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Potential Spectral Tuning of the Tapetum Lucidum in a Broadly Distributed Ungulate

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ABSTRACT

The tapetum lucidum, a biological reflector system found in the eyes of many vertebrate species, enhances visual sensitivity in low light conditions by reflecting light back through the retina. The structure of the ungulate tapetum fibrosum is unique, and its reflectance varies temporally in reindeer (*Rangifer tarandus*). We used reflectance spectroscopy to characterize the white-tailed deer (*Odocoileus virginianus*) tapetum fibrosum and to determine if reflectance varies spatially across the species' range. Our results revealed spatial variation in tapetal coloration and reflectance across parts of the white-tailed deer's range, with populations displaying distinct spectral profiles. Deer in the northern populations of Missouri, Minnesota, and Indiana primarily possessed more short-wave-reflecting tapeta, whereas deer in the Georgia population exhibited more mid-wave-reflecting tapeta and the tapeta of Texas deer appeared intermediate in reflectance. Our findings suggest that tapetal reflectance in white-tailed deer may be influenced by ecological and environmental pressures that vary spatially. These results suggest that local variations in the spatial and temporal dynamics of the tapetum fibrosum in ungulates may be an adaptive trait.

1 | Introduction

Many vertebrate species possess a biological reflector system in their eyes known as the tapetum lucidum (Schwab et al. 2002; Ollivier et al. 2004). The tapetum serves as a reflective surface at the back of the eye and provides a second opportunity for photoreceptor stimulation, enhancing visual sensitivity at low light levels (Schwab et al. 2002; Ollivier et al. 2004; Zueva et al. 2022). Vertebrate tapeta exhibit a diverse array of structures, organization, and composition (Ollivier et al. 2004; Zueva et al. 2022) with the choroidal tapeta being the most common (Schwab et al. 2002). Choroidal tapeta can be further divided into two separate classifications: tapetum cellulosum and tapetum fibrosum (Schwab et al. 2002; Ollivier et al. 2004; Zueva et al. 2022). Most mammalian carnivores possess a tapetum cellulosum composed of regular, cell layers containing

a variety of reflective materials (Schwab et al. 2002; Ollivier et al. 2004). In contrast, most ungulates possess a tapetum fibrosum composed of collagen fibrils suspended in fluid that create a reflective surface through diffuse and specular reflection (Schwab et al. 2002; Ollivier et al. 2004; Zueva et al. 2022).

Among mammals, carnivores exhibit the greatest reported variation in tapeta reflectance (Ollivier et al. 2004). Cellular variations in carnivore tapeta likely represent selective visual adaptations for specific wavelengths associated with the animal's ecology and their environment (Schwab et al. 2002; Fosbury and Jeffery 2022). Ungulate tapeta are often considered less remarkable in the variability of their spectral reflectance. However, reindeer (*Rangifer tarandus*), exhibit seasonal shifts in tapetum reflectance corresponding to not only the amount of reflectance, but also the specific wavelengths

Summary

- Reflectance spectroscopy revealed spatial variation in tapetum fibrosum coloration across the range of white-tailed deer (*Odocoileus virginianus*).

- Thus, tapetal reflectance may be shaped by ecological pressures and represent an adaptive function.

reflected (Stokkan et al. 2013; Fosbury and Jeffery 2022). The tapetum fibrosum of reindeer shifts from a gold-turquoise color with mid-wave reflectance in summer, a common color and reflectance observed across many ungulates, to a deep-blue color and short-wave reflectance in winter (Stokkan et al. 2013; Fosbury and Jeffery 2022). This seasonal shift in tapeta color and reflectance might be the result of coupled variations in the spacing and degree of order of the collagen fibrils related to the volume of interstitial fluid (Fosbury and Jeffery 2022). Reindeer are currently the only mammal known to undergo these seasonal changes, but the unique structure of the tapetum fibrosum means the tapeta of other species that experience prolonged changes in light environment may vary similarly (Fosbury and Jeffery 2022).

