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SHORT COMMUNICATION

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Axanthic-blue colour aberration in the American green treefrog *Hyla cinerea*: clues to the drivers of its spatial distribution

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Deviations from typical and evolutionary-selected colour patterns can occur in non-polymorphic species due to phenotypic expression of recessive genetic mutations, often triggered by evolutionary pressures or even anthropogenic factors (Ellegreen et al., 1997; Jablonski et al., 2014; Masselink & Tanaka, 2021). These chromatic aberrations frequently result in reduced survival and reduced reproductive success due to natural selection (Andrén & Nilson, 1981; Masselink & Tanaka, 2021), making them rare in natural populations (Jablonski et al., 2014; Henle & Dubois, 2017).

In frogs, albinism and leucism are the most extensively documented chromatic aberrations (Jablonski et al., 2014). A less frequently documented aberration is axanthism, which involves the absence of yellow or orange pigmentation (due to a lack of xanthophores), red pigmentation (due to a lack of erythrophores) and reflective or scattering pigmentation (due to a lack of iridophores) (Jablonski et al., 2014; Gould & McHenry, 2024). As a result, axanthic frogs typically exhibit one or a combination of the following colouration patterns: 1) a completely or partially blue body; 2) a grey or unusually dark body—though these cases can be difficult to distinguish from melanism (overproduction of melanin); and 3) a typical body colouration but with dark eyes (Jablonski et al., 2014).

We recently recorded an example of blue axanthism in an American green treefrog *Hyla cinerea* in Illinois (USA). This species, typically characterised by its lime-green dorsal surfaces, is widely distributed across the south-eastern USA. A single case of axanthism has previously been reported for this species (Cain & Utesch, 1976) but involved a non-blue form of this aberration. Consequently, our record represents the first documented case of this aberration for this species in Illinois. To further investigate the spatial distribution of this aberration and provide some, but not exhaustive, insights into the potential drivers of this phenomenon, we searched citizen science data for additional records of blue axanthism in *H. cinerea* and mapped them across the species native range, and in relation to land cover and human population density.

On 13 October 2024, at 15:08 h, we observed a juvenile axanthic-blue *H. cinerea* of unknown sex. It was found resting on a thin leaf at the edge of a clear, spring-fed lentic water body in Union County, southern Illinois (37° 34’29.2” N, 89° 26’22.8” W, WGS84) (Fig. 1A), at an air temperature of about 19 °C. The surrounding habitat consisted of low vegetation, sparse trees and a rocky outcrop (Fig. 1B). The dorsal surfaces of the individual were entirely cerulean blue,

Figure 1. American green treefrogs *Hyla cinerea* observed in southern Illinois (USA) - **A**. An axanthic-blue juvenile, **B.** Lentic water body habitat, and **C.** Typical-coloured juvenile specimen

with some scattered white dots bordered by greyish blue. The tympanic region was greyish blue. It exhibited a lateral white stripe extending from the jaw to the thigh, typical for the species, with some blue pigment extending below this line. The ventral surfaces were not directly inspected to avoid handling the specimen but, apart from the bluish vocal sac, appeared whitish, as is typical for the species. The eye was golden-brown, darker than the typical eye colour for the species. Besides the axanthic individual, three other juvenile *H. cinerea* were observed nearby, all displaying the typical lime-green dorsal colouration (Fig. 1C).

We compiled an additional 62 records (53 single localities) of axanthic-blue American green treefrogs by searching citizen science data from the Aberrantly Blue Frogs project at the online platform iNaturalist (https://www.inaturalist.org/projects/aberrantly-blue-frogs http://www.inaturalist.org) up until 31 December 2024. While we acknowledge that axanthism can manifest as a range of grey to dark body colours or darker eyes, we limited our search exclusively to blue-coloured individuals to ensure that we were examining a singular biological phenomenon (the lack of xanthophores) and avoiding the inclusion of melanism cases. The extent of blue colouration in these axanthic individuals varied widely, from isolated blotches on the typical green background, such as limited to the tympanum (e.g. https://www.inaturalist.org/observations/252558377), to scattered blotches (e.g. https://www.inaturalist.org/observations/252558376), and complete dorsal surface coverage, shown by our record. This approach highlights the valuable contribution of citizen science initiatives in documenting chromatic variation. Additionally, it provides an initial indication of the rarity of this particular aberration in natural populations of *H. cinerea* as records of blue axanthism found on iNaturalist accounted for only 0.1% of the total observations of this species at the time of the search (42,166 observations). This percentage is even likely overestimated, as aberrant blue frogs are probably more prone to be documented on iNaturalist, and some records may be repeated observations of the same individuals.

Through the mapping of citizen science records and the new record from Illinois, it is evident that most observations of axanthic-blue *H. cinerea* are concentrated near the edges of the species range (Fig. 2A). This pattern is only not corroborated in central Texas and southern Florida. Among the states where the species is native, the chromatic aberration has been recorded as follows (numbers of observations in parentheses): Alabama (2), Florida (6), Georgia (1), Illinois (2), Louisiana (2), Maryland (2), North Carolina (10), Oklahoma (1), South Carolina (3), Texas (3) and Virginia (21). However, nine other states within the species native range remain without records of this aberration: Arkansas, Indiana, Kentucky, Missouri, New Jersey, Pennsylvania, Mississippi, Tennessee, and West Virginia (Fig. 2A).

