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Effects of Unpaved Roads on Relative Abundance and Epigenetics of Early Successional Lizards

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EFFECTS OF UNPAVED ROADS ON RELATIVE ABUNDANCE AND EPIGENETICS OF EARLY SUCCESSIONAL LIZARDS

by

DAVID TEVS

(Under the Direction of Lance McBrayer)

ABSTRACT

Unpaved roads may provide uniform microhabitat characteristics and impart edge effects in the adjacent landscape that mediate environmental pressures acting on small vertebrates. These features may allow species that are associated with recent disturbance to persist in aging forest patches. Further, epigenetic mechanisms such as DNA methylation may provide these species the phenotypic plasticity necessary to occupy multiple habitats with different environmental conditions. To understand how small vertebrates use unpaved roads, the relative abundance and occurrence of Florida scrub lizards (*Sceloporus woodi*) and six-lined racerunners (*Aspidoscelis sexlineata*) were quantified using visual encounter surveys along unpaved roads bordered by habitat patches of different age classes and under different management regimes. To explore lizard microhabitat associations along unpaved roads, the proportion of several microhabitat characteristics within the road adjacent habitat (≤ 10m from road edge) were also quantified. In addition, *Sceloporus woodi* were captured from unpaved road and forest interior habitats (≥ 15m from road edge) to understand how small vertebrates use unpaved roads and DNA methylation to respond to environmental pressures. Variation in *Sceloporus woodi* diet, ectoparasite count, and DNA methylation was compared among 1) all road habitats, 2) all interior habitats, and 3) between road and interior habitats. Neither relative abundance nor occurrence of either species was affected by the road adjacent habitat. *Sceloporus woodi* relative abundance was positively correlated with the proportion of shade, whereas *Aspidoscelis sexlineata* relative abundance was positively correlated with substrate temperature. *Sceloporus woodi* from unpaved roads also had a more uniform diet and ectoparasite counts than those in stand interiors. Additionally, diet, ectoparasite exposure, and habitat type were correlated with DNA methylation changes within individuals, and three times as many differentially methylated regions were detected among lizards from forest interiors compared to lizards from unpaved roads. These results indicate molecular and non-molecular aspects of species biology are affected by unpaved roads. New habitat conditions along the road surface and in the adjacent landscape also appear to allow small disturbance dependent vertebrates to occupy previously inhospitable habitat. In doing so, these features may enhance connectivity and population viability in fragmented landscapes.

INDEX WORDS: Roads, Corridors, Florida scrub, Habitat disturbance, Forest management, DNA methylation, Epigenetics

EFFECTS OF UNPAVED ROADS ON RELATIVE ABUNDANCE AND EPIGENETICS OF EARLY SUCCESSIONAL LIZARDS

by

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MASTER OF SCIENCE

EFFECTS OF UNPAVED ROADS ON RELATIVE ABUNDANCE AND EPIGENETICS OF EARLY SUCCESSIONAL LIZARDS

by

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CHAPTER 1

VERGE HABITAT EFFECTS ON LIZARD RELATIVE ABUNDANCE

Introduction

Anthropogenic modifications to natural landscapes are a global phenomenon with implications for ecosystem health and viability. Roads and other similar features (trails, powerline clearings, etc.) are among the most ubiquitous of these modifications. The United States alone has over 4.5 million kilometers of paved roads and over 2.1 million kilometers of unpaved roads (USDT, 2018). Early research focused on quantifying the effects of road mortality resulting from vehicle collision (Dodd et al., 1989; Rosen and Lowe 1994). As the threats to ecosystem health imposed by habitat fragmentation have received greater attention (Andrews, 1990), research has shifted towards the effects of roads as barriers to population connectivity (Baur and Baur, 1990; Tucker et al., 2014; Floyd, 2015). However, much of the work focusing on the detrimental effects of roads has focused on paved roads. Unpaved roads typically have reduced likelihood of collision related mortality (McClure, 1951; Akrim et al., 2019) and are more likely to be traversed by some species (Robson and Blouin-Demers, 2013) compared to paved roads.

The degree to which unpaved roads affect local wildlife is likely driven in part by how the adjacent habitat (henceforth verge) is modified. Roads facilitate edge effects in the surrounding habitat by creating vegetation gaps that alter the abiotic habitat conditions within the verge (e.g., light penetration, wind movement, and humidity; Delgado et al., 2007b; Pohlman, 2007; Hamberg et al., 2010). Edge effects along the verge and the creation new microhabitat conditions along the road surface can generate favorable alternative habitat for organisms including invertebrates (Ries et al., 2001; Vasconcelos et al., 2006), small mammals (Brock and

Kelt, 2004), plants (Skinner, 2017), and reptiles (Delgado et al., 2007a; Kaunert and McBrayer, 2015). Unpaved roads can also facilitate dispersal of organisms, including amphibians (Brown et al., 2006) and small mammals (Bennett, 1990), through otherwise untraversable habitat. Edge-tolerant organisms, such as giant ameivas (*Ameiva ameiva*), may even occur in areas that were previously uninhabitable when unpaved roads are present (Sartorius et al., 1999). Edge facilitated species expansions may also prove detrimental to some organisms. Rates of parasitism by brown-headed cowbirds (*Molothrus ater*) on other grassland birds increase in areas with extensive anthropogenic edge habitat (Patten et al., 2006). Focusing efforts on understanding how edge-tolerant organisms use unpaved roads may help maximize the conservation value of these features for species that will most benefit.

Hereptofauna, particularly reptiles, are an excellent focal group to explore the effect of roads on wildlife. Many reptiles have a high tolerance for edge habitat (Carvajal-Cogollo et al., 2015; Schneider-Maunoury et al., 2016) and, in some cases, select road edge habitat over forest interiors (Delgado et al., 2007a; Kaunert and McBrayer, 2015). However, even within some reptile groups, there is variation in response to unpaved roads. Eastern hognose snakes (*Heterodon platirhinos*) show an aversion to crossing paved but not unpaved roads (Robson and Blouin-Demers, 2013), whereas eastern massasauga rattlesnakes (*Sistrurus catenatus*) show an aversion to crossing both paved and unpaved roads (Paterson et al., 2019). Given that unpaved roads are often necessary for land management, and reptiles can be sensitive to different land management practices (Shoemaker et al., 2009; McBrayer et al., 2020), the effects these features have on reptile communities may be further complicated when considering local management history.

North American National Forests contain thousands of kilometers of roads that serve recreation and land management practices including mining, logging, and prescribed wildfire. Many temporary natural-surface roads are created to transport goods and machinery for land management. Inherent differences in vegetation structure resulting from altered land management practices may confer different effects of unpaved roads on the surrounding habitat (Chen et al., 1999). The Ocala National Forest in central Florida USA has the two predominant habitat types, sand pine scrub and longleaf pine (LLP). Sand pine scrub is managed via clear-cut logging on ~40-year cycles, whereas LLP burned biennially to help conserve ecologically sensitive species. The sand pine scrub canopy is comprised primarily of sand pine (*Pinus clausa*), with a dense understory of regenerating oak shrubs (*Quercus spp.*) and palmettos (*Serenoa spp.;* Jackson, 1972; Greenberg et al., 1994). The LLP canopy is comprised primarily of loosely spaced longleaf pine (*Pinus palustris*) and an open understory of wiregrass (*Aristida stricta*) and turkey oak (*Quercus laevis*; Wells and Shunk, 1931; Jackson, 1972). With the exception of dense, mature stands of sand pine scrub, both habitats harbor many of the same herpetofaunal species that are known to use, and may benefit, from habitat along unpaved roads.

