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Original Article

Citizen science reporting indicates geographic and phenotypic drivers of road use and mortality in a threatened rattlesnake

Chaz Rhodes, Willard Haunfelder, and Bradley E. Carlson[*]

Department of Biology, Wabash College, Crawfordsville, IN 47933, USA
[*]Address correspondence to Bradley E. Carlson. E-mail: carlsonb@wabash.edu.
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Abstract

Roads may influence the selection of phenotypic traits of wildlife. In particular, the likelihood of vehicle collisions with wildlife may vary depending on body coloration in contrast to the road, which may be exaggerated by cultural attitudes toward the species. The timber rattlesnake *Crotalus horridus* is a threatened species that varies widely in coloration, and their color pattern could influence thermoregulatory use of roads and visibility to motorists. Moreover, better-camouflaged snakes may have higher road mortality in areas where environmental interest is lower and, perhaps, negative attitudes toward wildlife are more prevalent. We used citizen scientist observations of timber rattlesnakes from iNaturalist and categorized for each rattlesnake the surface they were on, its color pattern, and whether they were alive. We combined iNaturalist data with Google Trends data to characterize regional variation in environmental interest. We discovered that lighter-colored snakes were more likely to be found on roads, as were snakes further south, west, and on warmer days. Once on a road, coloration did not influence survival regardless of road type or environmental interest. However, snakes on asphalt roads or on southern roads were more likely to be found dead. The higher likelihood of lighter-colored snakes being found on roads suggests that they are at a greater overall risk of road death, potentially selecting for darker coloration. Citizen scientist behavior may at least partly underlie the influence of latitude on the results, however, and further work in the application of citizen science data to such research questions is warranted.

Key words: google Trends, iNaturalist, reptiles, roadkill, selection, snakes.

The widespread construction of roads for motor vehicle traffic is a major source of environmental change over the last century that can have significant impacts on wildlife populations. Within the United States, roads and roadsides cover about 1% of the land with negative ecological effects that extend to as much as 19% of the land area (or 1.8 million km^2; Forman 2000). Such effects include habitat fragmentation (Spellerberg 1998), reduced water quality (Brady 2012), traffic noise (Halfwerk et al. 2011; Tennessen et al. 2014), reduced gene flow and genetic diversity (Epps et al. 2005), and wildlife-vehicle collisions (Slater 2002; Hill et al. 2020). Furthermore, there is also early evidence of evolutionary responses to roads through physiological adaptations to tolerate road salt runoff (Brady 2012) and traffic noise (Tennessen et al. 2018). Anthropogenic environmental change in general can select particular phenotypes in wildlife, leading to rapid evolution in many wildlife populations (Palumbi 2001; Stockwell et al. 2003). For example, in mid-19th century England, the melanic form of the moth *Biston betularia* increased from 0% to 98% of the total population due to natural selection in response to industrial pollution (Clarke et al. 1985; Cook and Saccheri 2012). Wildlife-vehicle collisions could cause natural selection if the probability of using a road, or of being struck by a vehicle while on a road, depends on phenotypic traits. For instance, individuals that are better camouflaged against road surfaces may go unnoticed by drivers and be accidentally killed (DeGregorio et al. 2010). Moreover, the attitudes of motorists toward certain animals can determine whether they will attempt to avoid or even cause a collision (Crawford and Andrews 2016; Assis et al. 2020), and therefore the effect of phenotype (e.g., cryptic coloration) on road survival rates could depend on the motivations of motorists.

Snakes are commonly struck by vehicles, in part due to an attraction to warm road surfaces (Mccardle and Fontenot 2016). Snakes have been particularly maligned in many cultures, and though motorists in the United States are generally concerned about collisions with mammals and turtles, they hold more negative attitudes toward snakes on the road (Crawford and Andrews 2016), even intentionally striking them in some cases (Langley et al. 1989; Ashley et al. 2007; Secco et al. 2014). Snake coloration that is camouflaged on road surfaces could therefore be under natural selection in a way that depends on prevailing cultural attitudes toward snakes: specifically, more camouflaged snakes may survive better when motorists tend to strike them intentionally, whereas they would fare worse if they go undetected when motorists are trying to avoid them. Indeed, cultural perception of snakes has led to contemporary evolution in Japan, where populations of the Japanese mamushi *Gloydius blomhoffii* that are heavily hunted have evolved to be smaller with

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fewer vertebrae, and produce smaller and more offspring (Sasaki et al. 2009), a result of traditional cultural attitudes shifting against snakes (Sasaki et al. 2010). Additionally, thermoregulatory benefits or opportunities could jointly depend on geographic location and color pattern. For instance, cooler climates could favor more basking on roads, and especially so in lighter-colored animals that warm more slowly, increasing their use of roads.

In this study, we used citizen science data to evaluate patterns of road use and mortality in a widespread and variably colored snake. The timber rattlesnake *Crotalus horridus* is a threatened pitviper that varies dramatically from very pale to nearly black in coloration, with a higher frequency of dark morphs in northern and montane regions of its eastern United States range (Allsteadt et al. 2006). Both road mortality and human persecution have contributed to declines in timber rattlesnake populations (Rudolph et al. 1998; Andrews and Gibbons 2005; Olson et al. 2015), and roads in particular have been identified as creating barriers to gene flow and reducing genetic diversity (Clark et al. 2010; Bushar et al. 2014, 2015). Consequently, timber rattlesnakes are an ideal subject to examine road impacts and the potential sensitivity of these impacts to cultural attitudes. Moreover, darker color morphs (which would warm faster in the sun; Brown et al. 1982; Clusella Trullas et al. 2007) may benefit differently from basking on roads than lighter conspecifics, and the broad latitudinal range of this species also allows testing of the influence of climate differences on the likelihood of snakes using roads. We collected records of both living and dead timber rattlesnakes in a variety of habitats using citizen science observations from iNaturalist, a platform useful in conservation research in general (McKinley et al. 2017) and that has been specifically applied to studies of animal road mortality (Monge-Nájera 2018) and to animal color morph variation (Lehtinen et al. 2020), including that of snakes (Lattanzio and Buontempo 2021). We combined iNaturalist data on rattlesnake pigmentation (assessed from the photographs), live/dead status, geographic location, and substrate use (natural surfaces or various types of roads), with data on regional environmental attitudes. The latter was derived from internet search behavior data collected from Google Trends, which has previously been used to track environmental interest (Mccallum and Bury 2013; Ficetola 2013; Burivalova et al. 2018).

We specifically tested the following hypotheses: 1) Rattlesnake coloration will influence the probability of being found on a road and the probability of being killed while on a road. 2) Differences in cultural attitudes across regions will influence how color morph determines survival on roads, with better-camouflaged individuals (e.g., dark snakes on dark, asphalt surfaces) surviving at higher rates in areas with low environmental interest. 3) There will be broad latitudinal and longitudinal trends in road use and mortality, driven by diverse ecological differences among regions.