The spatial distribution of blue axanthism in *H. cinerea* may be influenced by several factors that are not mutually exclusive, but here our attention is focused on hypotheses related to natural population dynamics and anthropogenic influences. Regarding natural drivers, the higher frequency of axanthism records from populations located at the

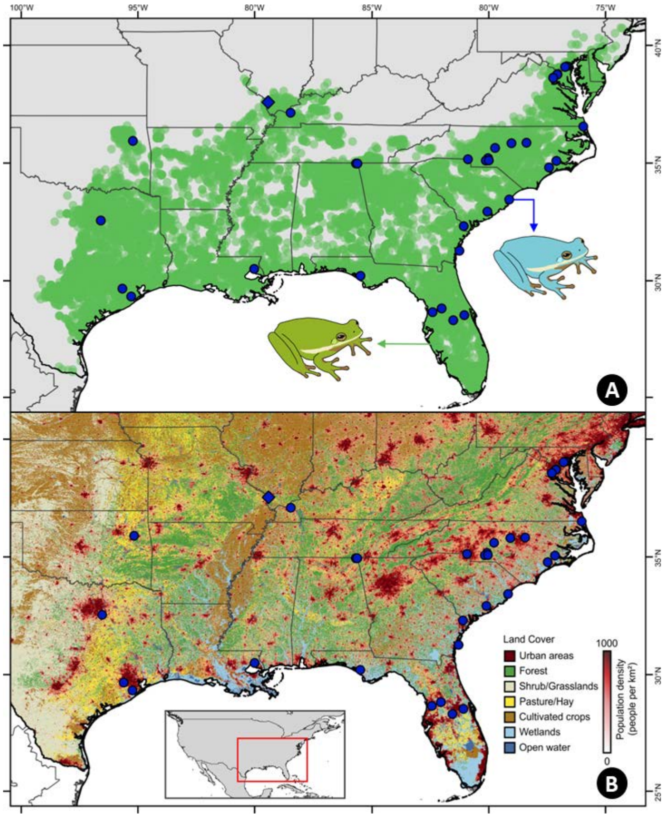

Figure 2. Geographic distribution of axanthic-blue American green treefrogs *Hyla cinerea* across the south-eastern United States (blue symbols), including our new record from southern Illinois (diamond) and citizen science contributions up to 31 December 2024 (iNaturalist.org; circles) - **A.** The distribution of axanthism compared to the species overall range (green symbols) based on all iNaturalist records, and **B.** The distribution of axanthism compared to land cover (sensu US Geological Survey, 2025) and human population density (sensu Pesaresi et al., 2024)

periphery of the species range supports the Centre-Periphery Hypothesis (reviewed by Pironon et al., 2017). This hypothesis posits that peripheral populations often exhibit reduced genetic diversity compared to central populations, due to factors such as restricted gene flow, smaller effective population sizes, lower habitat suitability and founder effects (Brown, 1984; Pironon et al., 2017). These factors can independently or synergistically enhance evolutionary processes such as natural selection and genetic drift, resulting in increased accumulation and fixation of mutations in peripheral regions (Eckert et al., 2008; Pironon et al., 2017). As a result, chromatic aberrations, including axanthism, are expected to be more prevalent in peripheral populations than in central ones. We also cannot rule out the effects of sampling bias in generating this pattern, as the regions with fewer records on iNaturalist are also more central to the species distribution (Fig. 2A). However, the fact that even well-sampled areas within this central region still lack records of this chromatic aberration seems to further support the Centre-Periphery Hypothesis.

Other researchers have proposed that anthropogenic factors, such as pesticide use and other chemical exposures, can increase mutation rates in species, potentially contributing to an increased occurrence of chromatic aberrations in natural populations (Ellegreen et al., 1997; Jablonski et al., 2014). However, no clear spatial correlation is observed between axanthic-blue cases of *H. cinerea* and agro-pastoral systems (sensu US Geological Survey, 2025), which typically employ large amounts of chemicals in their operations. This is particularly evident as the areas surrounding the largest expanse of cultivated crops and pastures within the species range lack any records of blue axanthism. On the other hand, a spatial correlation is apparent between axanthism cases and human population density (sensu Pesaresi et al., 2024), with a higher numbers of records reported in urban areas (Fig. 2B). Such a correlation is not entirely consistent, as densely populated central regions, such as Atlanta (Georgia), Birmingham (Alabama) and Jackson (Mississippi), as well as peripheral areas like Austin and San Antonio (Texas) and the coastal region of Florida, show no records of blue axanthism in this species (Fig. 2B). Such a correlative inconsistency between anthropogenic influence and blue axanthism in *H. cinerea* suggests that the observed association between higher axanthism case numbers and urban areas is more likely a result of sampling bias or genetic factors rather than conclusive evidence of any harmful effects caused by environmental pollution.

Considering this focus, we postulate that the occurrence of blue axanthism in *H. cinerea* is likely influenced by a combination of natural population dynamics and sampling biases. Although urban regions tend to yield more observations of axanthic blue individuals, this pattern is not consistent across the species range, as several densely populated regions within the species range lack records of this chromatic aberration. Thus, evolutionary forces, particularly those affecting peripheral populations, may play a more significant role in the increased incidence of this chromatic aberration at the edges of the species distribution. Nonetheless, we reinforce that these hypotheses remain highly speculative, and further research is needed as more data become available to allow for robust statistical testing. This would enable more explicit tests to confirm or refute these hypotheses, or propose alternative explanations.

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