits is often not explored. This is especially true for terrestrial systems where environmental light conditions are often not amenable for fluorescent signaling in contrast to, for example, aquatic habitats in which spectral properties of water promote functional roles for fluorescence. Despite the appeal of previously unknown ways in which coloration may drive evolution, the investigation of a putative role of fluorescence in communication must be tempered by a realistic understanding of its limitations. Here, we not only highlight and discuss the key body of literature but also address the potential pitfalls when reporting fluorescence and how to solve them. In addition, we propose exciting different research avenues to advance the field of tetrapod fluorescence.

Fluorescence is the optical phenomenon by which a chemical compound, a fluorophore, absorbs incoming short-wavelength light and re-emits this light at longer wavelengths. This is distinct from bioluminescence, where organisms chemically produce and emit light of various wavelengths, regardless of the ambient light. The visual appeal of vibrant, fluorescent colors to humans has led to the description of fluorescent patterns across the tree of life. These findings have led to breakthrough applications, such as the green fluorescent proteins (GFPs) used extensively in molecular biology (1). With so much known about how GFPs work, it is remarkable that the functional significance in the animals themselves remains unclear. However, impressive demonstrations of fluorescence in dark rooms with intense, unnatural excitatory lighting may encourage an outsized impression of their visual significance (Fig. 1). Indeed, many terrestrial tetrapods, in contrast to aquatic and marine animals, occupy habitats that are particularly suboptimal for the perception of weakly re-emitted fluorescent wavelengths. Furthermore, while the framework to investigate a potential function of fluorescence is well established in (marine) (in)vertebrates, this seems less so in tetrapods, as highlighted by an explosion of publications that describe fluorescence in tetrapods and often explicitly or implicitly link the presence of fluorescence with sexual selection and communication (2–9).

However, to determine whether fluorescence has a function in visual signaling, it is first necessary to consider how it is perceived. In other words, under which natural lighting conditions is the fluorescent animal viewed, and can the viewer detect and behaviorally respond to the fluorescence? An important consideration is the quantum yield of the fluorophore (i.e., the number of photons coming in versus those coming out), especially since many fluorescent biological materials emit only a tiny fraction of the photons they absorb. Under broad-spectrum lighting, such as the sun or moonlight that dominates natural scenes, fluorescent emission can easily be drowned out by reflected photons (Fig. 1). In water, the light spectrum becomes increasingly dominated by blue-green wavelengths with depth, thus limiting the waveband available for reflected coloration (10). By shifting part of this restricted waveband into scarce, longer wavelengths, fluorescence provides organisms with a possible avenue to produce color contrasts, increase conspicuousness, and perhaps reduce unwanted perception by potential receivers that are insensitive to longer-wavelength light. However, even in these conditions there is little evidence for biological fluorophores functioning in communication (11), and the bright, broad-spectrum photic environment of shallow water or terrestrial habitats makes this even less likely. Therefore, recent reports of fluorescence in all major terrestrial tetrapod clades, including amphibians (12), reptiles (3), birds (2, 4), and mammals (5–9) raise the fascinating question of its role (if any) in their lives. Here, we provide a framework to guide research tackling the functional significance of fluorescence in nature.

Communication

The intuitive appeal of fluorescence as a unique form of color-based communication must be tempered by a realistic understanding of its limitations. Indeed, fluorescence is so ubiquitous

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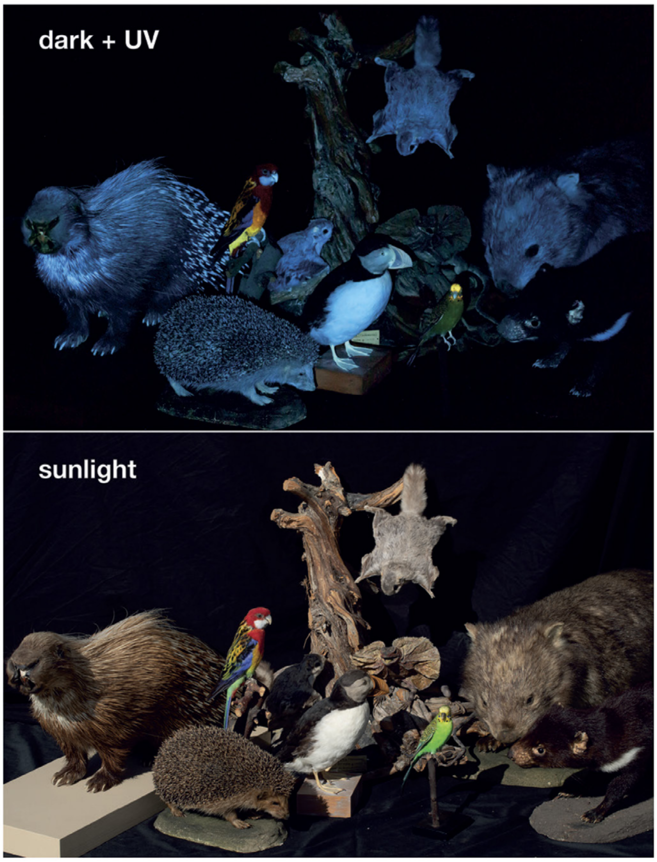