Materials and Methods

Rattlesnake data collection

We collected timber rattlesnake observation records from the iNaturalist website (http://www.inaturalist.org). These data represent contributions from diverse users of the iNaturalist platform (including members of the general public—"citizen scientists"—as well as professionals; Heberling and Isaac 2018). Records include one or more photographs of the organism encountered, location coordinates, dates of the observation and of uploading the record, and a field for verbal descriptions and comments on the observation, among other data fields (e.g., taxonomic information, observer name, etc.). Both users who submit observations and iNaturalist reviewers can suggest species identifications, based largely on the photographs. We downloaded data on all observations uploaded through 20 November 2020, restricted to records that were "research grade" (i.e., with broad agreement on species identification) and included geographic coordinates. For observations that contained multiple snakes (e.g., basking aggregations), we separated these records into multiple records with the same location information. We also examined records chronologically to readily identify and exclude duplications in cases where either the same user or multiple users of iNaturalist submitted repeated observations of the same individual snake (identified by the date/time, setting, color pattern, and often the pose of the snake). After accounting for multiple snakes in a single record and duplicate records of the same snake, we had a total of n = 2398 snakes observed with geographic coordinates included. In 58% of records, the geographic coordinates of the observation were obscured (either by the uploading user or automatically by iNaturalist to protect the location of at-risk species). Obscured coordinates are randomly drawn from a 0.2 × 0.2-degree rectangular area containing the true coordinates, which can offset locations by as much as 22 km (Jacobs and Zipf 2017). In our analyses, we treated obscured coordinates as representing the actual location of the snakes, as the measurement error introduced should be both random (unbiased) and small compared to the scale of the spatial patterns explored in the study (Jacobs and Zipf 2017). We also filtered out all observations with a stated positional accuracy error of ≥20 km, and all observations from before 2011, when iNaturalist became widely available, thus excluding old records entered with unreliable data.

For each observation, we used a custom script within the program R version 3.6.0 (R Core Team 2019) and utilizing the *jpeg* package (version 0.1-8) to view the primary linked image file associated with the observation. Similar to Allsteadt et al. (2006), we rated the intensity of melanization of the ground color (the region of the skin between the consistently dark-colored bands) on the dorsal surface of the trunk (midbody) of each rattlesnake on a 4-point, qualitative scale: 1) nearly white to very pale, highly contrasting with the dark bands; 2) lightly gray, brown, or yellow, contrasting with the dark bands; 3) gray, brown, or dark yellow, modestly contrasting with the dark bands; and 4) dark brown or nearly black, contrasting little with the dark bands (Figure 1). In some cases, the image appeared to be a close-up view of a small part of the rattlesnake or otherwise appeared difficult to categorize. If we could not find better images that included a view of the dorsal surface of the trunk on the webpage of the observation, then we did not score the coloration of those snakes (6% of observations were unrated).

These qualitative, image-based ratings of snake color entail many limitations. We cannot discount the possibility that coloration changes within individuals, perhaps in response to the substrates or body temperatures (Boback and Siefferman 2010), though this phenomenon is not known from this species. Continuous variation in coloration is reduced to qualitative categories, creating artificial cut-offs between similar colors, but this approach has been successfully used before to

Figure 1. Examples of timber rattlesnake color morphs as categorized in this study (1–4, from left to right). Photos were submitted by iNaturalist users under a Creative Commons CC BY-NC license. Photo credits, from left to right: Katie Bentley (2015, Georgia); Roderick Petersen (2020, Alabama); Milo Pyne (2015, Kentucky); Trevor Chapman (2018, Tennessee).

describe geographic color variation in this species (Allsteadt et al. 2006), and the large sample sizes should minimize the impact of ambiguous classifications. Differences in lighting conditions and cameras may influence the appearance of the snake's coloration. We attempted to account for and subjectively compensate for clearly unusual photographic conditions in our ratings; for example, a snake brightly lit by a flashlight at night may appear nearly white, so assessing the contrast between dark patches and background coloration may yield a more reliable color score. Image analysis software that analyzes color values in the image would be problematic to implement given these variations in photographic conditions, as well as variation in the orientations of the snakes and the regions of the body that are visible, favoring the subjective assessment method. However, we expect unaccounted-for error remains but that such error should be randomly distributed and unbiased with respect to the variables of interest. We also did not classify individuals by age, which can affect their color (Brown et al. 2007); however, we again do not anticipate that this biased any analyses we performed. Finally, different individual observers may rate color patterns differently. One of us (Bradley E. Carlson) performed all the color pattern ratings used in the final analyses. Furthermore, we measured the interobserver reliability of color scores assigned independently by 3 observers to the same random sample of 90 photographs. Using the R package *irr* (Gamer et al. 2019), we obtained an intraclass correlation coefficient (ICC) of 0.84 (95% CI: 0.78, 0.89; $P < 0.0001$), indicating a high concordance between scores assigned independently by different raters.

We also collected data on the live-dead status and background context from the photographs of each rattlesnake observation. Snakes were classified as either alive or dead, with the latter including animals that appeared mortally wounded or were described as having been injured/killed. We categorized whether a rattlesnake was on a confirmed road (paved surfaces or otherwise confirmed roads), whether it was on a possible road (including both confirmed roads and unpaved surfaces that may have been roads), and whether it was on a natural substrate (neither a confirmed nor a possible road; see Supplementary Table S1 for a complete description of all background contexts). Regardless of the background in an image, we counted snakes as being on confirmed roads if the user who submitted the record included a note indicating that it was initially found on a road or on the side of a road (including references to, e.g., "roadkill," "hit by a car," "AOR" [alive-on-road], and "DOR" [dead-on-road]). For some of our analyses below, we incorporate whether roads were paved with asphalt as a variable, because the darker coloration of asphalt roads compared to concrete or unpaved roads could determine how cryptic different rattlesnake color morphs appear and how quickly vehicles traveled, assuming that motorists on asphalt roads travel at higher speeds than those on unpaved roads. We did not score the backgrounds for unclear cases (i.e., snakes that appeared to have been moved from their original context, such as dead snakes placed in a bucket), and those observations were excluded from the relevant analyses. Tables 2–4 show the final sample sizes available for each analysis after excluding missing values of any variables included in the final model.

Environmental interest index

To quantify regional cultural differences in environmental interest, we used a "conservation culturomics" approach in which word use in digital text databases is measured (Ladle et al. 2016; Correia et al. 2021). Specifically, we analyzed the frequency of internet search term usage in the Google search engine using the online tool Google Trends (Correia et al. 2021). In previous work, Google Trends data have been used to characterize temporal trends in environmental interest (Mccallum and Bury 2013; Ficetola 2013; Nghiem et al. 2016). Importantly for this study, Google Trends can be used to quantify the popularity of specific search terms in a spatially explicit manner for several different sizes of regions (Cavanagh et al. 2014). For our research, we focused on metropolitan areas ("metros") within the United States. The metropolitan regions in Google Trends correspond to Nielsen Designated Market Areas (DMAs), clusters of contiguous counties based on regions that receive common broadcasts (television and radio) and therefore similar cultural influences (Cavanagh et al. 2014; Chae et al. 2015; Hall et al. 2020).

