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Investigation of Fluorescence in Selected Mammals of Arkansas

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Running Title: Fluorescence in Arkansas Mammals

Abstract

The adaptive value of fluorescence among the vertebrates has been studied most in fishes and birds, and only a few observations have been published regarding fluorescence in the pelage of mammals. Recently, reports of fluorescence in some marsupials, the platypus, and in flying squirrels have become available. We report the occurrence of fluorescent properties in some mammals from Arkansas. Most carnivores, bats, and rodents did not exhibit the property when viewed under UV light. However, opossums, rabbits, a weasel, muskrats, and moles showed substantial UV response, and a few other mammals showed minor fluorescence. Colors fluoresced included pink, green, and light cyan. Most species exhibited only 1 color, but the opossum responded with 2 colors. Potential explanations for positive responses to UV light include species signaling, mate assessment, predator avoidance, or prey location. Alternatively, the response may be an artifact without adaptive significance.

Introduction

When ultraviolet (UV) light is reflected from an object, the color is the same as the light projected (purplish), and if the color remains the same as it appeared in white light, the UV light was absorbed. Fluorescence is the property in which an object absorbs radiation of a shorter wavelength (higher energy) and emits a longer wavelength of lower energy, resulting in what is perceived as a different color. The result of UV fluorescence can be a glowing effect not visible to human eyes in white light. Human perception of UV light is limited, but many vertebrates see into the UV range (Bennet and Cuthill 1994). However, when fluorescence can make visible a color within the range of white light, an animal does not have to see into the UV spectrum, just the fluorescence itself (Marshall and Johnsen 2017). Though most mammals (with the exception of some primates) cannot discern colors representing the full spectrum in white light, many can detect UV light (Douglas and Jeffery 2014; McDonald *et al.* 2020), which opens some avenues of interpretation of adaptive use of fluorescence by mammals.

Still, little is known about occurrence of fluorescent properties in pelage of mammals, and most reported observations record the phenomenon in marsupials. Meisner (1983) in a published abstract with little detail, mentioned that the North American opossum (*Didelphis virginiana*) showed complex patterns of fluorescence, and Pine *et al.* (1985), noted that 24 of 32 species of New World didelphid marsupials fluoresced in UV light. Australian marsupials including Krefft's glider (*Petaurus notatus*), striped possum (*Dactylopsila trivirgata*), and long-nosed bandicoot (*Perameles nasuta*) exhibited different fluorescent colors when exposed to UV light (Reinhold 2021).

Among placental mammals, all species of North American flying squirrels (genus *Glaucomys*) fluoresce a pinkish hue under UV light (Kohler *et al.* 2019, Tumlison *et al.* 2019). Australian native rats including the fawn-footed mosaic-tailed rat (*Melomys cervinipes*) and the bush rat (*Rattus fuscipes*) also fluoresce, as well as the introduced black rat (*Rattus rattus*) (Reinhold 2021). Vivid red fluorescence also has been detected in the springhare (Pedetidae), an Old World placental mammal (Olson *et al.* 2021).

Even the monotreme (egg-laying) mammals recently have been shown to fluoresce a green to cyan color under UV light (Anich *et al.* 2021; Reinhold 2020). Explanations of the cause or purpose of the phenomenon in nature range from mere artifact to adaptations for navigation and orientation, species recognition, mate assessment, camouflage, and predator avoidance (Cronin and Bok 2016).

Methods and Materials

The purpose of this study was to determine whether pelage of any Arkansas mammal species

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fluoresces under UV light, and to offer insights and hypotheses based on our observations. We did not study whether whiskers or skin might have fluoresced. We shined light from a UVBeast[™] flashlight (385-395 nm) onto preserved specimens of mammals from Arkansas, housed in the Henderson State University Collection of Vertebrates, to determine if any form of fluorescence was emitted.

Specimens in this collection had been prepared between 1990-2021. Specimens were dry skins that had been preserved by skinning, fleshing, stuffing with cotton, and drying prior to storage in specimen cabinets. The fur had not been sprayed or treated with any chemical preservatives or insecticides. One alcoholic specimen had been preserved in 10% formalin, washed, and stored in 45% isopropanol. In a few cases, living specimens also were tested.