In Ocala National Forest, sand pine scrub and LLP harbor two lizard species that are associated with recently disturbed landscapes, Florida scrub lizards (*Sceleporus woodi*) and six-lined racerunners (*Aspidoscelis sexlineata*). Both are associated with habitat characteristics that proceed recent wildfire (e.g., bare ground; Mushinsky, 1985; Hokit et al., 1999), and become isolated when surrounded by large amounts of mature habitat (Schrey et al., 2011; Heath et al., 2012; Ragsdale et al., 2016; Orton et al., 2020). Both species are also capable of persisting in long-undisturbed stands of rosemary scrub (McCoy et al., 2012, 2013), likely due to greater availability of bare ground (Menges and Hawkes, 1998). Yet, densities are greatly diminished in the interior of mature stands of sand pine scrub (Greenberg et al., 1994). Many habitat characteristics present within early successional sand pine scrub (Greenberg et al., 1994; Greenberg, 2003; Kaunert and McBrayer, 2015) are also associated with unpaved road surfaces and the adjacent verge (Gesford and Anderson, 2007; Maynard et al., 2016), suggesting these features may allow *S. woodi* and *A. sexlineata* to occupy of mature sand pine scrub. *Sceloporus woodi* are already known to occur in greater density along roads bordered by recently disturbed sand pine scrub or LLP compared to forest interiors (Kaunert and McBrayer, 2015). However, no study has explored the density or occurrence of either species along roads bordered by mature sand pine scrub or among all verge types present with Ocala National Forest (i.e., all possible combinations of early successional sand pine scrub, mature sand pine scrub, and LLP).

This study quantified how verge habitat affects the occurrence and relative abundance of *S. woodi* and *A. sexlineata*. The degree to which each species is affected likely depends on how road verge characteristics vary among early successional sand pine scrub, mature sand pine scrub, and LLP. I tested hypotheses concerning how relative abundance and occurrence of both lizard species are affected by road verge type. 1) Lizard relative abundance and occurrence across verge combinations may reflect historical habitat associations of both species, with greater relative abundance seen in verges comprised more of early successional habitat. And 2) Lizard relative abundance and occurrence across verge combinations is uniform due to road edge effects homogenizing verge microhabitat characteristics. These data may show verge habitat varies with surrounding stand characteristics and the extent to which they allow early successional species to occupy long-undisturbed forest patches.

Methods

Road selection

Management history of stands with the Ocala National Forest was obtained from the U.S. Forest Service (Ocala National Forest Seminole Ranger District office; Kathy Bronson, pers. comm.). Single-lane forest management roads (e.g., those used to transport goods/machinery during logging) and off-highway vehicle roads (i.e., those used for dirt bikes, ATVs, etc..) were selected due to similarities in width, substrate, and traffic volume (personal observation). I sampled roads in the interior and perimeter of longleaf pine (LLP) and in early successional (ESS) and mature (MS) sand pine scrub stands. Six verge type combinations were studied based on the road adjacent habitat (henceforth verge habitat one/verge habitat two); ESS/ESS, ESS/MS, LLP/ESS, MS/MS, LLP/LLP and LLP/MS. Each verge combination was surveyed eight times, except for LLP/ESS which only existed in four locations (Appendix A). All surveys of the same verge combination took place in separate stands as delineated by U.S. Forest Service. Early successional sand pine scrub was classified as stands <6 years post disturbance, and MS was classified as ≥ 30 years post disturbance.

Visual Encounter Surveys

Herpetofauna relative abundance, occurrence, and diversity were estimated via visual encounter surveys. All surveys were conducted between 08:30 and 13:00 from 2 June to 13 August 2021 and from 6 June 2022 to 7 July 2022. To avoid excessive variation due to cloud cover, surveys were only conducted in <50% cloud cover. Surveys were conducted by two individuals each walking a transect along one of the two road edges in tandem for 10 minutes. The start of the road edge was defined as the point of contact where the surface of the road touches vegetation. Each surveyor scanned for two focal lizard species (*A. sexlineata* and *S.*

woodi) and any additional reptiles and amphibians along the road as well as the respective verge to their left or right. Any lizard that could be reliably identified from the road edge was assumed to be using some aspect of the road and was thus counted in the survey. When a potential species was heard before being seen, the transect time was stopped until the specimen could be visually identified. If the specimen could not be identified within 3 minutes of audible detection it was not counted, and the survey time resumed.

Microhabitat Sampling

Microhabitat characteristics were collected along each transect within five plots spaced equidistantly along each survey transect that were placed randomly on either road edge and extended perpendicular to the road surface (randomly 1-10m) towards the stand interior. Within each 1 square meter plot, the proportion of full sun, dappled sun, and full shade at ground level, as well as the proportion of three substrate types: open sand, litter, and course woody debris were estimated. The surface temperature of each substrate/light combination and estimated the proportion of coverage by herbaceous and woody vegetation was also measured. All estimations were taken from eye level of the same principal observer (DRT). In instances where vegetation covered part of the plot but did not alter the availability of substrate (e.g., large palm fronds), the proportion of vegetative cover did not detract from the substrate proportion.

Statistical analyses

Two analysis approaches were used to determine the effects of road verges on *S. woodi* and *A. sexlineata*. First, I tested whether presence/absence of *S. woodi* and *A. sexlineata* differed among verge combinations using G tests. Second, the Scheirer-Ray-Hare test was used to examine the effect of verge type on the relative abundance (number of encounters/10-minute survey) of *S. woodi* and *A. sexlineata*, as well as Shannon's diversity (H) corresponding with each survey. Sampling location was included as a random effect nested within each verge type in each Scheirer-Ray-Hare test. Spearman's ranked correlations were then used to determine the relationship between microhabitat conditions and lizard relative abundance, occurrence, and hereptofaunal diversity. Due to the number of tests, the Bonferroni correction was used to adjust *p* values in Spearman's ranked correlations.

To test how microhabitat characteristics varied among verge types, I calculated the mean proportion of each substrate, light, and vegetation type, as well as the mean temperature of each substrate/light combination contained in all five one-meter square sampling plots corresponding to a specific survey. Principal components analysis was then used to condense the proportion of all microhabitat conditions into axes that described major variation in each microhabitat condition among all verge combinations. Many surveys lacked various substrate/light combinations, resulting skewed distributions and missing data. Therefore, the Scheirer-Ray-Hare test was used to explore effects of verge type on the temperature of each substrate/light combination. Sampling location was included as a random effect nested within each verge type in these Scheirer-Ray-Hare tests. Course woody debris lacked enough observations for analysis of temperature under different light conditions, therefore the mean temperature of course woody debris under each light condition is reported but not included in any analyses.

Results

All verge combinations were surveyed for a total of 160 individual man hours, except for LLP/ESS which was surveyed for 80 individual man hours. This resulted in a total of 157 observations across 9 species (Table 1). Observations are dominated were dominated by A. *sexlineata* and *S. woodi*.

Differences in the per cent occurrence of *S. woodi* were detected between only two verge types (Appendix B; Figure 1). The occurrence of *Sceloporus woodi* was greatest along MS/MS roads, and lowest along LLP/MS roads yet were similar among all other verge combinations (Figure 1). The occurrence of *Aspidoscelis sexlineata* did not significantly vary among any verge combinations (Appendix B; Figure 1). Also, relative abundance of *S. woodi* (verge type: $H_{(0.05)(5)} = 4.161$, $P > 0.05$; sampling location: $H_{(0.05)(34)} = 35.656$, $P > 0.05$), relative abundance of *A. sexlineata* (verge type: $H_{(0.05)(5)} = 6.09$, $P > 0.05$; sampling location: $H_{(0.05)(34)} = 32.845$, $P > 0.05$), and Shannon's diversity (verge type: $H_{(0.05)(5)} = 0.539$, $P > 0.05$; sampling location: $H_{(0.05)(34)} = 41.121$, $P > 0.05$; Figure 2) did not differ between verge types and sampling locations.

Several correlations between microhabitat conditions and lizard densities were detected, yet none for Shannon's diversity. The number of *A. sexlineata* encounters was positively correlated with the temperature of sunny sand ($r = 0.5148$, $P = 0.0036$), shaded sand ($r = 0.5738$, $P = 0.0014$), dappled sand ($r = 0.6215$, $P = 0.0002$), sunny litter ($r = 0.5531$, $P = 0.0002$), shaded litter ($r = 0.4937$, $P = 0.0009$), and dappled litter ($r = 0.5930$, $P < 0.0001$). The number of *S. woodi* encounters was positively correlated with proportion of shade ($r = 0.475$, $P = 0.0011$).