We compiled a list of search terms that could suggest environmental interest or awareness derived from the combination of lists used in Ficetola (2013), Mccallum and Bury (2013), and Nghiem et al. (2016). We added to this list: "rainforest," "wildfire," "nature," "ecosystem," "solar energy," "national park," "snakes," "state park,","water quality," "recycling," and "hiking." For each metro area, we downloaded the Google Trends data for each search term over the period from 1 January 2016 (the last time Google altered its data collection system) through 20 November 2020 (the date up to which we downloaded iNaturalist observations). Google Trends data are presented on a 0–100 scale, with the metro area with the highest proportion of search activity for a particular term receiving a score of 100 and the relative search activity of other metro areas scaled to that value (Cavanagh et al. 2014). Ten of the terms we searched did not return values for some of the metro areas in which there were timber rattlesnake observations in the dataset, and these search terms were excluded. Mapping of scores led us to exclude "rainforest" and "national park," both of which had strong outlier metro areas, and "snakes," which exhibited a strong latitudinal trend that probably reflects greater snake diversity and abundance in the southern United States; "snakes" also showed little correlation with other search terms in a preliminary principal components analysis (see below). The final list of included terms is in Table 1, along with all excluded terms.

We performed a principal components analysis (PCA) on the Google Trends data for the selected terms. PCA can be used to reduce the dimensionality of a multivariate dataset to fewer axes of variation (called principal components [PCs]). These PCs function as new composite variables that capture covariation among the original variables, summarizing the larger dataset with minimal data loss. In this case, PCA was used to reduce Google Trends data on the 12 search terms to PCs that reflect the common patterns of covariation among the individual variables. The first principal component (PC1) explained 40.2% of the variation in the original data and appeared to be indicative of general environmental interest, as all terms either had moderate loadings in the same direction or, for a few terms, weak loadings that made them of little influence (Table 1). We used the PC1 values as a composite measure of environmentally related internet search activity, hereafter referred to as the "environmental interest PC score"; higher PC scores correspond to a tendency for high search activity in all search terms with more strongly positive loadings (Table 1). In analyses using the environmental interest PC score, we ascribed to a rattlesnake observation the score for the metro area whose centroid was nearest the point of the observation. PC2 is the composite variable orthogonal to PC1 that summarizes the second greatest amount of variation in the dataset, and it apparently reflects recreation-oriented environmental interest ("state park" and "hiking" load positively) versus scientific or applied concerns ("ecosystem," "pollution," and "environment" load negatively; Table 1). As it only accounted for 18% of the variation in the data and didn't reflect the variation of interest for our hypotheses, we didn't use PC2 in the analysis.

Table 1. Google Trends search terms used and principal components analysis (PCA) results. PC loading values indicate the strength and direction of the relationship between each individual search term and the composite PC score. The footnote lists search terms that were excluded due to missing values in some metro areas or extreme outliers in one or a few metro areas.

Search term	PC1 loading (40.2% of variance)	PC2 loading (18% of variance; unused)
Climate change	0.55	−0.04
Conservation	0.18	−0.02
Ecology	0.32	−0.15
Ecosystem	−0.07	−0.53
Environment	0.26	−0.29
Extinction	0.30	0.03
Hiking	0.42	0.34
Nature	0.36	0.03
Pollution	0.30	−0.36
State park	0.10	0.58
Wildfire	0.03	0.03
Wildlife	−0.004	−0.23

Excluded search terms: "biodiversity," "deforestation," "endangered species," "global warming," "invasive species," "national park," "orangutan," "rainforest," "renewable energy," "snakes," "solar energy," "sustainability," "water quality."

Covariate data collection

We also collected data on 2 additional covariates for use in particular analyses: mean temperature on the day of observation and human population density. Mean temperatures over the entire day of observation were expected to influence the probability of using a road, with sufficient heat being necessary for animals to move onto roads and to find them warm enough to be worth basking on. The risk of dying while on the road could be positively affected by the temperature if warm roads lead snakes to stay on them longer, or negatively if cooler temperatures produce slower movement rates off of the road. Temperatures averaged over the entire course of the day were expected to be generally reflective of broad patterns in temperature. We used the R package *climate* (Czernecki et al. 2020) to locate the nearest NOAA weather station for which temperature data were available for the date of observation and downloaded the mean temperature (in C). Distances of stations from recorded sites of observation averaged 86.6 km, and as such are indicative of regional and seasonal fluctuations in temperature rather than truly local conditions. The human population density was determined using the land area of each metro area and the 2010 census data associated with each metro (Appendix A of Federal Communications Commission 2016). In all analyses, population density is log-transformed due to the highly positively skewed distribution.

Statistical analysis

We performed the statistical analyses using generalized linear mixed models (GLMMs) with spatially autocorrelated random effects, as implemented in the R package *spaMM* (Rousset and Ferdy 2014). This approach was chosen for several reasons: 1) it permits both normal dependent variables (color morph) and binary dependent variables (live vs. dead and on-road vs. off-road); 2) it incorporates spatial autocorrelation among nearby observations; 3) it allows random intercepts for each metro area, accounting for potential pseudoreplication of environmental interest PC scores within metro areas (within which all PC scores are the same; Hurlbert, 1984); and 4) this package implements penalized quasi-likelihood approximations (PQL) during model fitting, which performs well for binary data (Rousset and Ferdy 2014). Across all analyses (described for each dependent variable separately below), our general model-fitting approach began with maximal models containing all fixed main effects and interaction terms of interest (including latitude and longitude, to account for broad geographic trends) and a Matérn spatial autocorrelation term (using true, great-circle distance between coordinates). When environmental interest PC scores were included as fixed effects, we included a random intercept term for the identity of the metro area that corresponds to the PC scores.

Then, we used a backward stepwise model selection procedure to evaluate the significance of fixed effects one at a time and simplify the model by excluding the least significant term in the model, repeating the process until no more terms in the model could be removed. We only evaluated lower-order interaction terms or main effects after the removal of higher-order terms within which they were contained. We used likelihood ratio tests (LRTs) to compare models differing only in the inclusion of the fixed effect under consideration. We conservatively retained all terms with $P < 0.10$ for the purposes of model selection, though we used $P < 0.05$ for identifying significant effects in the final (simplified) model. Spatial autocorrelation terms and metro area random intercepts were never removed. After arriving at a final model where no more terms could be removed, we report the beta coefficients (slopes) associated with each term and the LRT results for each term (comparing the final model to a model only lacking that term), though the main effects and lower-order interaction terms that participated in higher-order interactions could not be evaluated for significance in the presence of significant higher-order interactions. Tables 2–4 summarize all models described below and their results.