White-tailed deer (*Odocoileus virginianus*) are widely distributed across North and South America from the arctic to the tropics (Geist 1998). Among cervids, the white-tailed deer visual system is one of the most extensively studied (Newman and D'Angelo 2024), however, descriptions of their tapetum are limited. The tapetum of white-tailed deer, similar to other medium-large sized ungulates, covers a large portion of the superior eye-cup and exhibits a nearly horizontal inferior border (D'Angelo et al. 2008). Additional basic characteristics of the tapetum describe an azure-blue color centrally that transitions toward the periphery into shades of blue-green and yellow (D'Angelo et al. 2008). Given the broad distribution of white-tailed deer and the flexible nature of tapetum fibrosum reflectance, our objectives were to characterize the spectral reflectance of white-tailed deer tapeta across a larger portion of their range and to determine if spectral reflectance varies between populations.

2 | Methods

We obtained fresh whole eye globes from Georgia ($n = 5$; March 01, 2023), Indiana ($n = 7$; March 01, 2023), Minnesota ($n = 3$; April 26, 2023), Missouri ($n = 8$; March 14–15, 2023), and Texas ($n = 4$; March 24, 2023) during project-independent culling efforts by federal and state agencies of free-ranging (Georgia, Indiana, Minnesota, and Missouri) and captive (Texas) white-tailed deer (Figure 1). Culled deer were euthanized by sharp-shooting with a high-powered rifle. Immediately after death, we enucleated both eyes and transferred the fresh, whole globes to a buffered saline solution for < 36 h of storage at 24°C–26°C. Before measuring tapetum reflectance, the cornea, lens, and retina were removed. We placed the excised eye cups within a concave holder to prevent deformation and wrinkling. We conducted the reflectance measurements with an ILT950 Spectri-Light spectroradiometer (International Light Technologies,

Peabody, MA), neutral-white LEDs, and a UV bulb. Measurements were focused over the central area of the tapetum fibrosum approximately 45 mm from its surface. We then photographed the tapetum in raw format using a digital camera (Galaxy Z Fold4, Samsung Electronics CO., LTD.) under limited processing settings. Our imaging setup ensured illumination, distance, and camera settings (e.g., focal length, white balance, and ISO sensitivity) remained constant across the samples. All biological samples were processed and disposed of within the state or region of origin under the direction of the overseeing agency personnel. We present reflectance results as relative to the reflectance produced by a diffuse white standard used to normalize all reflectance data as a percentage of the total (range of 0 to 100). Conversion from red, green, blue (RGB) images to hue, saturation, and value was done using the rgb2hsv command in the MATLAB programming environment. We included both left and right eyes in our analyses because visual lateralization in ungulates supports the idea of functional asymmetry and inter-eye variation. As this study was exploratory, we chose not to aggregate eye measurements to retain potentially meaningful within-individual variation. When one eye was compromised by disease or trauma, only the viable eye was measured. We assessed potential differences in hue, saturation, and peak spectral reflectance using a Kruskal–Wallis test due to the non-normal distribution of the data. To conservatively verify the robustness of our results, we also fit a linear quantile mixed model (LQMM), treating individual deer as a random effect to account for repeated measures. Results from the LQMM verification supported the patterns identified by the Kruskal-Wallis tests reported below. All methods and procedures were conducted with prior approval from the University of Georgia Institutional Animal Care and Use Committee (no. A2023 01-014-Y1-A0).