Three PCA axes with eigenvalues greater than one were retained to describe variation in microhabitat among all verge types (Table 2). Principal component one was characterized by a gradient of increasing woody vegetation and a decreasing availability of sun. Principal component two was characterized by an increasing availability of open sand. Principal component three was characterized by a gradient of increasing herbaceous vegetation and shade. Density ellipses for each verge combination (Figure 3) show that verges comprised of only one habitat type (ESS/ESS, MS/MS, and LLP/LLP) are more separated along principal components one and two than verges comprised of multiple habitat types (ESS/MS, LLP/ESS, LLP/MS), suggesting this latter combination of verges have a more homogenous mixture of microhabitat conditions. The temperature of each substrate/light combination was unaffected by verge type or sampling location (Appendix C). Means for the proportion of microhabitat characteristics and temperatures of all substrate/light combinations are in tables 3-5.

TABLE 1. Sum of species encounters for six verge type combinations in Ocala National Forest. Data were collected in 160 minutes of searching for all verge combinations aside from LLP/ESS which was searched for a total of 80 minutes. Verge habitats on either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

Verge type	**Species**									
	Anolis carolinensis	***Anolis sagrei***	***Aspidoscelis sexlineata***	***Gopherus polyphemus***	***Hyla cinerea***	***Masticophis flagellum***	***Plestiodon spp.***	***Sceloporus woodi***	***Sistrurus miliarius***	**Total**
ESS/ESS	-	-	16	1	-	-	-	12	-	29
ESS/MS	1	-	10	-	-	1	1	15	1	29
MS/MS	-	-	9	-	-	-	-	18	-	27
LLP/LLP	1	-	10	1	1	-	-	13	-	26
LLP/ESS	-	-	9	-	-	-	-	5	-	14
LLP/MS	1	1	19	1	-	-	2	8	-	32
Total	3	1	73	3	1	1	3	71	1	157

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TABLE 2. Principal component loadings and eigenvalues corresponding to the proportion of each microhabitat characteristic among all verge combinations. Bolded values represent the primary loadings for each component.

	Principal component 1	**Principal component 2**	**Principal component 3**
Microhabitat condition			
Herbaceous vegetation	-0.18074	-0.34125	**0.67173**
Woody vegetation	**0.42524**	0.32581	-0.15836
Open Sand	-0.26318	**0.59859**	-0.18774
Litter	0.39031	-0.37459	-0.13453
CWD	-0.20434	0.30621	0.19881
Full sun	**-0.50548**	-0.20155	-0.28552
Dappled sun	0.29228	-0.2262	-0.3707
Full shade	**0.42686**	0.3053	**0.46031**
Eigenvalue	3.0425	1.8142	1.1607
Percent	38.031	22.677	14.508
Cumulative percent	38.031	60.708	75.216

TABLE 3. Mean (± S.D) proportions of light categories at ground level and substrate types contained within one square meter sampling plots corresponding to each verge type. Verge habitats on either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

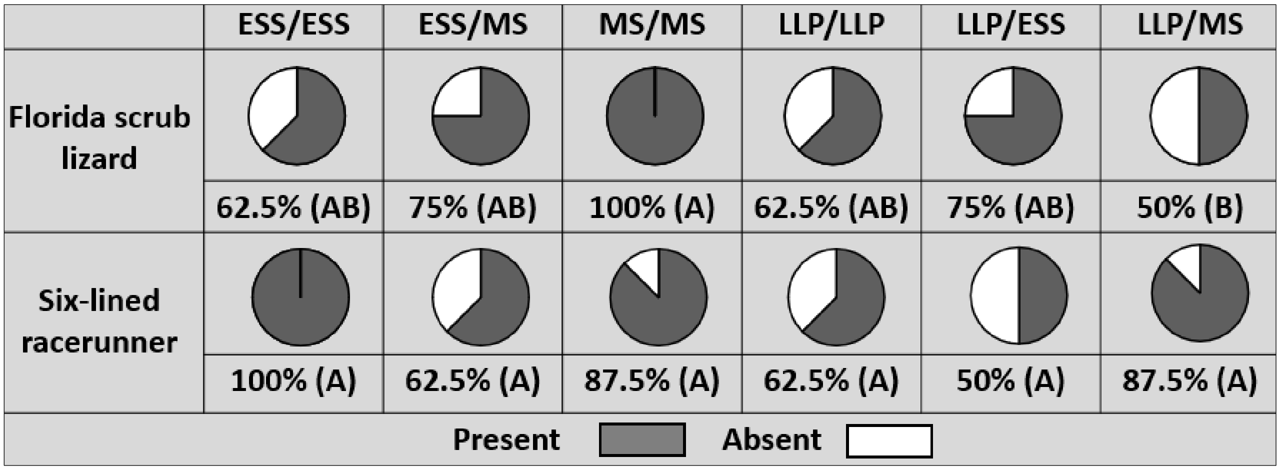
Verge type	Sun	Dappled sun	Full shade	Open sand	Litter	Course woody debris
ESS/ESS	41.45 ± 24.75	9.70 ± 5.53	48.83 ± 23.80	37.50 ±18.76	56.55 ± 21.07	2.95 ± 2.06
ESS/MS	36.38 ± 8.47	18.88 ± 6.51	45.25 ± 12.16	18.57 ± 11.47	62.71 ± 14.66	4.29 ± 2.21
MS/MS	14.08 ± 15.34	23.95 ± 8.66	61.83 ± 15.56	2.75 ± 6.61	84.75 ± 15.19	3.50 ± 2.62
LLP/LLP	53.83 ± 19.61	15.70 ± 9.31	29.95 ± 17.23	4.63 ± 3.96	68.33 ± 11.92	0.50 ± 1.07
LLP/ESS	49.15 ± 16.86	18.15 ± 6.19	32.65 ± 20.13	27.25 ± 17.40	51.25 ± 25.67	9.50 ± 6.86
LLP/MS	43.08 ± 20.93	13.03 ± 6.64	42.63 ± 19.72	9.33 ± 13.81	73.18 ± 16.63	3.13 ± 3.44

TABLE 4. Mean (± S.D) Proportions of herbaceous and woody vegetation contained within one square meter sampling plots corresponding to each verge type. Verge habitats on either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

Verge type	Herbaceous vegetation	Woody vegetation
ESS/ESS	12.85 ± 10.28	44.58 ± 17.19
ESS/MS	14.80 ± 7.62	38.03 ± 12.69
MS/MS	17.73 ± 15.13	47.38 ± 22.61
LLP/LLP	29.88 ± 12.29	11.10 ± 8.03
LLP/ESS	28.25 ± 12.80	9.65 ± 12.24
LLP/MS	25.68 ± 16.28	29.40 ± 14.01

TABLE 5. Mean (± S.D) temperature (°C) of each substrate/light category within one square meter sampling plots corresponding to each verge type. No significant differences were detected among any pairwise comparisons of verge type (test statistics found in Appendix C). Verge habitats on either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

Verge type	**Sand**			**Litter**			**Course woody debris**		
	sun	**dappled**	**shade**	**sun**	**dappled**	**shade**	**sun**	**dappled**	**shade**
ESS/ESS	40.14 ± 7.02	34.19 ± 4.18	31.43 ± 3.32	46.41 ± 9.66	35.67 ± 4.77	31.93 ± 3.36	50.42 ± 2.07	38.23 ± 4.66	33.65 ± 4.31
ESS/MS	35.28 ± 6.67	31.82 ± 4.69	29.03 ± 3.44	39.08 ± 6.92	32.06 ± 3.61	29.21 ± 3.27	58.70 ± 0	35.15 ± 7.85	39.50 ± 5.94
MS/MS	41.50 ± 20.51	33.23 ± 12.29	33.00 ± 0	40.34 ± 8.70	32.50 ± 4.70	30.12 ± 3.69	41.9 ± 0	35.98 ± 1.59	34.50 ± 2.83
LLP/LLP	40.60 ± 8.61	34.28 ± 6.24	32.45 ± 3.55	41.90 ± 8.14	32.56 ± 3.90	33.85 ± 4.72	-	-	-
LLP/ESS	32.55 ± 3.26	29.35 ± 2.98	27.28 ± 4.99	37.44 ± 6.09	31.8 ± 3.67	29.22 ± 3.59	46.20 ± 0	35.9 ± 0	-
LLP/MS	46.78 ± 5.97	35.73 ± 5.51	32.10 ± 4.76	44.99 ± 8.54	36.40 ± 3.86	32.65 ± 3.27	40.25 ± 4.17	36.7 ± 0	33.5 ± 0



