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Sexually Dimorphic Biofluorescence of the Postcloacal Gland in the Terrestrial Salamander, *Plethodon cinereus*

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Abstract.—**Recent research has documented widespread biofluorescence across amphibians. Among caudates in particular, representative species from 8 of the 10 families fluoresce under blue light excitation. Although fluorescence has been reported on the ventral surface of the tail in Eastern Red-backed Salamanders, *Plethodon cinereus*, nothing is known about the source or function of the fluorescence. This fully terrestrial salamander species has a broad geographic range, has complex mating behavior involving chemosensory and visual behaviors, defends territories from conspecific and heterospecific intruders, and is a model organism for studying many aspects of sociobiology. Our goal was to learn more about the source of fluorescence in *P. cinereus* and to explore demographic patterns of fluorescence. Additionally, we wanted to establish whether there is seasonal and geographic variation in fluorescence in this widespread salamander species. Through our examination of histological literature and close examination of photos of the ventral surface of tails under both white and UV light, we concluded that S1 glands, which comprise the postcloacal gland (PCG) in *P. cinereus*, are the source of fluorescence on the ventral portion of the tail. We found this trait to be highly sexually dimorphic, with males having significantly more fluorescent S1 glands both across seasons and localities compared to females. Additionally, we found that fluorescence only occurs in adult salamanders. Further, because the ventral surface of the tail is exposed during courtship and during territorial disputes, we hypothesize that male fluorescence of the PCG may function in the context of both mate choice and territoriality.**

Biofluorescence is widespread among animals and occurs when high-energy wavelengths of light are absorbed and then re-emitted at lower-energy wavelengths. Most biofluorescence has been documented in aquatic organisms, and it appears to be less common in terrestrial systems. However, recent studies provide more extensive documentation across broad taxonomic groups in amphibians (Lamb and Davis, 2020 reviewed therein; but see Thompson et al., 2019). For example, Lamb and Davis (2020) surveyed 11 anuran species from 5 families, 20 caudate species from 8 families, and 1 species of caecilian for biofluorescence. All amphibians examined fluoresced green to yellow (ca. 520–560 nm) in response to both ultraviolet (360–380 nm) and blue light (440–460 nm) excitation. Fluorescence appears to be widespread among caudate families with representatives from 8 of 10 families all showing fluorescence. Additionally, fluorescence was documented in caecilians and in unrelated lineages of frogs, suggesting that biofluorescence may have evolved early in the evolutionary history of amphibians.

Although biofluorescence is likely very common among salamanders, the mechanisms that produce fluorescence in response to UV and/or blue light excitation in salamanders are unknown. Based on mechanisms found in other amphibians, production may include chemical and structural components of dermal chromatophores (Bagnara and Obika, 1965), bony elements beneath the skin (Goutte et al., 2019), and proteins contained in glandular secretions (Lamb and Davis, 2020). For example, a class of fluorescent compounds called hyloins (derived from dihydroisoquinolinone) have been documented from some South American frogs (family Hylidae) and are found in both glandular secretions and lymph (Taboada et al., 2017a,b). Another study documented fluorescence in mucous-like secretions from caecilians in the genus *Typhlonectes* and in urine produced by salamanders in the genus *Dicamptodon* (Lamb and Davis, 2020). The function of fluorescence in salamanders is also not known, but in other groups may include intraspecific communication (reviewed in Macel et al., 2020), crypsis (Kohler et al., 2019, mammals), improved visual acuity (Taboada et al., 2017b, anurans), and sexual selection (Arnold et al., 2002, birds; Lim et al., 2007, salticid spiders). Hausmann et al. (2003) found that birds use UV reflective and fluorescent plumage in mate choice. The authors suggested that biofluorescence might be favored by natural selection if, for example, the signal is an indicator of a high-quality mate, or if the signal amplifies a specific mating behavior that allows individuals to judge with greater accuracy the quality of a specific courtship behavior.

Salamanders in the family Plethodontidae have complex mating behaviors involving chemosensory (Arnold, 1972) and visual signals (Organ, 1958; Verrell, 1997; reviewed in Staub et al., 2021) making them ideal species with which to examine the function of fluorescence in a behavioral context. More specifically, the small terrestrial salamander *Plethodon cinereus* is a model organism for studies of sociobiology, including sexual selection and territoriality (reviewed in Jaeger et al., 2016). This species has well-described aggressive and courtship behaviors that involve visual cues (Jaeger, 1984; Gergits and Jaeger, 1990; Dyal, 2006). Although *P. cinereus* is generally thought to be nocturnal, individuals venture forth from daytime retreats at dusk (Placyk and Graves, 2002) and have been observed on the surface of the forest floor in dim light (Cochran, 1911; Test, 1946). Thus, there are opportunities for red-backed salamanders to use biofluorescence in communication, and the large body of research on the sociobiology of this species should allow for interpretation of its function. Munoz (2018) documented biofluorescence in 45 individuals of *P. cinereus* from two counties in Pennsylvania during September and November of 2014. Of the 45 fluorescent salamanders captured, 33 were male, and only 12 were female. However, the number of non-fluorescing males and females was not reported, so it is unclear if the fluorescent signal is sexually dimorphic. Although Munoz (2018) reported fluorescence on the ventral surface of the tail, and mostly concentrated around the cloaca, his Figure 1 suggests that the fluorescence is concentrated posterior to the