3 | Results

Tapetum fibrosum from white-tailed deer populations distributed across the eastern U.S. were notably different in color and reflectance (Figure 1). In the northern populations (i.e., Indiana, Minnesota, and Missouri), tapetum color ranged from green-blue shades of turquoise to deep-blue depending on the individual. In contrast, tapetum colors from the population in Georgia were predominantly golden with some shades of turquoise, and tapetum colors from the population in Texas were dominated by green-blue shades of turquoise. Based on measurements of hue and saturation from individual tapetums (Figure 2), the three northern populations were significantly different from the population in Georgia ($p < 0.01$), whereas the population in Texas was not significantly different from the other populations in our study ($p > 0.05$). We did not find a significant difference in hue or saturation based on age ($p > 0.05$) or sex ($p > 0.05$) across our study populations. Peak spectral reflectance values exhibited a similar trend between the populations as those observed for hue and saturation, but with greater separation between certain populations. Above 400 nm, peak reflectance values from Missouri (453 nm $\pm$ 17.8; max = 503 nm, min = 434 nm) and Georgia (601 nm $\pm$ 47.8; max = 653 nm, min = 511 nm) appeared distinct, with the Missouri population exhibiting a peak reflectance significantly lower than all other populations ($p < 0.01$), while the Georgia
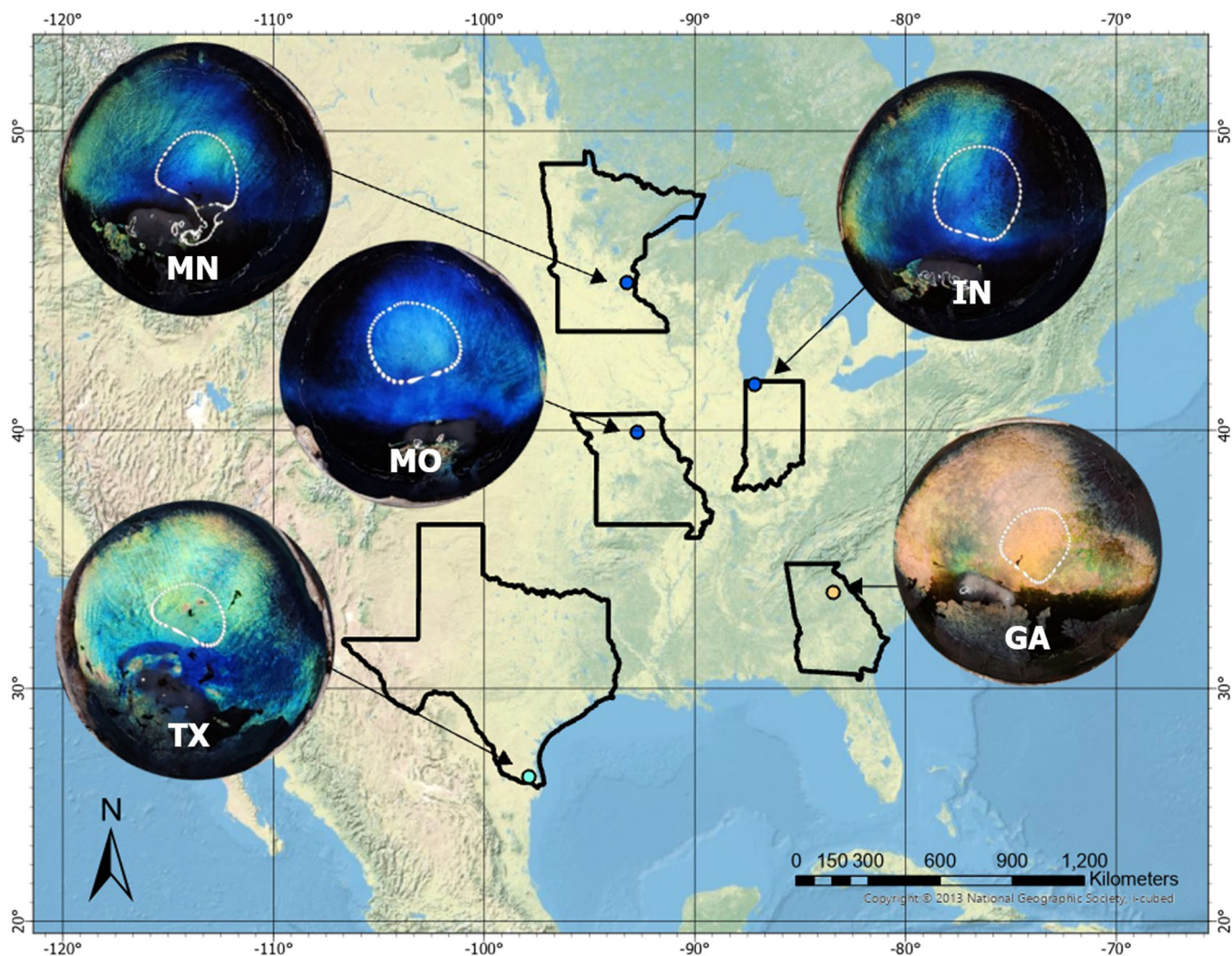
FIGURE 1 | Representative examples of tapetum fibrosum color from white-tailed deer (*Odocoileus virginianus*) populations in Georgia (GA), Indiana (IN), Minnesota (MN), Missouri (MO), and Texas (TX).
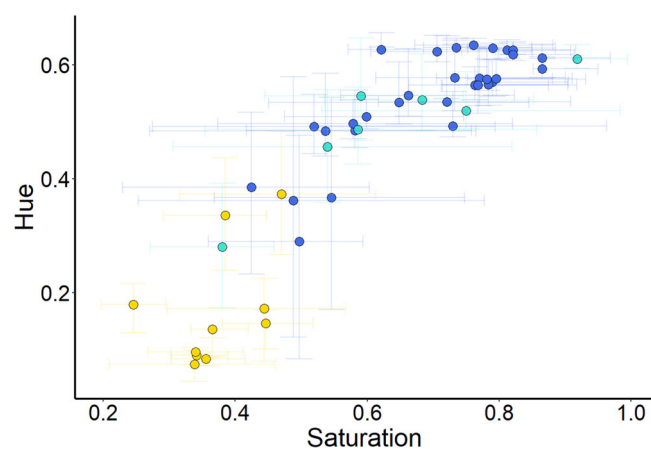