	ESS/ESS	ESS/MS	MS/MS	LLP/LLP	LLP/ESS	LLP/MS
Florida scrub lizard	62.5% (AB)	75% (AB)	100% (A)	62.5% (AB)	75% (AB)	50% (B)
Six-lined racerunner	100% (A)	62.5% (A)	87.5% (A)	62.5% (A)	50% (A)	87.5% (A)

FIGURE 1. Venn diagrams representing the proportion of surveys where Florida scrub lizards and six-lined racerunners were present (grey) and absent (white). Percentages correspond with the proportion of surveys within a particular verge combination where a species was present. Letters are read horizontally and indicate significance after Bonferroni correction. Verge habitats on either side of the road are separated by a “/”. LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

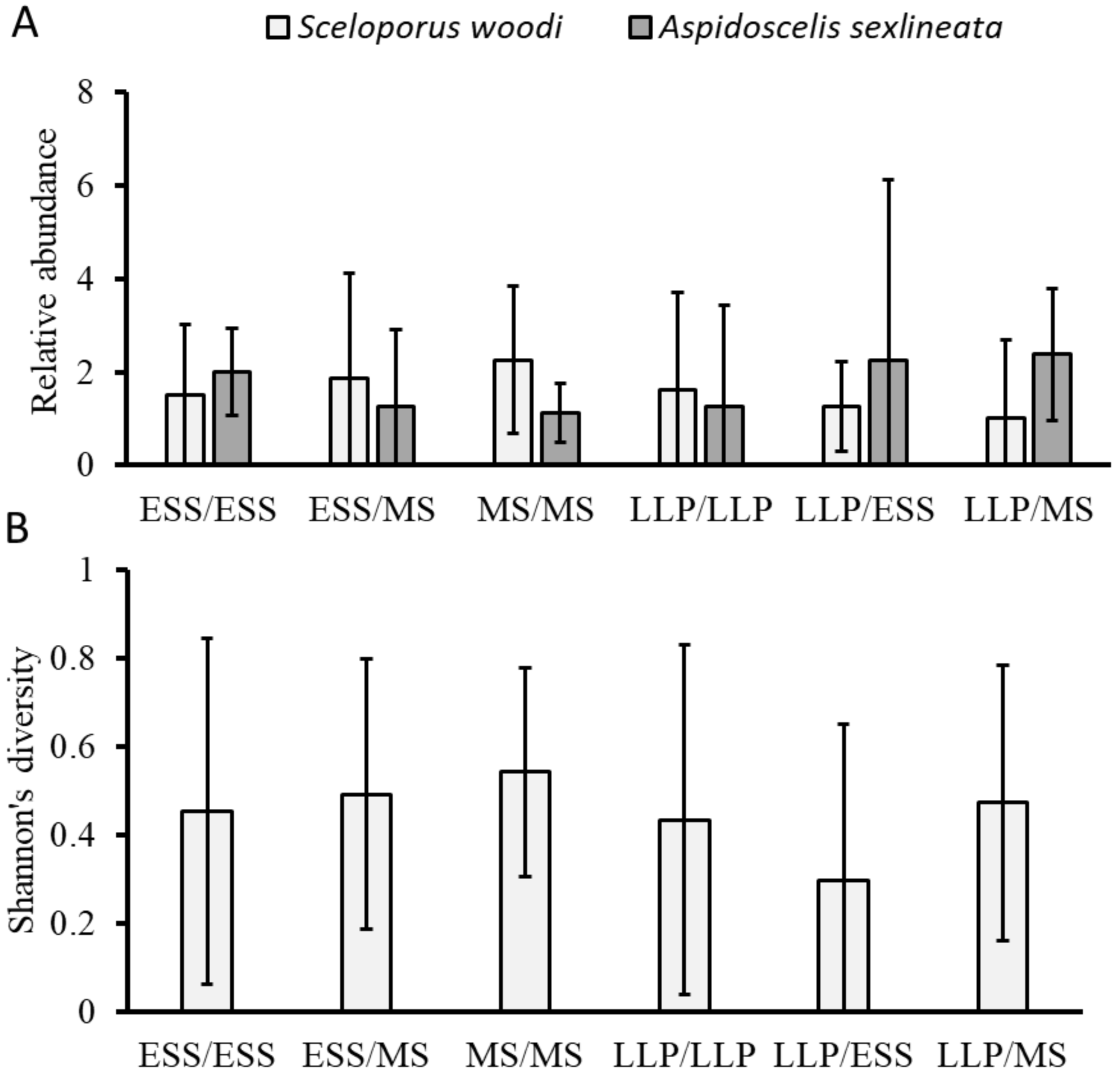


FIGURE 2. A) Relative abundance of *Sceloporus woodi* and *Aspidoscelis sexlineata* and B) Shannon's diversity across verge combinations in Ocala National Forest. No significant differences were detected between any comparisons. Relative abundance is measured in the total number of encounters during each 10-minute survey. Standard deviation is shown on each bar. Verge habitats on either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

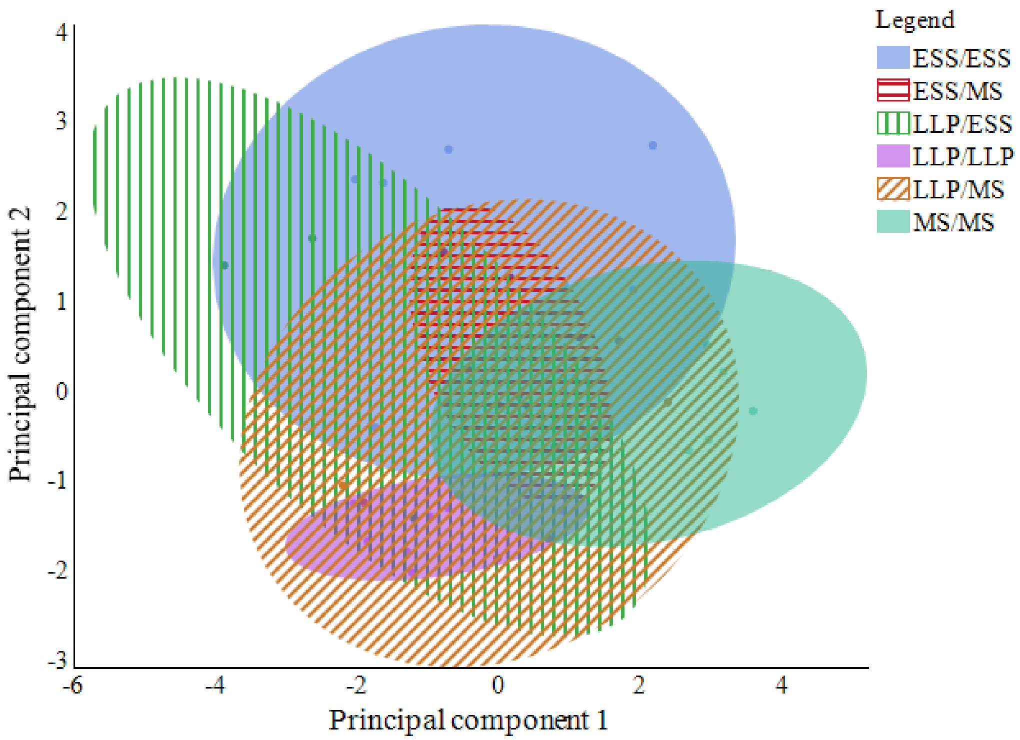


FIGURE 3. Plot of the first two principal components analyzing the proportion of measured microhabitat characteristics among all verge combinations. Density ellipses represent 95% confidence intervals for each verge type. Verge habitats on either side of the road are separated by a “/”. LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

Discussion

Occurrence and relative abundance of both species were found to be largely unaffected by verge type. Herpetofauna diversity was also unaffected by verge type. However, *Sceloporus woodi* were present along all MS/MS surveys, whereas *A. sexlineata* were present along all ESS/ESS surveys. While delineations were observed among verge types in the proportion of several microhabitat characteristics, there are also large portions of overlap in the density ellipses representing each verge type (Figure 3). This suggests that unpaved roads homogenize many of the microhabitat conditions that differ between ESS, MS, and LLP interiors. In doing so, unpaved roads appear to create equal opportunity for *S. woodi* and *A. sexlineata* regardless of disturbance or management history and allow both species to occupy habitats that have historically been viewed as unhospitable.