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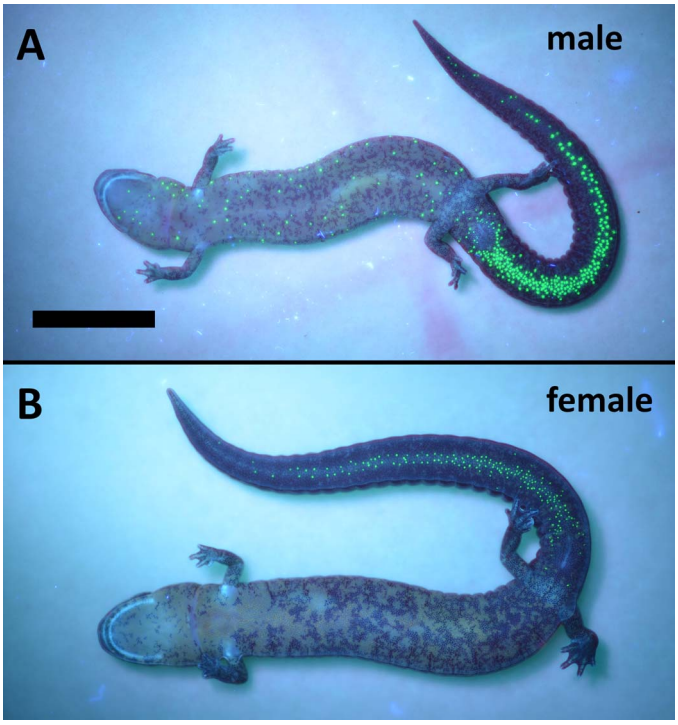

FIG. 1. Sexual dimorphism in UV fluorescence in *Plethodon cinereus*. Male (A) and female (B) in ventral aspect photographed under illumination from a 360-nm UV light source. We typically observed little to no UV fluorescence in female salamanders under 395-nm UV light, but this specimen fluoresced faintly under 360-nm UV light. The postcloacal gland, a macrogland made up of numerous S1 glands, is visible as fluorescent spots in the male salamander. Scale bar = 10 mm.

cloaca and is consistent with the location of the postcloacal gland (PCG) in *P. cinereus* (Simons and Felgenhauer, 1992; Simons et al., 1994). Cox and Fitzpatrick (2023) reported similar patterns of fluorescence from a small sample of *Plethodon metcalfi*. In their study, all males ($n = 12$) and approximately one half of females ($n = 14$) exhibited biofluorescence. Sexual differences in its intensity was most pronounced in the postcloacal region, but the source of fluorescence is unclear from their images. Because our understanding of biofluorescence in salamanders would benefit from additional research, we sought 1) to learn more about the source of biofluorescence in *P. cinereus*, 2) to determine if fluorescence is sexually dimorphic, and 3) to establish if biofluorescence differs in adults and juveniles. Finally, we wanted to examine the potential for seasonal and geographic variation in biofluorescence in this widespread salamander species.

MATERIALS AND METHODS

Seasonal and Demographic Variation in Fluorescence.—We sampled a single population of *P. cinereus* in Cuyahoga County, Ohio, United States monthly from December 2021 through November 2022 ($n = 332$ salamanders; mean = 41.5/mo; SE = 3.45). We refer to this locality as our reference site throughout the paper. Salamanders were not active at the surface in June, July, August, and January. We hand-captured salamanders (adults, juveniles, and neonates) by overturning rocks and coarse woody debris. Sex of male salamanders (greater than 32 mm snout-to-vent length [SVL]; Anthony and Pfingsten, 2013) was determined by snout morphology (Anthony et al., 2008) and the presence of whitish tissue around the vent (Gillette and Peterson, 2001; Rucker et al., 2021). Female salamanders (>34 mm SVL) were identified by presence of eggs, or if no eggs were visible, their more rounded snout and lack of white pigment surrounding the vent. To reduce the likelihood of recaptures, we were careful to sample in different areas within the same locality each month. The areas sampled were approximately 25 m^2 and were separated by 10–25 m. Salamanders were photographed and released at the point of capture. Images were taken of the ventral surface of each specimen following Wise and Buchanan (1992) within a darkened opaque box illuminated by an Escolite 395-nm handheld LED UV light. We used a Canon SX710HS digital camera with exposure, ISO, and white balance values set manually and equally for all photos. Distance from UV light to the specimen was 14.5 cm. We used a univariate analysis of variance (ANOVA) to examine the effect of sex and Julian date (season) on the numbers of fluorescent glands in animals from our reference site. Number of glands was the dependent variable in our analysis and sex and Julian dates were designated as fixed factors. All statistical analyses were conducted in SPSS version 28.0.