To test for geographic variation in average color morphs, we used maximum likelihood (ML) to fit a spatial GLMM with a normal (Gaussian) error distribution, color morph as a continuous dependent variable (an approximation from its ordinal rating scale), and latitude, longitude, and their interaction as independent variables. We ran this analysis both using all rattlesnake observations and using only those of snakes found off of roads. Next, we tested whether at a given location, rattlesnakes of different colors were more likely to be found on roads. We used PQL to fit a spatial GLMM with a binomial error distribution and the presence of the rattlesnake on a road (yes/no) as the dependent variable. The initial model included as continuous independent variables: latitude, longitude, color morph of the rattlesnake, temperature, color morph × temperature, color morph × latitude, color morph × longitude, latitude × longitude, and color morph × latitude × longitude. The interaction terms were included because we wanted to test whether the effect of color morph on road usage varied by temperature or among locations (e.g., whether dark-colored rattlesnakes on cooler days or in cooler northern climates show a lower rate of road use than their lighter-colored counterparts, who may be more likely to bask on roads to compensate for slower rates of warming than dark snakes). We ran this analysis 3 times using different criteria for whether a snake was considered on a road: whether the snake was on a possible road versus all other observations, whether the snake was on a confirmed road versus a confirmed non-road (excluding observations where it was uncertain whether snakes were on a road), and whether the snake was on an asphalt road versus all other observations (to compare rates of asphalt use in particular).

Finally, we tested the effects of rattlesnake color morph, geography, and environmental interest on the probability that rattlesnakes on roads were dead. Using only observations of snakes that were on roads, we used PQL to fit a spatial GLMM with a binomial error distribution and the status of rattlesnake on the road (live/dead) as the dependent variable. The initial model included the independent variables: latitude, longitude, color morph of the rattlesnake, environmental interest PC score, whether the road was asphalt paved, and the following interaction terms: latitude × longitude, color morph × environmental interest PC score, asphalt paved × environmental interest PC score, color morph × asphalt paved, and color morph × environmental interest PC score × asphalt paved. We also included as covariates temperature and human population density (as larger human populations may be associated with heavier traffic and lower survival). A random intercept term for the corresponding metro area was included to account for the non-independence of repeated values of environmental interest PC scores within metro areas.

We performed all analyses in R version 3.6.0 (R Core Team 2019). All code and data used are available at https://doi.org/10.6084/m9.figshare.16879270 (Rhodes et al. 2021).

Results

As in previous work (Allsteadt et al. 2006), there was a general geographic pattern in the color morph of timber rattlesnakes: darker snakes were more common in northern and eastern parts of their distribution (Figure 2). A significant latitude × longitude interaction indicated that the latitude effect was stronger further east, the result of stronger latitudinal trends in the eastern part of the distribution (Table 2A). This interaction term became marginally non-significant ($P = 0.07$) when the dataset was restricted to snakes found only on natural substrates (to account for possible detection biases on paved surfaces), but the geographic patterns were otherwise qualitatively similar (Table 2B).

We tested the probability an observed rattlesnake would be found on a road as a function of location and color morph (Figure 3A). Regardless of whether the criterion for road use was possible roads, confirmed roads, or asphalt roads, the 3-way latitude × longitude × morph interaction had $P < 0.05$, requiring its retention in the model (Table 3). The positive direction of these interaction terms indicates that although darker color morphs had a lower probability of being on roads overall, they became more likely to use roads further northeast in the rattlesnake's distribution (a combination of higher latitude and longitude values; Figure 3A). Because the model with a 3-way interaction is difficult to interpret and lower order terms cannot be statistically evaluated in the presence of the interaction term, we also ran the models with the 3-way interaction dropped. With models using both all possible roads and only confirmed roads, the final models selected included independent negative effects of latitude, longitude, and color morph on road use probability, along with a positive effect of temperature. That is, an observed rattlesnake was more likely to be found on a road if it was further south, further west, lighter in coloration, and on warmer days (Figure 3B–D). When we evaluated models of the probability of rattlesnakes being found specifically on asphalt (as opposed to all other substrates), the selected model was mostly consistent with the above analysis but did not include an effect of longitude (Table 3C).

Finally, we tested the probability a rattlesnake observed on a road would be dead, as a function of location, color morph, environmental interest PC score, and whether the road was paved with asphalt. Using all possible roads, we found a negative effect of latitude: snakes on roads further north were less likely to be found dead (Table 4A; Figure 4). There was also a significant interaction between environmental interest PC score and asphalt; the negative direction of this term indicated

Table 2. General geographic trends in timber rattlesnake color morphs, using either (**A**) data on snakes found on all types of substrates or (**B**) only snakes found on natural (non-road) substrates. In both cases, the result of the likelihood ratio test (LRT) required retaining the interaction term ($P < 0.10$) and therefore the significance of main effects of latitude and longitude were not evaluated. Positive model coefficients (β) indicate increasing melanization of rattlesnake ground color with increasing values of latitude (further north) or longitude (further east). Latitude and longitude were mean-centered such that their independent effects represent when the other value is at its mean.

Data used	Variable	LRT χ^2	LRT *P*-value	β
(**A**) All substrates	Latitude × longitude	6.49	0.01	0.004
(*n* = 2253)	Latitude	—	—	0.086
	Longitude	—	—	0.021
(**B**) Natural substrates	Latitude × longitude	3.32	0.07	0.003
(*n* = 1389)	Latitude	—	—	0.093
	Longitude	—	—	0.025

Table 3. Predictors of probability of timber rattlesnakes being found on a road, using either (**A**) all possible roads versus natural substrates, (**B**) confirmed roads versus natural substrates, and (**C**) asphalt versus all other substrates. Model terms are listed in order of removal during the backward stepwise selection procedure, retaining terms with $P < 0.10$. For models A and B, the 3-way latitude × longitude × morph interaction was initially retained. Due to the difficulty of interpretation in the presence of the 3-way interaction term, we also proceeded with the model selection after removing that term. Likelihood ratio test (LRT) statistics for lower order terms in models A and B are only calculated in the absence of the 3-way interaction term. For the final model (both including the 3-way interaction term and excluding it in models A and B), coefficients (β) indicate all terms that remained in the model and their effect on the probability of road use. Latitude and longitude were mean-centered, so all effects in the model are estimated at the mean value of these variables. Temperature was mean-centered and scaled such that a one unit change in value corresponds to one standard deviation on the original scale.