Occurrence and relative abundance of *S. woodi* among verge combinations support the role of unpaved roads in altering the normal habitat associations of this species. Given their historical association with early successional habitat characteristics (Greenberg et al., 1994; Hokit et al., 1999; Tiebout and Anderson, 2001), I expected greater occurrence and relative abundance of *S. woodi* along roads bordered by ESS or LLP. Yet 100% occurrence of *S. woodi* was observed along MS/MS roads and equal densities were observed across all verge combinations. Given the positive correlation between *S. woodi* relative abundance and the proportion of shade, it is possible 100% occurrence along MS/MS roads is driven by the greater availability of shade in these areas. Availability of shade and sand are the most important characteristics of all substrate/light combinations in informing *S. woodi* microhabitat associations (Tiebout and Anderson, 2001). The density ellipse for MS/MS roads was most strongly associated with positive values of principal component one (which corresponded with greater availability of shade; Table 2) but appeared unassociated with positive values of principal component two (which corresponded with greater availability of open sand; Table 2 and Figure 3). While we did not directly quantify the microhabitat conditions along the road surface, unpaved roads are often cleared of litter and debris to facilitate safe vehicular passage (Gesford and Anderson, 2007). Therefore, we propose that availability of shade in MS/MS verges and sand along the road surface allow *S. woodi* to depart from their historical habitat associations and occupy MS.

While occurrence and relative abundance of *A. sexlineata* conform more closely to previous expectations for this species, departures from their historical habitat associations were still detected. Given that *A. sexlineata* is typically associated with recently disturbed habitat (Mushinsky; 1985; Ashton and Knipps, 2011), greater relative abundance and occurrence were expected along roads bordered at least partially by ESS or LLP than those bordered at least partially by MS. While *A. sexlineata* occurred along 100% of ESS/ESS surveys, occurrence and relative abundance did not statistically differ among any verge combinations (Figure 1 and 2). These patterns are supported by previous accounts of *A. sexlineata's* high vagility and tolerance for a wide range of habitat characteristics (Clark, 1976; Mushinsky, 1985) allowing this species to maintain connectivity in long undisturbed stands of mature habitat (Schrey et al., 2011).

Variation in the proportion of microhabitat characteristics among verge combinations (Tables 2-4) may contribute to immigration but not persistence of *A. sexlineata* in previously uncolonized forest patches. The only verge combination with 100% occurrence of *A. sexlineata* (ESS/ESS) was most positively associated with principal component two where the highest positive loading microhabitat condition was open sand. However, *A. sexlineata* relative abundance was only correlated with substrate temperature, and substrate temperatures were uniform among all verge combinations (Appendix C and table 5). These correlations may reflect periods of increased activity, as *A. sexlineata* are more active on warmer substrates (Paulissen, 1988). Given this data, it is possible that warm, open sand is attractive habitat for dispersing *A. sexlineata* and may inform where dispersing individuals settle, and unpaved roads help this species persist after early successional habitat characteristics have faded.

The results of this study contradict previous work that described lower densities of *S. woodi* and *A. sexlineata* in MS (Greenberg et al., 1994). However, this discrepancy is likely due to different sampling methodologies. The present study exclusively targeted unpaved roads during sampling, Greenberg et al., (1994) appeared to target exclusively stand interiors. Interestingly, densities of both *A. sexlineata* and *S. woodi* along unpaved roads in Ocala National Forest are closer those described at Archbold Biological Station in Highlands County Florida, where time since fire did not affect relative abundance of either species (McCoy et al., 2012). This is likely due to differences in vegetation composition, as Archbold Biological Station is dominated by rosemary scrub opposed to the sand pine scrub of Ocala National Forest (Harper, 1921; Abrahamson et al., 1984). Compared to sand pine scrub, long undisturbed rosemary scrub offers much more open sand and light penetration (Menges and Hawkes, 1998). By creating a large expanse of bare sand along the road surface, unpaved roadways cutting through MS appear to modify the habitat in a way that more closely resembles that of rosemary scrub at Archbold Biological Station.

Occupation of mature roads by both species likely enhances connectivity in the fragmented habitat of Ocala National Forest. Many studies have speculated that unpaved roads bisecting stands of mature forest may act as dispersal corridors for *A. sexlineata* and *S. woodi* (Greenberg et al., 1994; Tiebout and Anderson, 1997; Tucker et al., 2014; Kaunert and

McBrayer, 2015), yet no study has explored how either species use these features in MS. Given that *A. sexlineata* possess greater vagility than *S. woodi* (Carpenter, 1959; Clark et al., 1999) and is more capable of maintaining connectivity when surrounded by long undisturbed habitat (Schrey et al., 2011), enhanced connectivity via dispersal along unpaved roads may not be as important for this species. However, *S. woodi* exhibit population structure among localities separated by several hundred meters of unideal habitat (Clark et al., 1999), suggesting unpaved roads may be of much greater importance for *S. woodi* in fragmented landscapes. Use of MS/MS roads may enhance connectivity of *S. woodi* in two ways. 1) MS/MS roads allow individuals to directly move between patches of early successional habitat. 2) MS/MS roads allow small populations to persist in the landscape between patches of early successional habitat and act as "stepping-stones" for gene flow to connect large populations. Small populations acting as stepping-stones to enhance connectivity of a larger population has been observed in a variety of systems, including corals (Miller and Gunasekera, 2017), birds (Saura et al., 2014), and mollusks (Crandall et al., 2012). Thus, similar effects may be taking place among *S. woodi* populations connected by MS/MS roads.

In conclusion, small single lane unpaved roads provide suitable conditions for populations of *S. woodi* and *A. sexlineata* to persist regardless of the surrounding habitat. This study also demonstrated that unpaved roads bisecting stands of MS offer viable habitat, despite the previous view that unhospitable conditions within MS negatively affect the relative abundance of these species (Greenberg et al., 1994). While they may not exist in MS under the canopy, unpaved roads allow *S. woodi* to disperse (or maybe persist) along roads in MS to other locations. Future studies may seek to explore how unpaved roads bisecting stands of MS enhance connectivity of these species. These data do not indicate whether the individuals observed along

MS/MS roads are simply dispersing through these habitats or are using these roads as true alternative habitat and persisting in the mature landscape. Nevertheless, these results indicate that unpaved roads homogenize landscape features necessary for small lizards.

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CHAPTER 2

DIET, PARASITE, AND EPIGENETIC EFEFCTS OF UNPAVED ROADS ON LIZARDS

Introduction

Anthropogenic landscape alterations are ubiquitous and can alter many biological aspects of species. Several anthropogenic landscape alterations, such as unpaved roads, powerline clearings, and firebreaks, generate edge habitat that can affect species distributions within the landscape. Edge effects created by unpaved roads influence sources of abiotic stress such as temperature, humidity, and light availability within the adjacent habitat (Delgado et al., 2007b; Pohlman, 2007; Hamberg et al., 2010). Edge-tolerant species often benefit from unpaved roads because they provide alternative habitat (Sartorius et al., 1999; Delgado et al., 2007a; Kaunert and McBrayer, 2015) and lower the risk of mortality from vehicle strikes compared to paved roads (McClure, 1951; Akrim et al., 2019). Non-road edge habitat can alter biotic sources of stress such as predator/prey dynamics (Andren and Anglestam, 1988; Hansen et al., 2019) and host/parasite relationships (Schlaepfer and Gavin, 2001; Patten et al., 2006). However, most previous wildlife studies on unpaved roads have focused on community structure or demography (Sartorius et al., 1999; Kaunert and McBrayer, 2015; Maynard et al., 2016; Schneider-Maunoury et al., 2016), with little attention being given to physiological or epigenetic traits. The response of local wildlife to the variation in environmental pressures between unpaved roads and stand interiors likely depends on the species’ natural habitat associations and capacity for plasticity.