Gland Identification.—We used the image processing program ImageJ (Schneider et al., 2012) to measure glands of two discrete sizes on the midventral surface of the tail of salamanders from Summit County, Ohio, United States, near our reference site. Salamanders were photographed in the laboratory in autumn (7 November 2022) when fluorescence is most evident. We used a Nikon D5100 DSLR in a darkened room under UV light from an Escolite 395-nm handheld LED UV light mounted 11 cm above the specimen. Additionally, specimens were photographed under white light (flash) to produce a clearer companion image for each UV-illuminated image. The camera was fitted with a 60-mm micro-NIKKOR 1:1 macro lens. The glands are visible in the skin under both white and UV light. In most males, larger glands fluoresce brightly when exposed to UV light (395 nm), smaller glands do not. In females, glands of about equal size either fluoresce or do not. We measured glands from images in 10 adult salamanders of each sex, randomly selecting 10 fluorescing and 10 nonfluorescing glands from each salamander. In most cases, glands were almost perfectly circular in appearance. A single linear measurement of diameter was taken across each gland at its widest point. To test for the effect of sex on sizes of the two gland types we used a general linear model (GLM) with sex designated as a fixed factor, SVL (snout to the anterior edge of the vent) was used as a covariate, and widths of the two gland types were dependent variables in the analysis.

Quantification of Fluorescence.—To estimate biofluorescence of individual salamanders, we imported digital photographs of the ventral surface of salamanders into ImageJ and measured SVL and tail length (including the vent). We identified an area on the tail equal to 1/3 of SVL for quantifying the glands in each image. This area stretched from the anterior edge of the vent posteriorly along the tail. We used this truncated area of the tail because many salamanders showed evidence of older tail breaks and, in regrown portions of the tail, glands take several months to repopulate (data not shown). Thus, we reasoned that the best way to account for previous (as well as recent) tail loss was to focus on an area of the tail that is gland-rich (Simons et al., 1994) and unlikely to be autotomized. This approach also standardized the area of the patch quantified for each salamander. We counted all glands in the designated area that were visibly excited by UV light.

Geographic Variation in Fluorescence.—Four populations from geographically distant portions of the range of *P. cinereus* were sampled in October of 2021 for a separate study on tail autotomy in this species. Adult salamanders were collected from Indiana (Parke County; $n = 19$), our reference site in northern Ohio (Cuyahoga County; $n = 37$), southern Ohio (Hocking County; $n = 29$), and eastern Pennsylvania (Lancaster County; $n = 27$). The populations sampled belong to mitochondrial clades described by Radomski et al. (2020). Specifically, our Pennsylvania and Indiana populations fall within the Northern clade, our northern Ohio population is within the Pennsylvania clade, and our southern Ohio population is in the Ohio clade. Only adult salamanders were used in this part of our study. These specimens were photographed in the laboratory using a Nikon D5100 DSLR as above. Exposure settings were manually adjusted as above to ensure consistency across photographs. We used a univariate ANOVA to test the effects of sex and location on number of fluorescent glands. Number of glands was the dependent variable in our analysis and sex and location were designated as fixed factors in this analysis.

Results

At all sampled sites visibly fluorescent glands were most obvious in adult male salamanders (Figs. 1 and 2). Fluorescence was concentrated on the ventral portion of the tail and was typically densest in the anterior 2/3 of the tail. In some cases, fluorescence extended to the tail tip. Fluorescence was also occasionally observed as scattered spots on the ventral trunk (as in Fig. 1), but only in males. At our reference site in northeastern Ohio, neonates (mean SVL = 20.0 mm; $n = 19$) and juveniles (mean SVL = 26.6 mm; $n = 76$) did not fluoresce, but a few large juveniles (or small adults) showed evidence of weak fluorescence. These larger juveniles were within 1 mm of our cutoff for adult sizes (three presumptive females and two presumptive males). Adult males (mean SVL = 36.3 mm; $n = 140$) were more fluorescent than adult females (mean SVL = 37.1 mm; $n = 97$; $F_1 = 50.78$, $P < 0.001$) at our reference site throughout the sampling period (Fig. 3; $F_7 = 6.25$, $P < 0.001$) and the difference between male and female fluorescence was at its greatest in September, October, and November (Fig. 3; date * sex interaction; $F_7 = 3.68$, $P < 0.001$). In total 77.1% of 140 males fluoresced and 33.0% of 97 females fluoresced at our reference site. Differences in fluorescence between males and females were consistent across sites sampled throughout the range of *P. cinereus*. Male salamanders had significantly higher numbers of fluorescent glands compared to females ($F_1 = 169.31$, $P < 0.001$), and this was true across all populations sampled (Fig. 4; $F_3 = 4.60$, $P = 0.005$). There was no significant sex * location interaction ($F_3 = 1.51$, $P = 0.217$).