Fig. 1. Fluorescence under different lighting conditions. Exposure of museum preserved specimens to unrealistic (only UV), and natural (sunlight) lighting conditions highlights the substantial effect of fluorescence in unnatural environments. Photos by M.J.B. under standardized camera conditions (only differing in exposure time). We thank Maria Mostadius for providing access to the zoological collection of the Department of Biology of Lund University.

in animal tissues that it should be considered the norm rather than the exception (9). Therefore, attributing specific functions to fluorescence requires exceptional evidence supported by a rigorous framework. Marshall and Johnsen (13) and Mazel (11) identified five conditions suggestive of a function in communication: A fluorescent compound i) with known excitation and emission wavelengths ii) is present in a visible body region and iii) exhibited in a suitable light environment, iv) producing a shift in coloration perceptible by potential viewers and v) eliciting a behavioral response in the receiver. This last criterion, in particular, is rarely tested, and more behavioral assays that manipulate fluorescence are needed (see refs. 2 and 14). Indeed, a recent study that investigates fluorescence in anurans marks a positive shift in research focus (14). At a minimum, physiological data describing the spectral response of photoreceptors (microspectrophotometry of visual pigments or electrophysiology of photoreceptors) should be coupled with visual modeling and discrimination experiments to shed light on the receivers' perceptual responses to fluorescent signals (15). Additionally, researchers should consider that fluorescence may act in combination with other adjacent or underlying color patches, either by mutually reinforcing total brightness, drawing attention to an accompanying informative signal, or providing context to receivers (16). We encourage researchers evaluating a putative fluorescent signal to strongly consider, and distinguish between, signal efficacy (i.e., its propagation and perception over environmental noise) and strategic design (i.e., its information content and effects on receiver behavior).

Certain terrestrial habitats can mitigate the dominant effect of reflectance over fluorescence, yet the evolution of specific sensory adaptations will still be required for fluorescence to play a role in communication. Particularly interesting are environments where the ambient light spectrum is dominated by shorter wavelengths, allowing longer-wavelength fluorescent signals to stand out. Examples of potentially favorable conditions for fluorescence may include organisms living in aquatic or dense green canopy habitats or animals that are active around the blue hour of twilight (8, 17). One way to evaluate this, and other sensory ecology related questions, is via phylogenetic comparative methods (PCMs), although we reiterate that standalone results do not inform us on the function or mechanisms of fluorescence. If fluorescence confers a selective advantage only in some environments, we would expect it to be correlated with specific ecological factors, such as crepuscular lifestyle or differences in habitat occupancy. PCMs can also be used to examine the coevolution between variation in fluorescent signal and variation in visual system of the receiver. Of specific importance is to increase our understanding about the visual perception abilities for a broader range of taxa, specifically, the relative contribution of rod (scotopic) and cone-mediated (photopic) sensitivities. Low-light color visual sensitivity data are not known for most tetrapods apart from some amphibians (18). Therefore, without the ability to discriminate colors in dim light, fluorescent signals could only be perceived as being achromatic and would lose much of their information value.

Predator Avoidance

Given that fluorescent species are often depicted as vibrantly colored against a dark background, it is perhaps counterintuitive that fluorescence could play a role in camouflage by decreasing the conspicuousness or background contrast of animals when viewed against natural backgrounds by nonintended receivers. While it is well established that the absorption of light to match the background can decrease conspicuousness, the conditions under which a fluorescent compound might be more effective, or equally effective but less costly, than a nonfluorescent compound require further elucidation. Factors such as the spectral properties of the environment, the intensity and directionality of ambient light, and the specific fluorescent properties of the compound in question are crucial in determining the efficacy and cost-effectiveness of fluorescence in camouflage strategies. Additionally, the energy losses associated with the conversion of light from lower to higher wavelengths may contribute to decreased overall brightness, potentially enhancing the effectiveness of fluorescence in specific scenarios (13). In the same vein, fluorescence may aid predator avoidance by enhancing pursuit-deterrent behavior (such as deimatic displays) or hindering the formation of a prey search image by increasing variation in appearance at the species level. Finally, fluorescence has been suggested as a form of Batesian mimicry (6) and aposematic signal (19), where similar habitat conditions may promote high-contrast fluorescent patterns in palatable and unpalatable species to avoid predation. However, again, these possible roles all still need to satisfy the conditions for fluorescent signaling outlined above, including perceptibility and elicitation of a behavioral response in potential receivers.