Data used	Variable	LRT χ^2	LRT *P*-value	*β* (including 3-way interaction)	*β* (excluding 3-way interaction)
(**A**) All possible roads	Morph × temp	0.52	0.47	—	—
(*n* = 2194)	Latitude × longitude × morph	4.14	0.04	0.006	—
	Latitude × longitude	0.01†	0.91†	−0.014	—
	Longitude × morph	0.20†	0.66†	0.007	—
	Latitude × morph	1.36†	0.24†	−0.045	—
	Latitude	3.93†	0.05†	0.031	−0.060
	Longitude	5.87†	0.02†	−0.056	−0.036
	Morph	4.38†	0.04†	−0.247	−0.155
	Temp	28.77†	<0.001†	0.277	0.278
(**B**) Confirmed roads	Morph × temp	1.90	0.17	—	—
(*n* = 2018)	Latitude × longitude × morph	6.40	0.01	0.008	—
	Latitude × longitude	0.00†	0.99†	-0.019	—
	Longitude × morph	0.12†	0.73†	0.009	—
	Latitude × morph	1.34†	0.25†	−0.052	—
	Latitude	6.32†	0.01†	0.025	−0.078
	Longitude	3.66†	0.06†	−0.053	−0.029
	Morph	9.08†	0.003†	−0.393	−0.251
	Temp	29.96†	<0.001†	0.311	0.311
(**C**) Asphalt roads	Morph × temp	0.98	0.32	—	—
(*n* = 2165)	Latitude × longitude × morph	4.67	0.03	0.008	—
	Longitude × morph	0.13†	0.72†	0.015	—
	Latitude × morph	0.28†	0.60†	−0.046	—
	Latitude × longitude	0.31†	0.58†	−0.015	—
	Longitude	0.01†	0.91†	−0.031	—
	Morph	8.72†	0.003†	−0.424	−0.265
	Latitude	19.17†	<0.001†	−0.051	−0.132
	Temp	22.59†	<0.001†	0.286	0.289

†Indicates values obtained by proceeding with backwards selection after removing the 3-way latitude × longitude × morph interaction, despite a *P*-value < 0.10 for the interaction term.

Table 4. Predictors of probability of timber rattlesnakes being found dead while on a road, using either (**A**) all possible roads or (**B**) only confirmed roads. Model terms are listed in order of removal during the backward stepwise selection procedure, retaining terms with $P < 0.10$. Coefficients (β) indicate all terms that remained in the model and their effect on the probability of mortality (positive values equating to increased probability of death), given that a snake is on a road. Latitude, longitude, PC1, human population density, and temperature were mean-centered, so all effects in the model are estimated at the mean value of these variables. PC1, density, and temperature were also rescaled such that a one unit change in value corresponds to one standard deviation on the original scale.

Data used	Variable	LRT χ^2	LRT *P*-value	β
(**A**) All possible roads	Latitude × longitude	0.04	0.85	—
(n = 792)	Longitude	0.13	0.71	—
	PC1 × asphalt × morph	0.17	0.68	—
	Asphalt × morph	0.56	0.46	—
	PC1 × morph	2.33	0.13	—
	Morph	0.16	0.69	—
	Density	2.33	0.13	—
	Temp	2.60	0.11	—
	Latitude	15.39	<0.001	−0.164
	PC1 × asphalt	5.20	0.02	−0.507
	PC1	—	—	0.386
	Asphalt	—	—	2.066
(**B**) Confirmed roads	Latitude × longitude	0.02	0.90	—
(n = 614)	Longitude	0.63	0.43	—
	PC1 × asphalt × morph	2.48	0.12	—
	PC1 × asphalt	0.49	0.49	—
	PC1 × morph	1.07	0.30	—
	PC1	0.42	0.52	—
	Asphalt × morph	1.86	0.17	—
	Morph	0.16	0.69	—
	Density	2.88	0.09	−0.196
	Temp	4.17	0.04	0.189
	Latitude	19.76	<0.001	−0.170
	Asphalt	47.03	<0.001	1.757

that with increasing environmental interest, the (otherwise positive) effect of asphalt on death tended to weaken (Table 4A). In other words, the higher risk of death for snakes on asphalt-paved roads was partly reduced in areas with greater environmental interest. The positive effect of asphalt on the probability of death could be in part attributable to the fact that asphalt-paved surfaces were almost certainly true roads, whereas other (non-paved) surfaces that we classified as "possible roads" could include some non-road surfaces with inherently low death rates. We therefore also performed the analysis using only confirmed roads, and again the positive effect of asphalt on death rates was highly significant (Table 4B). Additionally, there remained a negative effect of latitude, but the interaction between environmental interest PC score and asphalt was no longer significant. Finally, on confirmed roads, there was a non-significant ($P = 0.09$) negative effect of human population density and a significant positive effect of temperature on road mortality risk.

We noticed that for many of the 361 observations from the state of Texas, the comments left by the submitter of the record suggested that they had a particular interest in documenting roadkilled specimens (e.g., referring to salvaging the specimen with a permit). Notably, a "Roadkills of Texas" project has successfully encouraged the reporting of roadkilled animals in the state (https://www.inaturalist.org/projects/roadkills-of-texas). Because the existence of this project could affect the likelihood of dead snakes being reported, and Texas represents the western and southern limits of the timber rattlesnake distribution, we thought it was possible that data from Texas could bias the analyses. We therefore re-ran the above analyses for probability of being found on a road and probability of dying on a road (using all possible roads) with observations from Texas excluded. The conclusions of these analyses were largely similar, except that the effect of longitude on the probability of road use became non-significant ($P = 0.09$) and there was a significant positive effect of temperature on the probability of dying, which was not the case for all possible roads when including Texas data (Supplementary Table S2). Other variables were similar in significance, magnitude, and direction of effect (Supplementary Table S3), and we therefore focus our interpretation of our findings on the entire dataset, including observations from Texas.

Discussion

Using citizen science observations in the iNaturalist platform, we evaluated the role of color morph and geography on timber rattlesnake road use and road mortality. Our consistent chief finding across different subsets of the data were that snakes that were lighter in color and/or further south were more likely to be found on roads. However, among snakes that were on roads, only southern location and asphalt pavement