When we compared our linear measurements to published values for gland sizes in *P. cinereus*, we found strong congruence with sizes of serous 1 (S1) glands and the smaller mucous glands. In males (mean SVL = 37.3 mm ± 0.56), large fluorescent S1 glands averaged 0.239 mm (±0.008) and mucous glands averaged 0.113 mm (±0.002) in diameter. In females (mean SVL = 37.9 mm ± 0.54), fluorescent S1 glands averaged 0.120 mm (±0.004) and mucous glands averaged 0.112 mm (±0.0014) in diameter. Sever and Siegel (2015) report a mean mucous gland height of approximately 0.080 mm (extrapolated from their Fig. 2), which is similar to our mean of 0.112 mm. Similarly, literature values for individual S1 glands making up the postcloacal gland measure approximately 0.20–0.24 mm in diameter (extrapolated from figures in Simons and Felgenhauer, 1992 and Simons et al., 1999), whereas our presumptive S1 glands averaged 0.24 mm in males. Gland height should be comparable to our linear diameter measures because S1 and mucous glands are globular in shape in the postcloacal region (Simons et al., 1999). Differences in gland sizes between sexes were not influenced by SVL (S1 glands: $F_1 = 0.075$, $P = 0.787$; mucous glands: $F_1 = 2.604$, $P = 0.125$). Males had significantly larger fluorescent S1 glands compared to females ($F_1 = 152.49$, $P < 0.001$). Sizes of mucous glands did not differ between males and females from our sample ($F_1 = 0.009$, $P = 0.927$).

Discussion

When exposed to 395-nm UV light, males of the Eastern Red-backed Salamander, *P. cinereus*, fluoresced much more brightly than females. Juveniles and neonates did not fluoresce under UV light. We present evidence that the source of the fluorescence is the S1 glands described by previous researchers, the first such evidence of a glandular source of fluorescence in Caudata. The sexually dimorphic pattern was observed across four spatially distant localities. Fluorescence was most evident in both sexes in autumn, but this seasonal peak was more prominent in males.

Gland Description.—Hecker et al. (2003) described two types of granular serous glands in *P. cinereus*, the serous 1 (S1) and serous 2 (S2) glands. These two glands have distinct functions: S1 glands are thought to function in chemical communication and S2 glands function in nutrient storage and in an antipredator context. S1 serous glands are simple granular acinar glands and are located in the dermis on the ventral surface of the tail and surrounding the cloaca (Hecker et al., 2003). These glands are larger than S2 glands, which are most densely concentrated on the dorsal surface of the tail; both glands are much larger than mucous glands, which can be found scattered among chromatophores throughout the integument (Simons et al., 1994; Hecker et al., 2003). In *P. cinereus*, S1 glands are collectively considered a macrogland, which is located posterior to the cloaca on the ventral tail and is referred to as the postcloacal gland (PCG; Simons et al., 1994). The postcloacal gland is used by both sexes to place territorial scent marks on substrates and on fecal pellets through a behavior known as the "postcloacal-press" (Jaeger et al., 1986; Jaeger and Gabor, 1993; Simons et al., 1994, 1997, 1999). In our specimens, sizes of large fluorescent glands relative to smaller nonfluorescent glands are consistent, at least in males, with size differences between the only common gland types, mucous and S1, that are found in abundance in the ventral skin of the tail (Hecker et al., 2003). Based on the size, appearance, and location of fluorescent areas on the ventral surface of tails observed in our study, we propose that the sources of fluorescence are S1 glands that constitute the PCG in *P. cinereus*.

An unanswered question remains. Why were the fluorescent glands of female *P. cinereus* so much smaller than those of males in our sample? Hecker et al. (2003) found that large S1 glands made up 25% and 17.5% of glands in the ventral tails of male and female *P. cinereus*, respectively. The authors did not report differences in the sizes of the S1 glands, but they describe them as equally developed in both sexes. Alternatively, Smith (1963) reported that large glands in the ventral tail of *P. cinereus* were most visible in reproductively active males, and Thurow (1956a) reported similar dimorphism in *P. cinereus* as well as in other species of *Plethodon* (Thurow, 1956b; 1964; 1966). Thurow (1956a) noted that large ventral tail glands in *P. cinereus*