The light was held about 15-30 cm from the specimens (depending on the size of the area we desired to illuminate) and images were taken with a Samsung Galaxy S7 phone camera. We immediately compared images taken and our visual perceptions of colors, and determined that the colors were perceptibly the same. Specimens were examined dorsally and ventrally, and for species with thicker fur, we parted the fur in order to reveal any fluorescence in underfur.

Results

We detected no fluorescence under UV light in any specimens of Chiroptera (species examined: Perimvotis subflavus (tricolored bat, n=7), Lasiurus borealis (eastern red bat, n=12), L. seminolus (Seminole bat, n=3), Aeorestes (=Lasiurus) cinereus (hoary bat, n=3), Lasionycteris noctivagans (silverhaired bat, n=2), Eptesicus fuscus (big brown bat, n=8), Corynorhinus rafinesqii (Rafinesque's big-eared bat, n=3), and Tadarida brasiliensis (Brazilian free-tailed bat, n=5)). Most Carnivora also revealed no fluorescence (species examined: Procyon lotor (raccoon, n=12), Neovison vison (American mink, n=6), Spilogale putorius (spotted skunk, n=2), Mephitis mephitis (striped skunk, n=3), Urocyon cinereoargenteus (gray fox, n=7), Vulpes vulpes (red fox, n=2), Canis latrans (coyote, n=2), and Lynx rufus (bobcat, n=5).

Flying squirrels fluoresce a pinkish coloration, especially on the white ventral pelage (Kohler *et al.* 2019; Tumlison *et al.* 2019). In our examination of another small squirrel, *Tamias striatus* (eastern chipmunk, n=12), a few specimens showed a mild pinkish appearance under UV light on ventral fur, but we believe the effect is too little to warrant further

comment. Similarly, Castor canadensis (North American beaver, n=5) presented with mildly greenish guard hairs. Otherwise, most of the rodents we examined did not display fluorescent properties. These included: Geomys breviceps (Baird's pocketgopher, n=20), Microtus pinetorum (woodland vole, n=5), Neotoma floridana (eastern n=19), woodrat, Ochrotomys nuttalli (golden mouse, n=5), Oryzomys texensis (Texas marsh rice rat, n=3), Reithrodontomys fulvescens (fulvous harvest mouse, n=9), Sigmodon hispidus (hispid cotton rat, n=16), Mus musculus (house mouse, n=20), Marmota monax (woodchuck, n=10), Sciurus carolinensis (eastern gray squirrel, n=31), and Sciurus niger (eastern fox squirrel, n=23). Interestingly, fluorescence is known in bones and teeth of fox squirrels (Dooley and Moncrief 2012), and was witnessed in skeletal material within our collection, but the effect was not detected in pelage.

Fluorescent forms We found fluorescence in pelage of several species of mammals, some of which have been only vaguely mentioned in previous literature, and some here for the first time.

Didelphis virginiana (Virginia opossum, n=14). Three live adults and 3 dispersing juveniles (observed 24 April 2021) found by chance and illuminated with the UV light at night, glowed pink (Figure 1) with moderate to intense fluorescence in all 6 specimens. The dorsal underfur showed bright pink in 2 of the 8 museum specimens of opossums we examined, and a mild response was seen in another, under UV illumination. Seven of the 8 fluoresced noticeably pink on the ventral hairs (Figure 2).

Opossums sometimes have a whitish to yellowish throat patch, and in some individuals this patch continues as a streak down the thorax. Those patches fluoresced a light cyan color, making the area much brighter in UV. The patch was evident in 7 of 8



Figure 1. Image of a live opossum about 2 m distant from the UV light. On this individual, the underfur fluoresced a bright pink color, making the animal glow in the illumination. White hairs on the face fluoresced only mildly. All purplish coloration on the opossum and substrate resulted from reflectance of UV light.