FIGURE 2 | Measurements of hue and saturation for each of the white-tailed deer (*Odocoileus virginianus*) in Indiana (IN), Minnesota (MN), Missouri (MO), Texas (TX), and Georgia (GA). Hue and saturation values are based on the HSL (Hue, Saturation, Lightness) color system. The IN, MN, and MO eyes were significantly different ($p < 0.01$) from GA, while TX was not significantly different from either grouping ($p > 0.05$). The bidirectional error bars are the standard deviation.

population exhibited a peak reflectance significantly higher than all other populations ($p < 0.01$). The remaining northern population in Indiana (473 nm $\pm$ 5.3 SD; max = 476, min = 460) and Minnesota (475 nm $\pm$ 28.6; max = 522 nm, min = 445 nm) did not possess a peak reflectance significantly different ($p > 0.05$) from the Texas population (509 nm $\pm$ 70.0; max = 675 nm, min = 446 nm). Above 400 nm, the relative reflectance measured was similar across all populations (Figure 3a), however, the Georgia population exhibited a slightly higher relative reflectance at peak reflectance than all other populations ($p < 0.01$). Below 400 nm, the peak reflectance was similar across all populations ($p > 0.05$), while the relative reflectance below 400 nm varied between populations with the Georgia and Texas populations exhibiting significantly higher relative reflectance than the Indiana and Missouri populations ($p < 0.01$). The Minnesota population exhibited significantly higher relative reflectance than Missouri ($p < 0.05$; Figure 3b). Finally, four fetuses from two deer culled in Minnesota possessed a moderately reflective tapetum fibrosum with spectral characteristics similar to adults in the population, although the mean peak reflectance was lower at 446 nm compared to 475 nm in adults.
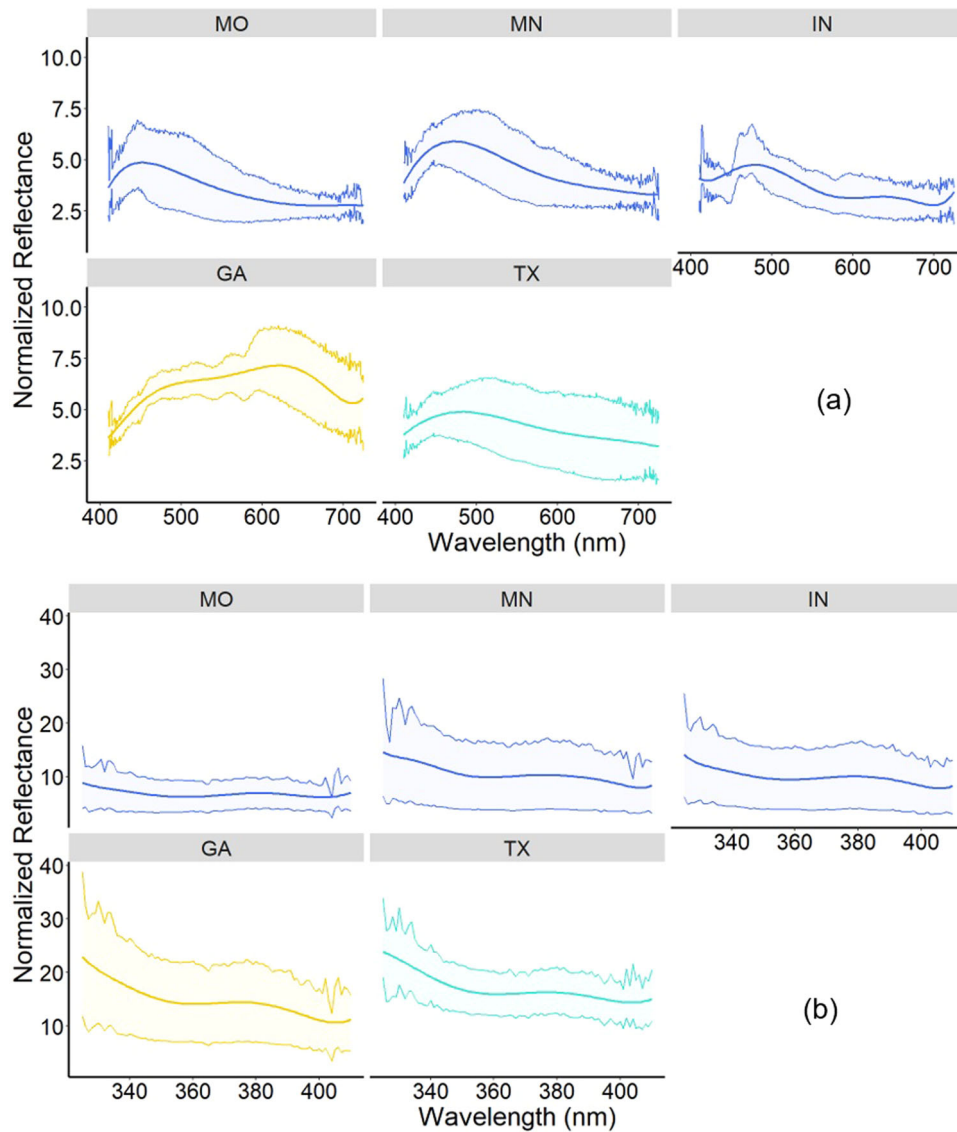
FIGURE 3 | (a) Reflectance spectra from 410 to 700 nm and (b) 320 to 410 nm from white-tailed deer (*Odocoileus virginianus*) populations in Georgia (GA), Indiana (IN), Minnesota (MN), Missouri (MO), and Texas (TX). Means are represented by solid lines within the shaded standard deviation ribbon.