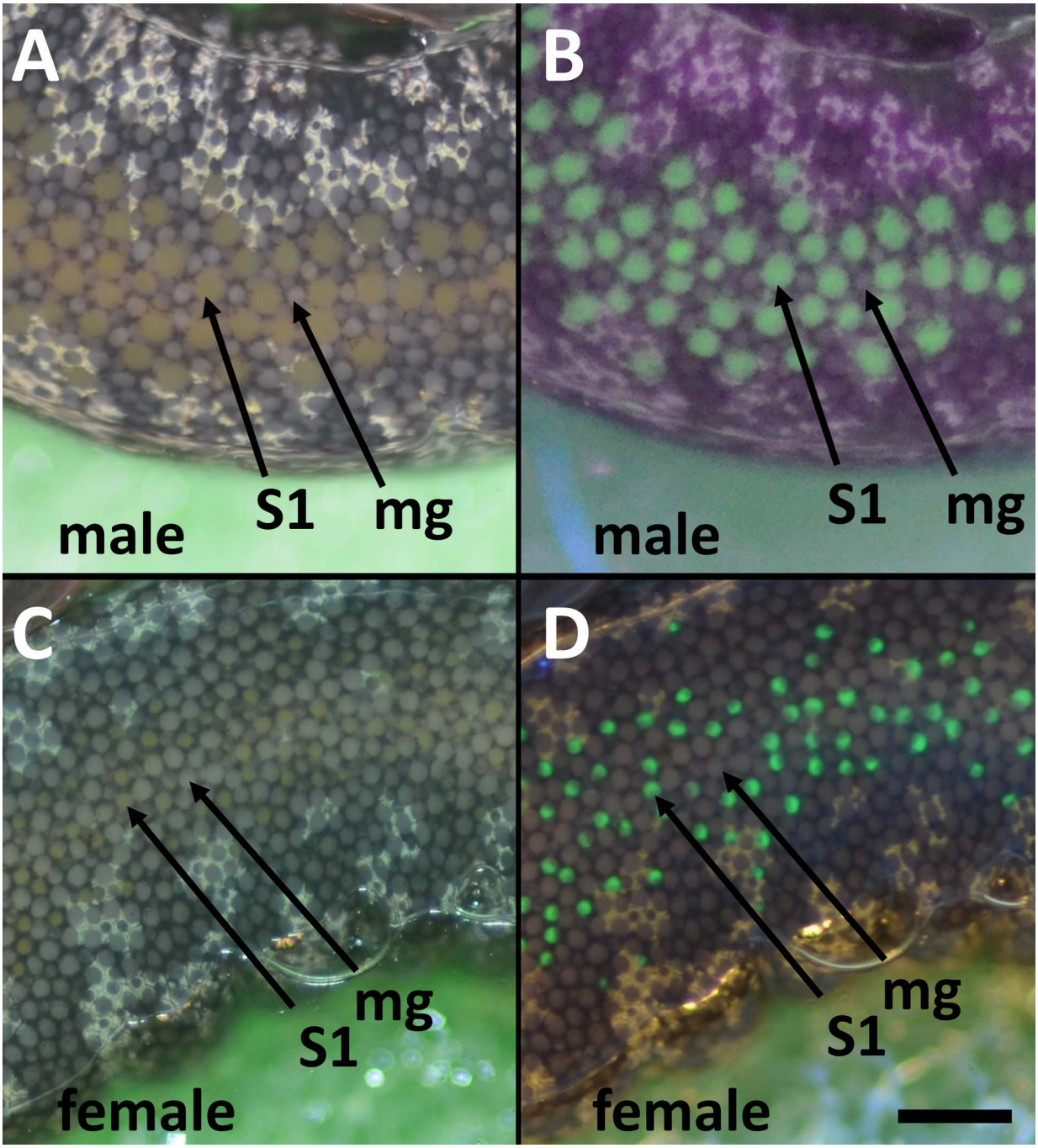

FIG. 2. Close crop of a ventral portion of the gland cluster just posterior to the cloaca in a male (top, A and B) and a female (bottom, C and D) salamander under white (left) and UV (right) light. The male salamander was photographed under 395-nm UV. The female salamander was photographed under 360-nm UV illumination to enhance fluorescence. Large fluorescent glands in the male are visible under white light as golden yellow orbs. These same glands fluoresce brightly under UV light. In the female salamander, fluorescent glands are similar in size to nonfluorescent glands. S1, simple granular acinar glands; mg, mucous gland; scale bar = 1 mm.

produced an orange- or gold-colored secretion. The large S1 glands in our samples can be easily seen through the skin with a dissecting microscope, and the orange coloration of the glands is evident under white light (Fig. 2a, c). Note that this coloration is visible in both females (Fig. 2a) and males (Fig. 2c), but it is much more evident in males. Note also that it is the orange-colored glands that fluoresce in both sexes. Our data suggest that, in *P. cinereus*, the PCG of males is made up of larger S1 glands than that of females, a size difference found in other glands in related species (Rollins and Staub, 2017; Trame et al., 2022). Additionally, because we found large differences in the numbers of fluorescent glands between the sexes (discussed in the following), our data also suggest that either females have fewer S1 glands in the tail, or fewer of their S1 glands fluoresce. These sexual differences in PCG morphology may suggest a duality of function in males, wherein territorial males and territorial females (sensu Horne and Jaeger, 1988) use the PCG to scent mark, but only in males is the gland complex a target of sexual selection.