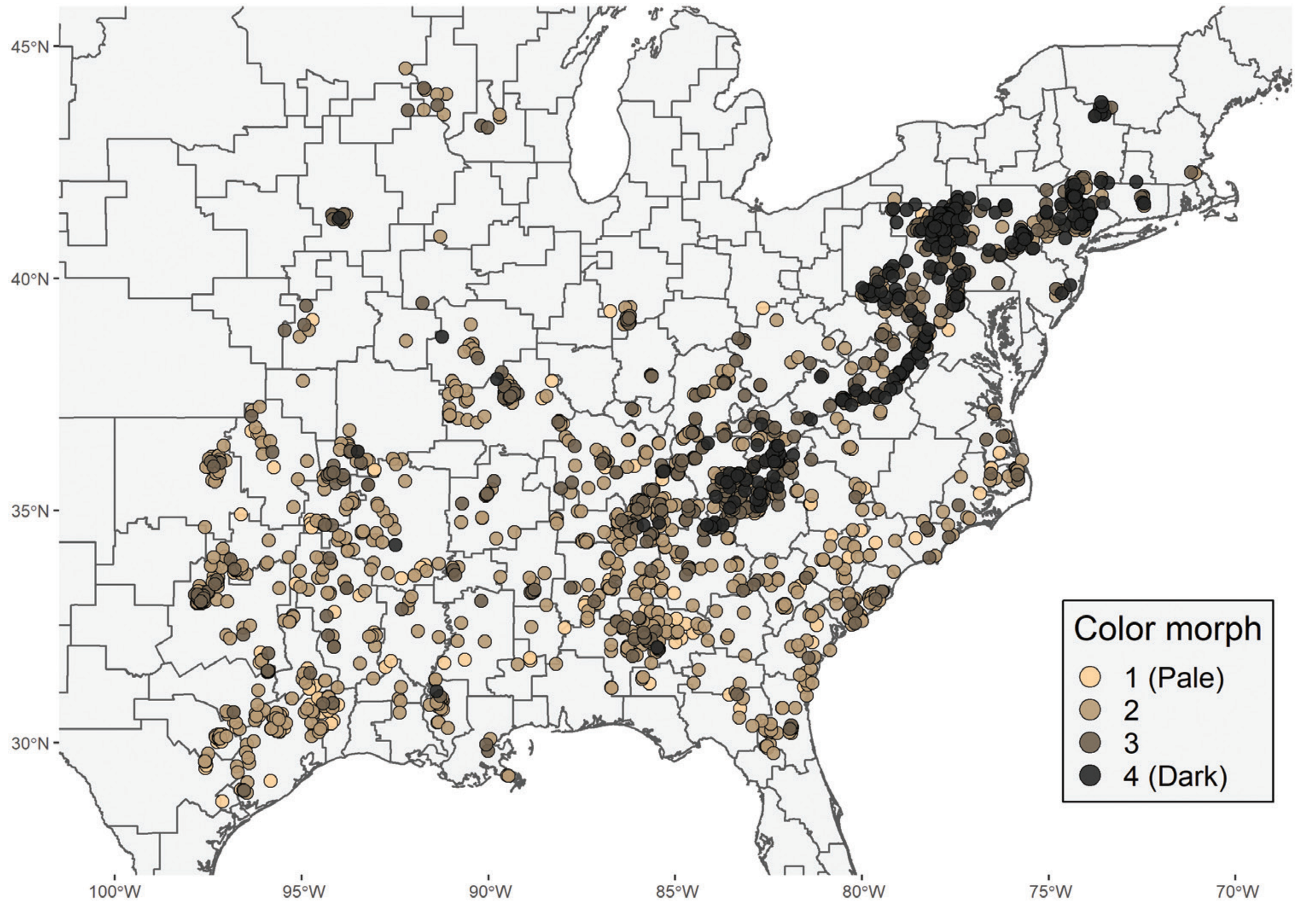

Figure 2. Occurrence map of timber rattlesnake color morphs used in the analysis. Polygons mark the borders of metro areas (DMAs).

increased apparent mortality risk. Together, this suggests that roads could exert selection against light-colored snakes, both within populations and across the range of the species: both road use and mortality were elevated in the south where the rattlesnakes were also generally lighter colored. However, no evidence indicated a role of crypsis interacting with environmental attitudes to shape mortality risk, given the lack of effects of color morph and environmental internet search activity on mortality once snakes were already on roads.

Timber rattlesnakes that were lighter in coloration were more likely to be found on roads as opposed to natural substrates, regardless of the criteria for designating a surface as a road. This effect may have been somewhat influenced by geography (as revealed by significant latitude × longitude × morph interaction terms), but the general pattern appears robust. Though lighter color correlated with lower latitudes, where road use was also higher, the effects of latitude and coloration were modeled as independent predictors of road use. The effect of snake coloration on road use could be due to thermoregulation opportunities. Road use in reptiles is often attributed to basking behavior, as roads are frequently exposed to more sunlight and have surfaces that warm readily and retain heat (Shine et al. 2004; Mccardle and Fontenot 2016). In support of this, our results indicated higher road use probability when mean daily temperatures were higher. We interpret this as indicating both seasonal differences (with cooler weather limiting snake movement) as well as warmer conditions heating the roads more and enhancing the benefits of using the roads. Additionally, on confirmed roads, higher temperatures were associated with a higher probability of death, which may indicate snakes stayed on the roads longer to take advantage of the warm surface. The increased reflectance of lighter coloration reduces warming rates during basking, so road use may be especially valuable to lighter-colored snakes that otherwise could experience difficulty achieving preferred temperatures (Brown et al. 1982). Indeed, darker timber rattlesnake morphs are generally associated with conditions in which thermoregulation may be more difficult (more mature forests and higher latitudes and elevations), suggesting that the dark coloration assists them in achieving warm body temperatures (Reinert 1984; Allsteadt et al. 2006). It is also possible that other phenotypic traits correlated with coloration could influence the propensity to cross roads. For example, a substantial body of work in other animals identifies relationships between melanin-based coloration and aspects of behavior relevant to road use (Ducrest et al. 2008; San-Jose and Roulin 2018). For example, bolder burrowing owls (*Athene cunicularia*) tended to select breeding sites closer to roads (Carrete and Tella 2010), and behavior in multiple bird species has been linked to melanin (Mateos-Gonzalez and Senar 2012; van den Brink et al. 2012a, b). We are not aware of any evidence that melanization in timber rattlesnakes, or any other snake species, is linked to behavior nor that individual behavioral differences could affect road use in snakes, but this could be a valuable avenue of research. A speculative possibility is that the reduced stress sensitivity commonly associated with melanistic animals (Ducrest et al. 2008) could yield less freezing behavior in darker timber