4 | Discussion

Spectral tuning of ocular media including the tapetum lucidum, likely plays an important role in the acquisition of visual information relevant to a species' ecology (Schwab et al. 2002; Stokkan et al. 2013; Fosbury and Jeffery 2022; Newman and D'Angelo 2024). Given the unique flexibility of the tapetum fibrosum in ungulates, spectral tuning of this ocular media might occur both spatially and temporally depending on ecological and environmental pressures. Our results suggest spatial variation of tapetum fibrosum color and reflectance exists in white-tailed deer. The mean peak reflectance of the northern populations coincides with the blue peak reflectance (444–486 nm) reported for reindeer in winter (Stokkan et al. 2013; Fosbury and Jeffery 2022). Uniform blue tapeta denote a traditionally uncommon presentation of mammalian tapetum coloration (Johnson 1900, 1968; Ollivier et al. 2004), and its presence in non-arctic white-tailed deer populations is of

research interest. Additionally, the mean peak reflectance of the Georgia population coincides closely with the peak reflectance values reported for reindeer in summer (541–625 nm) (Stokkan et al. 2013; Fosbury and Jeffery 2022), while the mean peak reflectance of the Texas population appears intermediate to reindeer tapeta from winter and summer. Additionally, color-based comparisons of hue and saturation identified a similar trend with the tapeta of the northern populations appearing distinct from the Georgia population. It is important to note that all samples in this study were collected during the spring, rather than near the solstices when seasonal extremes in tapetal reflectance might be most pronounced. Consequently, we cannot determine whether the observed differences reflect persistent population level (i.e., spatial) variation, seasonally driven plasticity (i.e., temporal variation), or a combination of both. For example, the intermediate characteristics observed in the Texas population may represent a transitional state, while the more "summer-like" measurements in the Georgia population