Figure 2. Comparison of the ventral pelage of an opossum (*Didelphis virginiana*) under white light (top) and UV (bottom). The whitish underfur produced a pinkish fluorescence under UV, but a midventral white streak produced a cyan color. In the black and white print version of this image, little difference is seen, but colors are vivid in the online version of this paper.

individuals we examined, and the response was most intense in specimens with darker yellow patches (as seen in white light). Yellow hairs in lab rats (Norway rats) similarly have been reported to fluoresce brilliantly (Rebell *et al.* 1956).

Other white hairs on the opossums, such as on the head, did not show this degree of response. Caution must be used in examination of greenish to cyan coloration, especially in the genital area, because urine remaining on hairs can also provide this response. However, the patterns we describe on the opossums were far anterior and limited to only the otherwise white hairs.

Scalopus aquaticus (Eastern mole, n=19). Besides 17 dry skins, we also examined an untreated frozen specimen and a specimen that had been fixed in 10% formalin and preserved in 45% isopropanol. Regardless of state of preparation, all specimens produced a vivid dull-greenish response, which was evident on dorsal and ventral perspectives (Figure 3). This indicated that preservation in fluids does not necessarily denature the effect, and that the effect likely did not result from museum preparation of the skins.

The upper shaft of hairs of moles has an expanded spatulate shield region, which was the only portion of the hair shaft that fluoresced. Examination under a dissecting microscope further revealed that the tips of the hairs were the primary locations of the greenish effect.



Figure 3. Dorsal and ventral perspective of pelage of the Eastern mole (*Scalopus aquaticus*) comparing appearance in white and UV light. All of the pelage fluoresced a greenish to cyan color when UV light was projected from the right angle. The widened spatulate tips of the hairs were the only parts of the hair shaft that fluoresced. In the color images, fluorescing green hairs can be seen, but the purplish hair at the periphery of the specimen is reflecting UV. Changing the angle of the UV source also changed which hairs reflected and which fluoresced.

Blarina carolinensis (southern short-tailed shrew, n=10). All of these shrews emitted a greenish fluorescence similar to that of the moles, but to a much lesser degree. The effect was most pronounced when the light was held vertical or posterior to the shaft. The mole hairs also reacted in that manner.

Rattus norvegicus (Norway rat, n=2).We detected green fluorescence scattered over the postcranial pelage of both specimens (Figure 4). Similar fluorescence was reported for a congener, the black rat (*Rattus rattus*; Reinhold 2021), and this fluorescence in white lab strains of the Norway rat is caused by Kynurenine (Rebell *et al.* 1956, 1957; Rebell 1966).

Ondatra zibethica (muskrat, n=3). Superficial examination of muskrat skins did not detect any fluorescence. Parting of the thick underfur, however, revealed a yellow-green response (Figure 5). The effect was limited to the posterior dorsolateral portions of the pelage, and the fluorescent part to the basal half of hair shafts of underfur hair, allowing the brown distal half

of the hairs to obscure the grayish basal half of the lower shaft.



Figure 4. Norway rats (*Rattus norvegicus*) showing greenish-cyan fluorescence on the postcranial pelage. The head area reflected UV light so appears purplish.



Figure 5. Comparison of muskrat (*Ondatra zibethica*) fur under white and UV light. Fluorescent effect occurred only on the posterior dorsolateral positions on the 3 specimens examined. The darker gray portions of the hairs fluoresced, whereas the lighter gray upper portions reflected UV light (showing purple).

Mustela frenata (Long-tailed weasel, n=2). One of the 2 weasels examined produced a greenish response to UV light. The head area did not fluoresce, whereas the post-cranial brownish pelage (under white light) emitted a greenish hue under UV. In white light, the head and body were not evidently different in coloration. The fluorescence was especially distinctive when compared with skins of their near relatives, mink, which are only a slightly different shade of brown but did not fluoresce (Figure 6).

Latham (1953) found no fluorescence in the longtailed weasel and ermine (*M. erminea*), both of which remained a dull brown under UV light, but least weasels (*Mustela rixosa* – now *M. nivalis*) fluoresced 'a vivid lavender color'. We believe it more likely that the brown color indicated absorption of UV light, and the fluorescence was actually reflectance of the purplish (lavender) UV light. However, Toussaint *et al.* (2021) argued for the occurrence of lavender fluorescence in the ermine, though spectroscopic analysis did not reveal porphyrins (also, their specimen was in white winter pelage vs. the brown summer pelage described by Latham (1953)).