Species may use molecular mechanisms of plasticity, such as DNA methylation, to respond to variation in environmental pressures between unpaved roads and interior habits. For example, edge effects can manifest changes in both dietary composition and host/parasite relationships (Schlaepfer and Gavin, 2001; Wilkin et al., 2009), both of which can elicit changes in DNA methylation (Lea et al, 2016; Hu et al., 2018). Individuals may change DNA methylation patterns in response to external environmental pressures and use these changes to mediate phenotypes to cope with the pressure (Hu et al., 2018; Kilvitis et al., 2019). These responses occur by changing the relationship between DNA and transcription machinery (Bossdorf et al., 2008) and, consequently, this precipitates changes in gene expression (Holliday, 2006). Changes in gene expression arising from altered patterns of DNA methylation can affect organism physiology (Baerwald et al., 2016; Kubota et al., 2016) and morphology (Cubas et al., 1999). These changes can also help organisms cope with the challenges associated with novel landscapes (Liebel et al., 2013; Hu et al., 2019), suggesting they may also play a role in helping species respond to anthropogenic habitat alterations, such as the creation of unpaved road edge habitat.

Wildfire-maintained ecosystems are an optimal setting to explore how animals use unpaved roads and DNA methylation to respond to environmental variation. Many species that evolved in tandem with sporadic wildfire now depend on distinct post-fire successional stages (Greenberg et al., 1994). In Ocala National Forest (ONF) of peninsular Florida, wildfire suppression and commercial logging have reduced the availability of early successional post-fire habitat. However, the two predominant habitat types in ONF, sand pine scrub (SPS) and longleaf pine (LLP), are interspersed by many unpaved roads that offer open sand and ample light penetration similar to post wildfire habitats (Greenberg et al., 1995; Greenberg, 2003; Gelford and Anderson, 2007; Maynard et al., 2016). By mimicking habitat conditions associated with recent wildfire, unpaved roads in SPS and LLP create beneficial habitat for early successional species (Kaunert and McBrayer, 2015), yet variation in vegetation communities and management history may precipitate different environmental pressures between unpaved roads and stand interiors in each habitat.

In ONF, SPS is predominantly managed for timber production via clearcut logging followed by roller chopping on ~40year cycles. This separates SPS into early successional scrub (ESS) and mature scrub (MS) categories. As ESS ages, open sand is replaced by leaf litter and a dense understory of oak shrubs (Greenberg et al., 1994,1995). Recently, management of several stands of SPS has shifted from logging to prescribed fire to conserve species (e.g., Florida scrub jays), but these stands have not existed long enough to reach maturity. Aside from a decreased availability of woody debris in recently logged ESS due to higher decomposition rates resulting from burial of logs during roller chopping, few differences in habitat structure have been reported between these two management styles (Greenberg, 1994, 1995). Longleaf pine is exclusively managed via biennial prescribed fire to help conserve ecologically sensitive species. Compared to SPS, LLP has a more open understory dominated by wiregrass (*Astrida stricta*) with widely spaced long leaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*; Wells and Shunk 1931; Jackson 1972). These differences in vegetation structure and management history can alter many ecological factors (McBrayer et al., 2020).

Florida scrub lizards (*Sceloporus woodi*) occupy both the SPS and LLP habitats of ONF. This species has historically been associated with recent habitat disturbance (Hokit et al., 1999; Tiebout and Anderson, 2001) and experiences genetic isolation when surrounded by large expanses of mature forest (Schrey et al 2011; Tucker et al 2014; Orton et al., 2020). However, patches of early successional habitat and unpaved roads may both act as “stepping-stones” to maintain genetic connectivity (Heath et al., 2012; Kaunert and McBrayer, 2015; Orton et al., 2020). Also, *Sceloporus woodi* have altered DNA methylation patterns as time since fire increases, possibly as a compensatory mechanism for the decreased genetic diversity in isolated long unburned habitats (Tevs et al., in review). In ONF, *S. woodi* experience intersexual and inter-habitat (ESS and LLP) differences in DNA methylation (Tevs et al., unpublished data), possibly driven by differences in thermal opportunity between habitats and in thermal preference between sexes (Neel and McBrayer, 2018; Anderson, 2021). While they reportedly occur in lower densities in MS interiors than ESS interiors (Greenberg et al., 1994), recent work suggests *S. woodi* occupy unpaved roads in MS in equal numbers to ESS and LLP (Tevs et al., unpublished data). Unpaved roads also harbor more lizards than stand interiors of ESS and LLP (Kaunert and McBrayer, 2015). However, previous work reporting variation in other aspects of *S. woodi* biology, such as diet and ectoparasite count, between ESS and LLP did not include MS and did not account for variation among lizards occurring along roads and in stand interiors (Williams and McBrayer, 2015; Orton et al., 2019).

This study seeks to understand how diet, ectoparasite count, and DNA methylation are affected by unpaved roads and management history in ONF, as well as how diet and ectoparasite count affect DNA methylation within *S. woodi*. Several hypotheses are offered regarding expectations for these patterns (Figure 4). 1) Lizards along unpaved roads of ESS, MS, and LLP will display fewer differences in DNA methylation, diet, and ectoparasite count than those within stand interiors. 2) Lizards along roadways will have different DNA methylation patterns, diet, and ectoparasite count than those within stand interiors. 3) Logging and wildfire ESS management will elicit different DNA methylation patterns, diet, and ectoparasite count to individuals. 4) Ectoparasite count and diet will affect DNA methylation patterns among individuals due to the physiological stress these variables impart. Exploring how unpaved roads and management history (ESS vs. MS vs. LLP and burned ESS vs. clearcut ESS) affect DNA methylation will address the underlying molecular mechanisms governing the response of the Florida scrub lizard to anthropogenic habitat modifications. Furthermore, this study will quantify how habitat modifications (i.e., unpaved roads) and anthropogenically modified disturbance patterns generate local environmental pressures on constituent species.