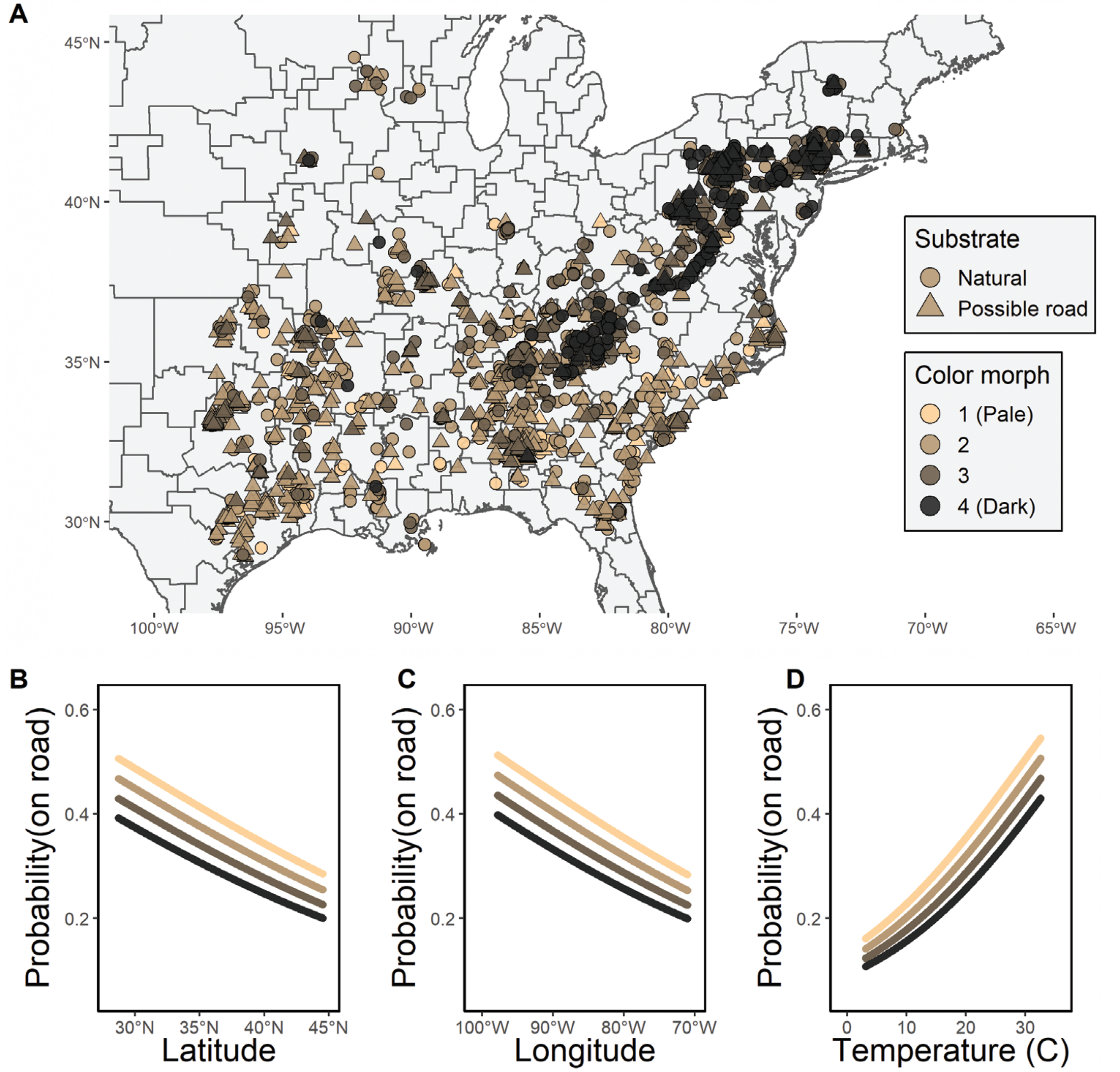

Figure 3. Timber rattlesnake use of possible roads as a function of location (latitude and longitude), color morph, and mean daily temperature. (**A**) Indicates occurrences of snakes found on roads versus natural substrates, with color morph indicated by point color and presence on a possible road indicated by point shape. (**B–D**) Depict the effects of latitude, longitude, and mean daily temperature, respectively, together with color morph, on the probability of a snake being found on a possible road. Probabilities in **B–D** are predicted values from the statistical model that excluded interaction terms (see Table 3A). Line colors in **B–D** reflect different color morphs, as in **A**.

rattlesnakes, resulting in them spending shorter times on roads and thus being less frequently observed on them.

Alternatively, it remains possible that the apparent effect of color on road use probability is an artifact of opportunistic data collection by citizen scientists. Notably, coloration was affected by an interaction between latitude and longitude (Table 2), a pattern that appears to represent the association between darker coloration and the increased elevations of the Appalachian Mountain range (Figure 2), where cooler climates are expected to favor melanism for its thermoregulatory benefits (the thermal melanism hypothesis; Allsteadt et al. 2006), though other factors (e.g., predation pressure) could also influence geographic variation in color. There may be fewer roads in higher elevation regions, and stopping a vehicle to photograph snakes along narrow and curving roads in areas of complex terrain can be difficult and dangerous (BEC, personal observation). Together, these factors could reduce the probability of iNaturalist observations on roads in the areas in which darker snakes are most common. The effect of color morph on road use was significant while accounting for broad geographic patterns (Table 3), but there may be finer-scale differences between the habitat types of darker and lighter snakes, specifically in terrain. Because the conservation status of timber rattlesnakes precludes the ready availability of high-resolution data on the specific locations of the snakes in this study, the possibility of location-based sampling bias is