Figure 6. Comparison of mink (*Neovison vison*) and weasel (*Mustela frenata*) pelage under white light (top pair) and UV light (bottom pair). The mink skin is on top of both pairs. Note both are variants of brown in white light. Under UV, the mink largely reflects the light, creating a purplish color, whereas the weasel fluoresces a green hue.

Sylvilagus floridanus (Eastern cottontail, n=8). The dorsal pelage of the hind feet of cottontails is white under normal light, but emits a brighter light cyan coloration under influence of UV (Figure 7). Further, the brown hairs on the bottom of the foot fluoresced greenish. Other white hairs on the rump and venter also become brighter in UV. Eastern cottontails use alert postures and jumping sequences during interactions with females and rival males, and these involve lifting the hind section of the body above the plane of the shoulders (Marsden and Holler 1964), which exposes the surface of the hind foot and the tail region. Submissive postures hold the body close to the ground, largely hiding the foot and hindquarters. Fluorescence in this area might serve to amplify the display. Swamp rabbits (Sylvilagus aquaticus, n=7) have brownish hairs on top of the hind foot, which did not noticeably fluoresce although other white hairs of the area did show a brighter pale cyan to white.

Discussion and Conclusions

The value of fluorescence in the subphylum Vertebrata has been tested in a few species, but its



Figure 7. Comparison of dorsal surfaces of the hind foot of a cottontail rabbit (*Sylvilagus floridanus*) under white light (top) and UV light (bottom). The images show both hind feet of 1 specimen. Pale cyan fluorescence in UV light brightens the white hairs and increases the contrast with the brown hairs of the foot, which appear purplish due to reflectance of UV wavelengths.

purpose only hypothesized for many others. A primary question is whether natural fluorescence functions as a signal, or is merely a by-product of pigment structure or life history (Arnold *et al.* 2002). For example, fluorescence in marine turtles could be an artifact of diet including organisms that fluoresce, or presence of fluorescent algae on the carapace, but because males showed more intense effects, there could be an ecological role (Gruber and Sparks 2015).

Some tests have identified various adaptive purposes of UV fluorescence or UV vision. Blue tits are sexually dichromatic birds in UV light, which can be used in mate choice (Hunt *et al.* 1998). Parrots also use fluorescence for sexual signaling and mate choice (Arnold *et al.* 2002; Pearn *et al.* 2001). Many reef fishes use red fluorescence to enhance visual communication (Michiels *et al.* 2008), but they also can use it as camouflage if the background also fluoresces (Sparks *et al.* 2014). Fluorescence in the platypus may reduce visibility to UV-sensitive predators (Anich *et al.* 2021), but foraging animals may incorporate UV cues (reflectance and absorbance) from the environment, or of food items, into their foraging strategy (Honkavaara *et al.* 2002).

Fluorescent frogs may enhance their visibility to other frogs at twilight (Taboada *et al.* 2017). Some butterflies adaptively dupe bird predators by use of UV light to focus attacks on the eyespots on the back of their wings, thus avoiding fatal head grabs (Oloffson *et al.* 2010). Given all the possible interpretations to explain vertebrate ability to see UV light, or to fluorescence and thus make the UV visible, detailed studies are needed to examine any adaptive hypothesis for each mammalian species determined to fluoresce. If adaptive, fluorescence should adjust invisible UV light into the visible spectrum to some advantage. Based on our observations, we offer some recommendations and considerations for future study.

Kohler *et al.* (2019) suggested a possible link between fluorescence and nocturnality. All of the mammals we found to fluoresce are crepuscular to chiefly nocturnal. However, many nocturnal species did not fluoresce, including all bats and most rodents.

Moles are subterranean and have tiny eyes, thus ability to detect UV would seem to be of little value. Glösmann *et al.* (2008) reported that European moles could see UV light and offered an adaptive explanation as the ability to detect leakage of light where tunnel systems might need repair. Thus, an adaptive purpose of UV vision is possible, but this possibility would not explain a purpose for green fluorescent pelage that we observed in all specimens of moles.