Potential for Visual Perception of Fluorescence.—Although the function of biofluorescence is unknown in salamanders, researchers have speculated that salamanders may visually perceive

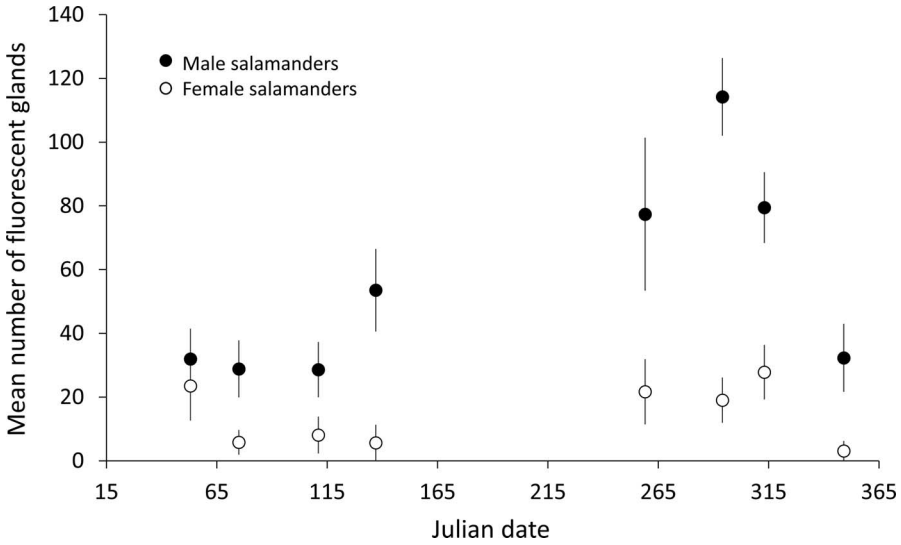

FIG. 3. Seasonal sexual dimorphism in UV fluorescence of adult *Plethodon cinereus*. Mean number of visibly fluorescent glands is indicated for each collection date. Bars are SE. Glands were counted in the ventral portion of the tail (1/3 of SVL) when exposed to 395-nm UV light.

fluorescence of other individuals (Munoz, 2018; Lamb and Davis, 2020; Macel et al., 2020; Thomas et al., 2022). Chen et al. (2008) found that salamanders lost blue sensitive cones (after metamorphosis) while increasing size, density and sensitivity of green rods, a transition that corresponds to a shift from shallow aquatic to dim terrestrial habitat. Therefore, wavelengths of green light emitted from green biofluorescence in terrestrial salamanders should be visually perceived by conspecifics and perhaps heterospecifics (Chen et al., 2008). Additionally, green light is abundant in forest shade because chlorophyll preferentially absorbs red and blue wavelengths, so under a full canopy, the ambient light tends to be depleted in those wavelengths and more abundant in intermediate green and yellow wavelengths (Endler, 1993). If *P. cinereus* also has green rods, it is possible that they could aid in the visual perception of green wavelengths common in forest habitats, as well as those emitted from the fluorescent postcloacal glands in males. *Plethodon cinereus* individuals are known to use visual displays in territorial contests (reviewed in Jaeger et al., 2016), and there is some evidence that both visual and chemical cues can be used to differentiate familiar from unfamiliar conspecifics (Kohn and Jaeger, 2009). If salamanders can visualize the emitted fluorescent wavelengths, it is possible that fluorescence of the PCG may function in a territorial and/or mating context. We argue below that, despite glands being present on the ventral surface and typically in contact with the substrate, there are many opportunities for visual inspection of the PCG in reproductive and competitive contexts.

Sexual Dimorphism.— Our data clearly show that fluorescence of the postcloacal gland (PCG) in *P. cinereus* is sexually dimorphic, with males having larger diameter and greater numbers of fluorescent S1 glands compared to females. These differences between sexes were consistent among geographically distant localities of three mitochondrial lineages (Radomski et al., 2020), suggesting that the trait is widespread across populations of *P. cinereus*. Juveniles in our study lacked fluorescent S1 glands,

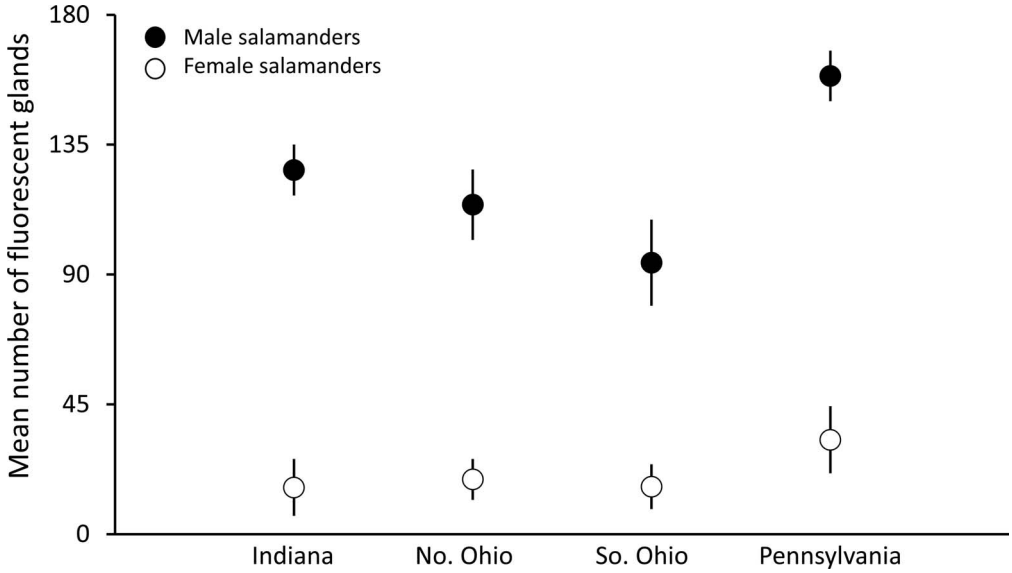

FIG. 4. Geographic variation in UV fluorescence of adult *Plethodon cinereus* in autumn. Mean number of visibly fluorescent glands is indicated for each locality. Bars are SE. Glands were counted in the ventral portion of the tail (1/3 of SVL) when exposed to 395-nm UV light.