could indicate an early seasonal shift relative to the more northern populations. Although our inferences may be limited due to sampling constraints, we think certain environmental factors might explain the variation in tapetum fibrosum reflectance we observed. When considered together, the populations with the most similar reflectance values (Indiana, Minnesota, and Missouri) were located at $\geq 40°$ latitude and in areas of low to moderate canopy cover. In contrast, the more distinct Georgia population resides at $< 35°$ latitude and in an area of high canopy cover. Interestingly, the intermediate Texas population has environmental characteristics shared between the Georgia population and the northern populations with a geographic location $< 35°$ latitude and low canopy cover. Latitude might influence spectral tuning of tapetum fibrosum because of associated differences in daylight length, twilight duration, and seasonal ground cover (e.g., snow). Blue enriched tapeta would be beneficial in environments with extended twilight or nocturnal periods, as well as in areas of regular snowfall because short-blue wavelengths are abundant in these light environments. On the other hand, nocturnal-forest environments are enriched in the middle-green wavelengths (Veilleux and Cummings 2012), and the turquoise-gold reflectance of white-tailed deer tapeta in Georgia might be highly adaptive for their environments even during winter. These results suggest latitude and vegetation type might influence tapetum fibrosum reflectance of white-tailed deer.

Despite representing the most southerly site of the northern population, we measured the lowest mean peak reflectance in the Missouri population. We think this might be a result of the variation in nocturnal illumination levels across our study populations. Light pollution (Figure S1), or the alteration of night-time illumination caused by anthropogenic sources of light (Falchi et al. 2016), has known effects on animal behavior and visual physiology. Among our study sites, only the Missouri site at approximately $10 \, \mu cd/m^2$ experiences an artificial brightness level $< 90 \, \mu cd/m^2$ (Falchi et al. 2016). The blue shift observed in reindeer may result from extended pupil dilation in low-light conditions, which modifies ocular drainage, alters eye fluid balance, and triggers draining of the interstitial fluid from the tapetum fibrosum (Fosbury and Jeffery 2022). Thus, light pollution might influence white-tailed deer tapeta reflectance because pupil dilation decreases with increasing brightness, so deer at sites with greater light pollution experience less pupil dilation at night. The impacts of light pollution on ungulate visual ecology are not well-studied, however, in other species, light pollution can interfere with orientation abilities, disrupt circadian rhythms, and alter species occurrence patterns (Ciach and Fröhlich 2019). The advantages and disadvantages of the white-tailed deer's tapetum reflectance likely depend on their light environment. Future research into the effects of night-time illumination on tapetum characteristics would provide valuable insights into the visual ecology of deer in anthropogenically altered habitats.

The tapeta of all studied populations reflected UV wavelengths, reminiscent of the UV saddles observed in reindeer by Fosbury and Jeffery (Fosbury and Jeffery 2022). UV sensitivity in reindeer has been noted for its potential importance in foraging behaviors (Tyler et al. 2014; Dominy et al. 2023) and predator detection (Hogg et al. 2011; Stokkan et al. 2013). The sensitivity of white-tailed deer to UV light (Cohen et al. 2014), coupled with the reflectance of UV wavelengths by their tapeta, suggests that UV light may play a functional role in their ecological interactions and adaptations as well. The role UV light plays in white-tailed deer visual ecology remains unclear, though foraging behaviors, predator detection, and conspecific signaling might be guided by UV light (Newman and D'Angelo 2024). Future studies exploring the specific role and impact of UV-mediated visual functions would clarify these ecological relationships for white-tailed deer.

While our results are largely descriptive, they suggest spatial variation of tapetum fibrosum reflectance across the range of white-tailed deer might be influenced by environmental factors of latitude, vegetation type, and light pollution. Unfortunately, an investigation of potential temporal variation in white-tailed deer tapetum reflectance fell outside our realm of inference. However, we think the spatial variation characterized here, and its similarities to an ungulate species that undergoes temporal shifts in tapeta reflectance, highlights intriguing possibilities to explore the potential spatial and temporal dynamics of the tapetum fibrosum in broadly distributed ungulate species. Further investigation might elucidate whether the spatial and temporal tuning of the tapetum fibrosum is a widely distributed characteristic of ungulates, as well as the breadth of spatial and temporal variations present within species. Additionally, future work could explore whether such changes are mediated solely by physiological responses or whether longer-term processes, such as epigenetic regulation, might also contribute.

Ethics Statement

All methods and procedures were conducted with prior approval from the University of Georgia Institutional Animal Care and Use Committee (no. A2023 01-014-Y1-A0).

Data Availability Statement

All data for this study are available in the Supplementary Information accompanying this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.