Fluorescence appeared only in the basal portions, and in small areas, of muskrat underfur. Underfur hairs in muskrats have a long brown upper shaft, which does not fluoresce and also covers the reactive basal parts of the shaft. How moles or muskrats might adaptively use their obscured fluorescence is particularly unclear. Any adaptive value might be related to factors other than intraspecific interactions. However, we examined dry specimens of muskrats. As a wetland species, their fur often is wet, which causes hairs to matt and may allow the hidden fluorescent area to become exposed, and thus an adaptive possibility exists.

Underfur hairs of opossums typically have a short black tip and a long white to grayish remainder. The pink glow produced on some opossums was visible on that entire whitish portion of the shaft. Pine *et al.* (1985) noted the same fluorescent portions for other didelphid marsupials. All 6 of our live adult and juvenile opossums seen during springtime fluoresced dorsally, whereas only 2 of the 8 museum specimens did so (though most fluoresced ventrally).

Shrews showed a minor UV reaction of greenish color similar to that of the moles. Arguably, the taxonomic relationship between these mammals may indicate a shared response due to phylogeny. However, mink and weasel also are phylogenetically related but none of our sample of mink reacted to UV light, whereas 1 of 2 weasels produced a strong response. The head fur of that weasel absorbed UV but the body fluoresced a distinctive green. Additional specimens should provide better insight, but we hypothesize that the presence and distribution of UV coloration may differ among ages or stages of molt. Some species of owls can be aged via examination of which feathers fluoresce, because younger feathers following molt have more porphrins so fluoresce brighter, and older feathers less (Weidensaul et al. 2011). Further, Bollinger (1944) noted that fur of the brushtail possum (Trichosurus vulpecula) was more fluorescent when in the new growth phase. Thus, in species where UV response is inconsistent across specimens, we argue that age effects or stages of molt might be examined to explain such observations.

Our observations of fluorescence deep within the fur of muskrat might be related to patchy or wavy molt patterns (Ling 1970) with newer hairs fluorescing whereas older hairs do not, and have no other adaptive value. Seasonal molt in some species, regardless of age, also may relate to variation. The adaptive shift between white winter fur and brown summer fur of several arctic mammals is well known. Hypothetically, similar seasonal adjustments in hair pigmentation to utilize UV might be expected.

Small sample sizes in many studies prevented examination of data stratified by age and sex, which might reveal patterns, but good sample sizes used by Kohler *et al.* (2019) in flying squirrels and Olson *et al.* (2021) in springhares found no variation by age, sex, location, or time of collection.

Mammals we observed fluoresced green, cyan, and pink. Moles appeared green both dorsally and ventrally, but opossums showed pink dorsal to ventral underfur, and cyan in the white patch of the throat and mid-venter. Different fluorescing colors on the same individual could mean different signals, multiple effects to achieve 1 signal, only 1 may be adaptive, or both may simply be artifacts from pigmentation in the pelage. Our samples were too small to compare sexes and ages, but not all opossums showed the same intensity, or even presence, of fluorescence.

Time and method of preservation may affect pelage of prepared skins of mammals. Labile pigments in some hairs may change after death (Pine *et al.* 1985), and if those fluoresced while the animal was alive, studies of museum specimens may not reveal the property. Chemicals used in preparation of wet specimens or tanning solutions may alter pigment structure and remove or reduce fluorescent properties. Specimens preserved in alcohol after fixation in formalin may be less likely to retain the effects, though our 1 alcoholic mole specimen retained its fluorescent effects. We present data from museum specimens that did retain fluorescent properties, but we note that some museum specimens may have variably lost or retained the property. For example, all of the live opossums we examined fluoresced dorsally but not all museum preparations did so. Pine *et al.* (1985) and Olson *et al.* (2021) also suspected that fluorescence might be brighter in live animals, and might degrade in museum specimens over time.

Finally, it should be noted that the best test of fluorescence is by use of fluorescent spectroscopy to determine wavelengths of perceived responses. We have provided new observations that warrant further study with more technical projects.

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