despite the fact that Simons et al. (1994) reported active simple acinar glands (i.e., glands containing secretory product) in the postcloacal region of juveniles (one neonate of unknown sex, one subadult female). It is not yet known how the glands of juveniles change as they reach maturity and fluorescence is expressed.

Terrestrial plethodontid salamanders perform nose tapping (NT) behavior during which they tap their snouts to a surface to sense chemical stimuli that convey information about sex, quality of potential mates and their territories, and fighting ability of territorial residents and intruders (Mathis, 1990; reviewed in Jaeger et al., 2016). *Plethodon cinereus* individuals are known to NT substrates (Jaeger and Gergits, 1979), fecal pellets (Jaeger, 1984), competitors such as conspecifics (Gergits and Jaeger, 1990), other species of salamanders (Lancaster and Jaeger, 1995), and even centipedes in laboratory contests (Hickerson et al., 2004). Odors travel up the nasolabial grooves, which extend from the upper lip to the lateral corner of each external naris. These grooves function as capillary tubes, pulling odors into the nares, which stimulates the vomeronasal sensory epithelium in the brain (Dawley and Bass, 1989; Graves, 1994). One interesting question related to the role of fluorescence in communication in Eastern Red-backed Salamanders is whether the chemical information garnered by NT, and sex-specific behaviors like fecal squashing (Walls et al., 1989; Jaeger and Wise, 1991), is actively advertised by the sender via the visual signal of fluorescence. If so, fluorescence could provide an additional signal modality for advertising male quality to potential mates (Candolin, 2003; Jaworski et al., 2018). Chouinard (2012) designed experiments to test if female *P. cinereus* could assess differences in the quality of prey consumed by males through sources other than fecal pellets. Previous studies found that females engage in fecal squashing, which likely provides cues about diet quality (e.g., chitinous content of prey), but that these cues are not actively advertised by the sender and may be a byproduct, or indirect signal, of male quality rather than an honest signal. Chouinard (2012) obtained odors from body washes of males fed high- versus low-quality diets to eliminate confounding factors of prey odors in feces and other odors associated with male substrates. He also examined the amount of protein in the mental and PCGs of males fed different-quality diets. Gravid females were able to determine differences in male diet quality based on body wash alone, and high-quality males had significantly more protein in both types of signaling glands compared to low-quality males. Changes in the diets of males may therefore be associated with changes in production of the protein signal and may serve as honest signals of not only male quality, but also quality of his territory. If intensity, brightness, or number of fluorescent glands in PCGs of male *P. cinereus* is indicative of the amount of protein in the glands, it may serve as a secondary honest signal that is assessed visually by females. Such signal variation as described by Chouinard (2012) might also explain within-population variation in number of fluorescent S1 glands observed among individuals in our study.

There are two behaviors that occur during courtship of *P. cinereus* that present opportunities for females to examine fluorescence of the PCG. Dyal (2006) reported novel courtship behaviors in females of *P. cinereus* in which they engage in "cloacal nudging." Nudging occurs when the snout is in contact with or pressed against the cloacal region of a courting male. A second courtship behavior involves tail arching and undulation by males. Gergits and Jaeger (1990) observed 10 courting pairs (4 of which resulted in insemination) on 15 October 1974. When a male crossed a female's pheromonal trail, "he nose-tapped with side-to-side motion, aligned himself with the trail, and followed it precisely during all 10 interactions. Upon approaching the female, the male sometimes nose-tapped the female's body and then always moved in front of her, which blocked the female's movement. At this point, the *male arched his tail upward just distal to the vent, and, with the tip still on the ground, began to wiggle his tail from side to side*" (italics ours). If the female remained in place, courtship proceeded, but if she moved away the male reoriented himself in front of the female and began the arched tail-wagging behavior again. Because cloacal nudging and tail arching expose the fluorescent portions of the tail to would-be mates, the two behaviors provide support for the notion that fluorescence is a signal of mate quality. However, whether fluorescence correlates to measures of male quality is an untested hypothesis.