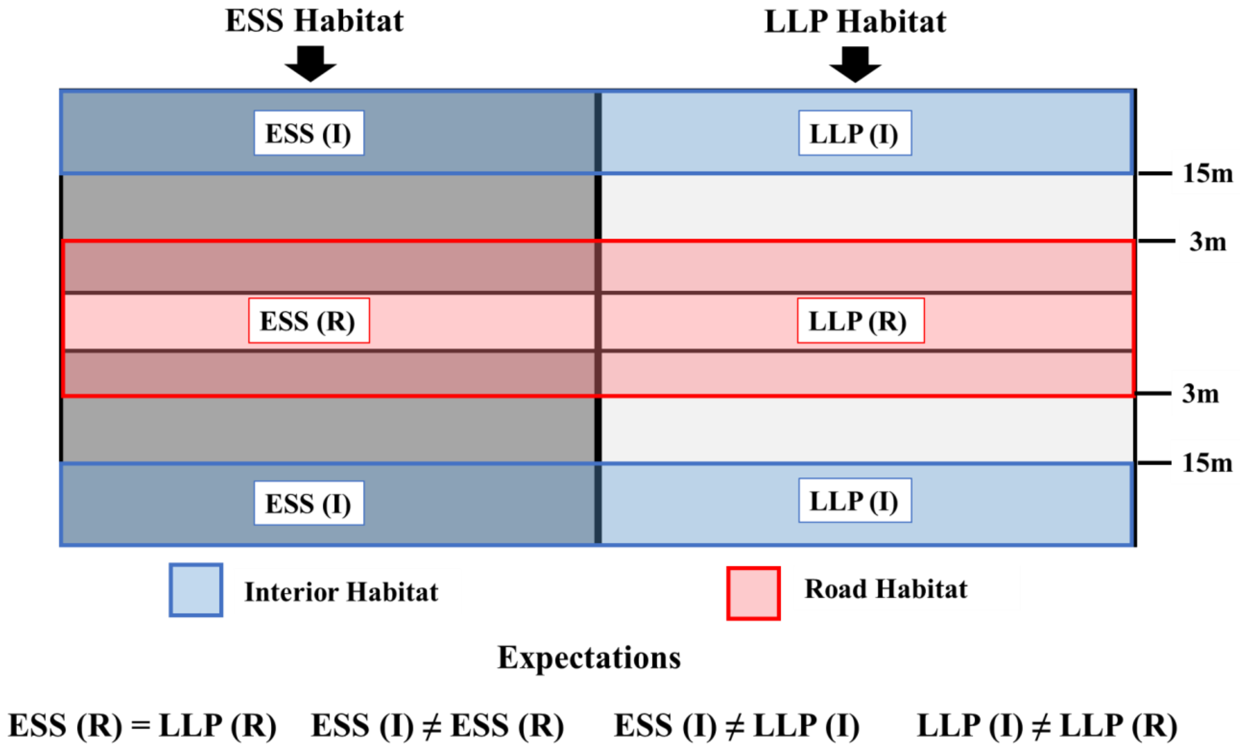

FIGURE 4. Diagram outline hypotheses concerning environmental pressure diversity between unpaved road and interior habitat. Unpaved roads are expected to provide more unform habitat regardless of the surrounding landscape and thus will confer less differences in diet, ectoparasites, and DNA methylation onto lizards between ESS and LLP than the interiors of these same habitats. ESS = Early successional sand pine scrub. LLP = longleaf pine. R = road habitat. I = interior habitat.

Methods

Lizard Sampling

Lizards were collected from June-August of 2021 and 2022 by hand or noose from roads of ESS (burned and clearcut), LLP, and MS, as well as from interiors of ESS and LLP within separate forest stands as delineated by U.S. Forest Service. Stands ≤ 5 years post disturbance are defined as ESS, while stands ≥ 30 years post disturbance are defined as MS. Road habitat included the road surface plus the 3 meters extending into the surrounding habitat. Interior habitat is defined as being ≥ 15m away from the road edge. Only recent and long undisturbed sand pine scrub were targeted because the correlation between DNA methylation and time since disturbance is largely driven by differences between these two disturbance categories (Tevs et al., in review). Exclusively males were targeted to avoid intersexual variation confounding DNA methylation patterns (Tevs et al., unpublished data). Following ectoparasite assessment and stomach flushing, a subset of lizards (n = 81) was anesthetized and euthanized using MS222 (Conroy et al., 2009), and a section of the liver tissue was removed from the same lobe of each individual for DNA methylation screening.

Within six hours of capture, lizards were stomach flushed to quantify diet (n = 190; Legler and Sullivan, 1979), and inspected for ectoparasites (n = 112). All ectoparasites on the entire body of each lizard were counted using a 10x jeweler’s lens. Stomach contents were stored in 70% ethanol until contents could be separated to taxonomic order. Because Hymenopterans were previously reported as the most abundant group in *S. woodi* diets (Williams and McBrayer, 2015), this order was split into four groups: larval forms, winged forms, and two terrestrial morphospecies. As higher taxonomical classification of terrestrial hymenopterans often requires fully intact specimens (Glasier et al., 2013) splitting this group based on morphological variation allowed for capture at least a portion of the variation present. I delineated each morphospecies based on the longest measurement of the head, with measurements < 1mm belonging to morphospecies one and measurement > 1mm belonging to morphospecies two. For each lizard, all prey items belonging to single group were counted and had their total volume measured using liquid displacement. I pooled morphospecies for volumetric assessment because few individuals were fully intact, and the body parts of each morphospecies could not always be reliably distinguished.

DNA Methylation Screening

Variation in DNA methylation among individuals was screened using epiRADseq (Schield et al., 2016). EpiRADseq is a ddRADseq protocol that replaces *MspI* for the methylation sensitive *HpaII* (Mascher et al. 2013; New England Biolabs, Ipswich, MA). *HpaII* fails to cut the target sequence when the restriction site is modified by DNA methylation. DNA methylation levels are assessed by examining read-count variation, with fewer read counts of a fragment equating to more DNA methylation and zero counted fragments indicating 100% DNA methylation at that site. Fragments are then assembled into a pseudo-reference genome. Following restriction digest, Ion Torrent IonXpress double-stranded barcoded adaptors and y-adaptors were ligated. Emulsion PCR was performed following manufacturers protocols of the Ion PGM-Hi-Q-View OT2-200 kit on the Ion Express OneTouch2 platform. Finally, the resultant fragments were sequenced following manufacturers protocols of the Ion PGM-Hi-Q-View Sequencing 200 Kit using an Ion 316v2 BC Chip.

I used Ion Torrent PGM using Torrent Suite version 4.4.3 to perform initial quality control and demultiplex runs. Fragments were trimmed to 50bp for assembly of the pseudo-reference genome using Geneious Prime v. 2021.2.2. Individual fragments were mapped with

BWA Galaxy Version 0.7.17.4 (Li and Durbin, 2009, 2010), and featureCounts Galaxy Version 1.6.4+galaxy1 (Liao et al., 2013) was used to determine read counts of fragments for 50 bp bins.

Analysis of DNA methylation patterns followed two approaches. First, to determine how unpaved roads affect DNA methylation relative to stand interiors, edgeR (Galaxy Version 3.24.1+galaxy1; Robinson et al., 2010; Liu et al., 2015) was used to compare read counts in each 50bp bin among road (ESS, MS, and LLP) and interior (ESS and LLP) habitats, between roads and stand interiors of ESS and LLP, and between management styles of ESS (clearcut and burned) using a FDR of 0.05. Correcting α using FDR reduces the risk of type II error resulting from the large number of tests performed in edgeR. After collapsing significant adjacent bins, it was determined if differences in read counts between bins indicated more or less methylation and significant fragments that were shared among comparisons were identified. Second, edgeR was used to compare read counts among lizards belonging to the first and fourth quartiles for ectoparasite count, dietary volume, and volumetric dietary diversity. Comparing quartiles increased the chance that any detected differentially methylated loci were attributable to diet and ectoparasite count. Only volumetric dietary variables were analyzed because they more accurately represent the nutritional content of the diet.

Statistical Analysis

The first goal was to determine if roads affect diet and ectoparasite count for individuals among habitat categories (ESS, MS, and LLP). First, Ectoparasite count was highly skewed and was square root transformed in all models. Then, separate ANOVAs were used to compare the effects of all roadway habitats, roads and interiors of ESS and LLP, and interiors of ESS and LLP on ectoparasite count, total number of prey items consumed, total volume of prey items consumed, total number of prey groups consumed, and Shannon’s diversity (count and volume).

In each of these ANOVAs the effect of sampling location was included as a random effect nested with the comparison of interest (e.g., sampling location nested with habitat type when comparing all road habitats). Tukey's HSD was then used to further determine variation among all three road habitat types. To determine differences in the proportion of different prey groups in *S. woodi*, the proportion of each prey group relative to the total number and volume of all prey items within each habitat was calculated. The five prey groups within each habitat that comprised the greatest proportion of the total number and volume of prey items were then determined.

The next goal was to quantify how variation in ESS management (burned or clearcut) affects diet and ectoparasite count of individuals among habitats. Replication of lizards in the interiors of burned ESS was lacking. Therefore, road and interior lizards were pooled for these analyses. All dietary variables (total number of prey items consumed, total volume of prey items consumed, total number of prey groups consumed per individual, and Shannon's diversity) and ectoparasite count were compared using ANOVAs with the effect of sampling location nested with management category (burned or clearcut).