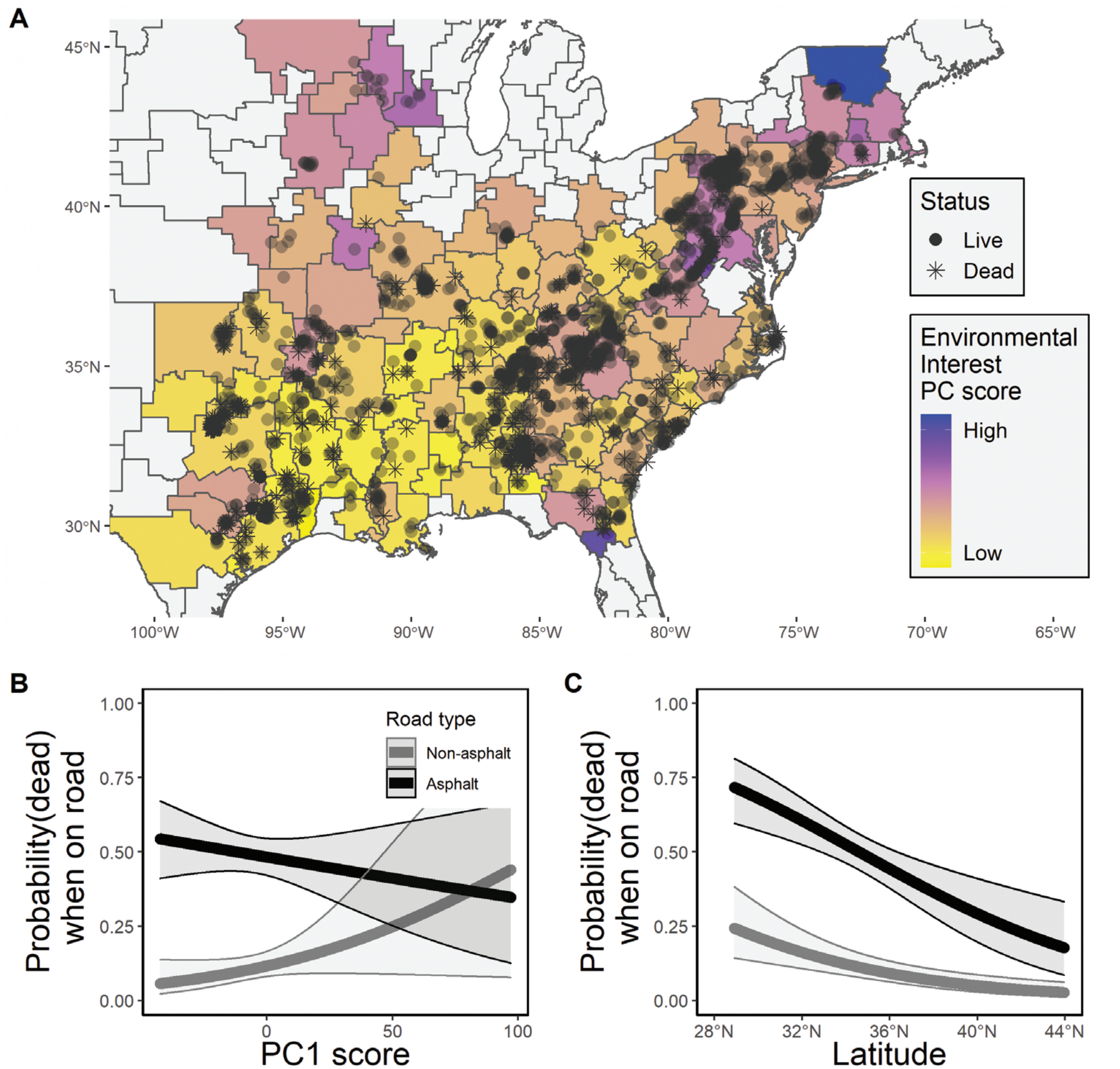

Figure 4. Timber rattlesnake death on roads as a function of location (latitude and longitude), road type (asphalt or non-asphalt), and environmental interest (PC1 scores). (**A**) Indicates occurrences of snakes found on roads, with live/dead status indicated by point shape and the environmental interest scores of metro areas indicated by color. (**B**) Depicts the interactive effect of environmental interest and road type (asphalt paved versus non-asphalt paved) on the predicted probability of a snake being found dead, given that it was found on a possible road. (**C**) Depicts the effect of latitude on the probability of a snake being found dead, for both asphalt-paved and non-asphalt paved roads. Both **C** and **B** include 95% prediction intervals calculated from the "intervals()" function in the R package *spaMM*, omitting random effects. Line colors in **C** are as in **B**.

difficult to assess and remains a general challenge for analyzing citizen science-based data.

The reason for the higher proportion of rattlesnakes found on roads in the southern portions of their range is unclear. In general, access to the warmer temperatures of the road surfaces would be less necessary in warmer climates. However, within a site, warmer temperatures are associated with greater use of roads for thermoregulation in a diverse community of snakes (Mccardle and Fontenot 2016), suggesting greater thermoregulatory value for roads under warmer conditions, which is consistent with our observation of warmer temperatures increasing road use in timber rattlesnakes. This might be reflected at larger geographic scales as well, with southern timber rattlesnakes benefitting more by roads that have been warmed by the higher solar radiation and, perhaps, greater contrast with the thermal properties of natural substrates. If road use and therefore mortality rates are in fact higher further south, there is the potential for southern timber rattlesnake populations to be more sensitive to roads, leading to declines and consequent food web impacts due to the loss of predatory snakes (e.g., Bestion et al. 2015). Alternatively, snakes across the range may be equally likely to use roads, and the greater probability of iNaturalist reports of snakes on roads in the south could reflect geographic biases in citizen scientist behavior. Searching for snakes on roads has long been known as an efficient strategy for finding them, by both professional scientific collectors and by amateurs and enthusiasts (Shine et al. 2004). The greater abundance and diversity of reptiles in the southern United States may increase overall encounter rates with snakes and, therefore, increase interest in participating in the activity. This would produce a larger number of specimens found on roads compared to opportunistic encounters during other activities (e.g., hiking in less disturbed habitats). The data available make it difficult to differentiate actual latitudinal differences in road use from apparent differences due to observer bias. Similarly, the higher proportion of dead snakes on roads in the south may reflect either observer willingness to investigate and document roadkill or actual differences in mortality risk on the roads. The latter could be caused by motorist behavior, vehicle speeds, or the low visibility of snakes using roads during the night in areas where daytime temperatures are too high. Future study of citizen science behavior should explore possible geographic biases in how reptiles are encountered and documented.

Higher road use was found in lighter-colored snakes and, perhaps, snakes further south (where they are generally lighter colored), and this was coupled with reduced apparent survival on roads in the south. This pattern of lighter-colored snakes using roads more and being more common in the south where road mortality is higher would generate greater road mortality in lighter morphs if their probability of being killed when they are on a road is the same as or greater than darker conspecifics. We had hypothesized that the color of the snakes could influence their mortality rate while on roads, primarily due to differences in the visibility of the snakes to motorists, with darker snakes presumed to be most cryptic while on asphalt-paved roads. Whether more cryptic snakes survived better would depend on the motivations of the motorists: if motorists would attempt to avoid collisions with snakes of which they were aware, cryptic coloration would increase mortality. We therefore anticipated that regional environmental attitudes, as assessed through Google search engine activity, would interact with road type (asphalt or non-asphalt) and snake color to influence mortality rates of snakes on roads. This was not the case. There were no significant interaction terms between road type and snake color, and the only significant term that included the principal component scores for environmental interest indicated a tendency for the greater mortality of snakes on asphalt paved roads to converge with non-asphalt paved roads in regions of higher environmental interest (Figure 4B). The higher overall mortality of the rattlesnakes on asphalt paved roads is unsurprising, as these generally will allow higher vehicle speed and greater amounts of traffic than unpaved roads.

Higher rates of road use and similar rates of mortality while on roads would be expected to produce selection against light coloration in timber rattlesnakes, shifting the average color patterns in the species toward greater melanization. The short time span of our dataset and the long lifespans of timber rattlesnakes (Brown and Simon 2018) prevent the detection of temporal trends in average color pattern in this study and perhaps in general, though it may be fruitful to test whether coloration has changed over time using museum collections (e.g., MacLean et al. 2019) or new surveys of historically studied populations (e.g., Karell et al. 2011). If selection is indeed favoring darker colored snakes due to road mortality, the evolutionary response may be reduced or reversed by climate change. As climatic warming occurs, selection should favor lighter coloration (but see Roulin 2014), counter to the apparent benefits of dark coloration for reducing road mortality. We encourage evaluating these predictions through continued long-term monitoring of melanization in wild populations of timber rattlesnakes and other color-variable/polymorphic species.

We noted above that there may be sampling biases associated with the likelihood of observers recording snakes and/or roadkill in particular regions of the timber rattlesnake range. This is in addition to other unknown factors that may generate sampling biases using data collected through citizen science. For example, rattlesnake encounters may be of more interest and more likely to be documented in areas where snakes, and in particular venomous snakes, are less common. The biases we identified, along with other potential biases, represent a constellation of factors that could cloud inferences or generate spurious correlations, a well-recognized problem in citizen science that can be addressed with careful planning and standardization of sampling effort (Isaac et al. 2014; Altwegg and Nichols 2019; Ditmer et al. 2021). The use of a coordinated, pre-planned effort was not available here and would be unlikely to yield nearly such large sample sizes.

Nonetheless, we believe our analyses identify an interesting pattern that is consistent with theoretical expectations (lighter-colored timber rattlesnakes used roads more often) and could lead to an evolutionary response to roads (reduced frequency of lighter-colored snakes due to elevated risk of road mortality). Further exploration of this pattern with systematic surveys across the species range would be valuable for understanding and predicting the response of this at-risk species to ongoing environmental change and would provide an opportunity to examine the concordance between citizen science data collection and more controlled research studies.

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Conflict of Interest statement

The authors have no conflict of interest to declare.

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