Seasonality.—Seasonal variation in fluorescence provides another line of evidence that the PCG in male *P. cinereus* might be an important signal of male quality and mate choice by females. We found that fluorescence in males peaked in October, declined through late fall, and was at its lowest in March and April. This peak in fluorescence falls within the extended mating season reported by other researchers (Sayler, 1966; Werner, 1969; Sever, 1997; Sever and Siegel, 2006) which lasts from October to April, and corresponds to the pattern of hypertrophy in the breeding season and atrophy outside the breeding season in courtship glands in other plethodontids (Rupp and Sever, 2018; Trame et al., 2022). Perhaps more importantly, a study examining seasonal variation in testosterone levels in Eastern Red-backed Salamanders found that levels peaked in September and October (Church and Okazaki, 2002) which corresponds to our peak in fluorescence of the PCG. Thus, seasonal variation in fluorescence coincides with observed fall mating, with a peak in testosterone, and with aggressive interference by extra-pair males (Gergits and Jaeger, 1990). If intensity of fluorescence is in fact a true signal of male quality or fighting ability, we predict that highly fluorescent males may be more successful in courting females and repelling rival males. This prediction could be tested by comparing fluorescence of paired and unpaired males in the field.

Male–Male Communication.—An alternative function of fluorescence of the postcloacal gland (PCG) in *P. cinereus* may be its use as a signal in male–male communication. Postcloacal nudging is a behavior originally observed in males (Jaeger and Gabor, 1993) and may function in a territorial context. Jaeger and Gabor (1993) observed both residents and intruders "shoving" their snouts under the tails of other males as if lifting the tail to position the snout at the PCG. They found that chemosensory investigation is predominantly directed at the portion of the body containing the PCG, and that early evaluation of pheromones between residents and intruders lessens the likelihood of escalation to biting, especially by intruders. Although it is unclear why residents would nudge the PCG of intruders, Jaeger and Gabor (1993) proposed that the gland may convey information beyond territorial ownership. Perhaps the gland contents provide the assessor with information regarding the identity of kin, neighbors (Jaeger, 1981), and sex, or assessment of fighting ability of the intruder (Wise, 1991). We suspect that cloacal nudging behavior also reveals fluorescence of the PCG and that gland fluorescence may convey similar or related information to opponents during male–male interactions. Whether variation in fluorescence correlates to important traits such as fighting ability and resource holding potential remains to be tested.

Wise et al. (2004) examined the effect of tail condition (salamanders with or without tails) on scent marking and found that scent-marking chemicals may convey important information about the resource-holding potential of residents and that these chemicals are produced by the PCG. If fluorescence of those glands serves as a visual signal of scent-marking chemicals, partial or complete tail loss for residents would not only inhibit chemical production and deposition of the scent mark, but also the accompanying visual signal of the resident's fighting ability, or other status. Wise and Jaeger (1998) found that in resident–intruder contests, with both visual and olfactory information available to opponents, tailed intruders were less aggressive toward tailed residents compared to those without tails. They suggested that tail loss may lead to decreased defensive ability of residents and may result from loss of the tail as a component of aggressive displays like all-trunk raised (ATR). We suggest that because residents without tails lack a fluorescent signal, it is also possible that intruders were emboldened during intrusion into residents' territories and responded with increased aggression.

Jaeger (1984) described a suite of agonistic visual displays performed by *P. cinereus* in territorial contests. The behavior ATR was defined as a "look big" threat posture in which the animal "extends the legs downward such that the head and entire trunk are raised above the substrate. . .occasionally with the trunk arched. *The tail may be entirely raised or distally resting on the substrate*" (italics ours). Jaeger and Schwartz (1991) subsequently redefined ATR as a gradational series of postures signaling increasing threat (ATR1–ATR5). They suggested the arching of the trunk and the raised tail as "integral components" of the behavior rather than random variables. ATR3 includes the addition of the tail lifted from the substrate, and during ATR5 the trunk is arched and the tail is lifted simultaneously. Jaeger and Schwartz (1991) found that intruders spent more time in submissive behaviors as residents increased intensity of ATR, suggesting that intruders recognize the gradational threat signals. Because ATR3 and ATR5 expose the fluorescent PCG of male red-backed salamanders for visual inspection by rival males, the fluorescence may provide an additional signal related to fighting ability or resource holding potential. Thus, opponents may use a combination of scent marks deposited on the substrate and the visual signals of body position and fluorescence to assess potential competitors.

Conclusions

Biofluorescence in amphibians has only recently been described, and there are many unanswered questions regarding its function. Seasonal, demographic, and geographic patterns of biofluorescence in the postcloacal gland described here suggest that fluorescence is widespread in *P. cinereus* and, because fluorescence is sexually dimorphic (and most apparent in males), it is likely associated with courtship behavior and possibly male–male competition. Mate choice is heavily dependent upon territory holdings of resident males in this species (reviewed in Jaeger et al., 2016), so the two contexts (mating and competition) are difficult to separate experimentally. It is possible for fluorescence of the PCG in males of this species to serve a dual function as both an honest signal of mate quality and as a signal of fighting ability in territorial contests. Additional research should focus on whether woodland salamanders can visually perceive biofluorescence, whether fluorescence of males correlates with measures of male quality and fighting ability, and how widespread fluorescence is in the genus and family.

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