Noncommunicative Functions

Fluorescence could also be directly selected for its role in several noncommunicative contexts. For instance, in invertebrates, fluorescence may aid spatiotemporal orientation by producing self-stimulating visual cues that animals could use to infer their position in the water column, their exposure to natural light, or daily variations in the spectral composition of sunlight (20). Additionally, fluorescent proteins can counteract oxidative stress, as recently demonstrated in sea anemones (21). Other biological functions such as thermoregulation, photoprotection, or improving signal-to-noise ratio in visual sensory input could also benefit from the absorptive property of fluorescent compounds. These ideas remain largely untested in tetrapods and conditions for these functions may indeed be less favorable compared to those in invertebrates (but see ref. 22), because tetrapods are likely to be influenced by a combination of physiological, ecological, behavioral, and evolutionary factors, which may differ from those encountered in invertebrate organisms.

Lack of Function

Despite (and due to) its widespread occurrence in biological materials, fluorescence may lack a specific function in most cases and constitute an indirect by-product of selection on other traits. For instance, widespread fluorescence in tetrapod bony outgrowths (e.g., teeth and protective skull tubercles; 3, 23, 24) is in most cases best explained as a by-product of apatite and proteins, some of the main components of these outgrowths. Likewise, Toussaint et al. (8) suggest that most fluorescence in mammals is likely a by-product of the heme biosynthesis pathway, where porphyrins (potentially in toxic quantities) are excreted to the epidermis for breakdown. Similarly, the red iridescence observed in European hedgehogs may be incidental and produced by bacteria, without a biological function (5). Most notably, some of the highest mammals' fluorescence is observed in fossorial moles (25) with limited or no vision. Until compelling evidence comes to light, lack of function should be considered as the default explanation for tetrapod bone and keratin fluorescence.

A Bright Future

Regardless of its function, numerous intriguing questions remain regarding the nature and implications of fluorescence. The mechanisms of fluorescence production and the underlying factors regulating excitation and emission wavelengths are in many cases (outside of those used for human applications) unknown. While several studies have identified multiple fluorophores responsible for tetrapod fluorescence (such as porphyrines, psittacofulvins, guanine, pteridines, and carotenoids), it is unlikely that this restricted set explains all instances of fluorescence (e.g., as in ref. 17). Porphyrins are a candidate pigment class that might be more common than previously appreciated in mammals, as they are responsible for fluorescence in many birds but are only known from a few mammals. Additionally, carotenoids, common in birds, have only been found in a handful of mammal species (26). This raises questions about the existence of other elusive pigments triggering fluorescence in mammals. One study suggests that mammal fluorescence may be the result of structural color (7), although there is neither evidence supporting this strong claim nor a plausible mechanism for it. In synthetic fluorophores, photonic structures can alter the emission spectra (27) but these structures alone, however, cannot create fluorescence without a fluorophore. Nevertheless, keratin, for example, can serve as a scaffold for fluorophores, and amino acids in its protein structure can result in fluorescence (9).

Fluorescence in nature is fascinating but requires research more rigorous than scouring natural history collections with UV lights for additional fluorescent species. Instead, we need to design holistic studies, that use a combination of large datasets and hypothesis-driven comparative analyses, well-designed behavioral experiments, and a better understanding of how these signals are perceived and processed. In addition, once the cellular mechanisms of fluorescence are understood, researchers can use comparative genomic approaches commonly used to identify the genetic mechanisms of other types of coloration. Finally, elucidating the chemical and ecological functions of fluorescence may inspire the development of biomaterials such as sustainable and energy-efficient displays or lighting systems that maximize energy conversion and minimize energy waste. In doing so, we will be able to confirm the use of fluorescence as a signal in natural contexts and to identify its genetic, cellular, developmental, ecological and mechanisms that shape evolution of this unique optical phenomenon, while leveraging this understanding to develop innovative materials.

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