Results

Lizards along roads were more similar in diet compared lizards in stand interiors, which were more variable between habitats. Lizards in LLP interiors consumed a greater number of prey items (habitat: $F_{1,31.846} = 15.7195$, $P = 0.0004$; sampling location: $F_{5,30} = 0.0963$, $P = 0.9921$) but had lower dietary diversity by prey counts than those in ESS interiors (habitat: $F_{1,17.531} = 18.2608$, $P = 0.0005$; sampling location: $F_{5,30} = 0.2757$, $P = 0.9228$). However, lizards in the interior of both habitats consumed a similar volume of prey items (habitat: $F_{1,7.2772} =$

0.3149, $P = 0.5916$; sampling location: $F_{5,30} = 1.3490$, $P = 0.2712$), a similar number of prey groups per individual (habitat: $F_{1,13.092} = 2.3336$, $P = 0.1504$; sampling location: $F_{5,30} = 0.4189$, $P = 0.8318$), and had similar volumetric dietary diversity (habitat: $F_{1,8.567} = 1.0354$, $P = 0.3368$; sampling location: $F_{5,30} = 0.8887$, $P = 0.5009$). However, none of these dietary metrics differed among unpaved roads among all three habitats (ESS, MS, and LLP; Appendix D).

The diets of lizards along the road compared to those within stand interiors differed within ESS but not LLP (Table 6). Lizards in ESS interiors consumed a greater number of prey groups per individual (road vs. interior: $F_{1,13.374} = 6.1760$, $P = 0.0269$; sampling location: $F_{10,58} = 0.6664$, $P = 0.7504$) and had greater counted (road vs. interior: $F_{1,13.031} = 7.2894$, $P = 0.0182$; sampling location: $F_{10,58} = 0.7380$, $P = 0.6859$) and volumetric (road vs. interior: $F_{1,13.539} = 6.0623$, $P = 0.0279$; sampling location: $F_{10,58} = 0.6369$, $P = 0.7726$) dietary diversity than those along ESS roads. However, lizards along the road and interior of ESS consumed a similar number (road vs. interior: $F_{1,16.521} = 0.0060$, $P = 0.9394$; sampling location: $F_{10,58} = 0.3592$, $P = 0.9591$) and volume (road vs. interior: $F_{1,11.205} = 2.3555$, $P = 0.1526$; sampling location: $F_{10,58} = 1.8007$, $P = 0.0808$) of prey items. However, none of these dietary metrics differed between lizards inhabiting unpaved roads and stand interiors of LLP (Appendix D). Unpaved roads appear to alter the principal prey groups between unpaved road and interior habitat of ESS and LLP. The volume of only two prey groups were equally important among all road and interior habitats (adult Coleopterans and Hymenoptera formicidae morphospecies 1). The number of two prey groups were equally important to all road and interior habitats (adult Coleopterans and Hymenoptera formicidae; Table 7).

Ectoparasite count differed for individuals among habitats along the road surface but not within stand interiors. Road adjacent habitat (ESS, MS, or LLP) affected ectoparasite counts on

S. woodi (habitat: $F_{2, 12.269}$ = 4.4396, P = 0.0354; sampling location: $F_{11,64}$ = 3.2440, P = 0.0014; Figure 5), with lizards along MS roads harboring more ectoparasites than those along LLP roads (P = 0.0359), but no difference was detected in ectoparasite count between MS and ESS roads (P = 0.8042) nor between ESS and LLP roads (P = 0.0707). Also, no differences were detected in ectoparasite count between roads and interiors in ESS (road vs. interior: $F_{1,6.0877}$ = 0.32687, P = 0.1199; sampling location: $F_{6,39}$ = 2.9161, P = 0.0190) or LLP (road vs interior: $F_{1,6.931}$ = 3.2687, P = 0.6560, P = 0.4449; sampling location: $F_{6,35}$ = 2.9102, P = 0.0208), or between interiors of ESS and LLP (habitat: $F_{1,4.0131}$ = 0.2.8934, P = 0.1639; sampling location: $F_{4,28}$ = 2.4021, P = 0.0736).

Management of SPS affected *S. woodi* diet but not ectoparasite count. Lizards in clearcut ESS consumed a greater number of prey groups per individual (management: $F_{1,24.991}$ = 0.4.5727, P = 0.0424; sampling location: $F_{7,61}$ = 0.9532, P = 0.4732) and had greater dietary diversity by prey counts (management: $F_{1,21.581}$ = 5.2728, P = 0.0318; sampling location: $F_{7,61}$ = 1.1512, P = 0.3440) than those in burned ESS. However, lizards in burned and clearcut ESS did not differ in total number (management: $F_{1,51.218}$ = 0.6020, P = 0.4414; sampling location: $F_{7,61}$ = 0.3627, P = 0.9204) or volume (management: $F_{1,15.593}$ = 2.0723, P = 0.1698; sampling location: $F_{7,61}$ = 1.8390, P = 0.0959) of prey items consumed, volumetric dietary diversity (management: $F_{1,37.498}$ = 3.7417, P = 0.0606; sampling location: $F_{7,61}$ = 0.5732, P = 0.7749), or ectoparasite count (management: $F_{1,3.1802}$ = 2.3614, P = 0.2169; sampling location: $F_{3,42}$ = 3.1741, P = 0.0338).

DNA methylation patterns were more similar among lizards occupying unpaved roads compared to those occupying forest interiors of the same habitat type (Table 8). Lizards on roads had fewer significant differences in DNA methylation compared to lizards from habitat interiors. Lizards within ESS interiors differed from LLP interiors at 296 loci (296 hypermethylated and 0 hypomethylated), 64 of which were shared with other comparisons (Figure 6a). Lizards along ESS roads differed from those along LLP roads at 95 loci (92 hypermethylated and 3 hypomethylated). Lizards along ESS roads differed from those within ESS interiors at 115 loci (103 hypermethylated and 12 hypomethylated). Lizards along LLP roads differed from those within LLP interiors at 80 loci (78 hypermethylated and 2 hypomethylated).

Lizards along the road in MS and LLP are more similar in DNA methylation than any other road habitat comparison (Table 8). Lizards along MS roads differed from those along LLP roads at 41 loci (4 hypermethylated and 37 hypomethylated) and from those along ESS roads at 323 loci (12 hypermethylated and 311 hypomethylated). The comparison between MS and ESS roads also shared 8 loci with the comparison between ESS and LLP roads, and 32 loci with the comparison between MS and LLP roads (Figure 6b). Scrub management also appears to affect DNA methylation in *S. woodi*, with 97 differentially methylated loci detected between burned ESS compared to clearcut ESS (55 hypermethylated and 42 hypomethylated).

Both diet and ectoparasite exposure are associated with changes in *S. woodi* DNA methylation (Table 8). Lizards with low dietary volume (i.e., first quartile) differed at 38 loci (10 hypermethylated and 28 hypomethylated) from those with high dietary volume (i.e., fourth quartile). Lizards with low dietary diversity differed at 12 loci (2 hypermethylated and 10 hypomethylated) from those with high dietary diversity. Lizards with low ectoparasite counts differed at 33 loci (7 hypermethylated and 26 hypomethylated) from those with high ectoparasite counts.

TABLE 6. Mean (± S.D.) of total volume (mL) of prey items consumed, total number of prey items consumed, total number of prey groups consumed per individual, Shannon's diversity (count and volume), and ectoparasite count between roads and stand interiors of each habitat type and between management styles of early successional sand pine scrub. ESS = Early successional sand pine scrub. MS = Mature sand pine scrub. LLP = longleaf pine. Roads and interiors are pooled in burned and clearcut ESS.

		Total volume	**Total number**	**Prey groups/individual**	**S. Diversity (count)**	**S. Diversity (volume)**	**Ectoparasite count**
ESS							
	Road	0.13 ± 0.08	7.29 ± 12.04	2.80 ± 1.43	0.73 ± 0.51	0.80 ± 0.51	93.31 ± 42.72
	Interior	0.10 ± 0.11	5.95 ± 3.31	3.68 ± 1.38	1.10 ± 0.43	1.07 ± 0.36	60.06 ± 53.05
	Burned	0.14 ± 0.10	6.25 ± 8.57	2.79 ± 1.45	0.74 ± 0.54	0.81 ± 0.53	104.12 ± 44.30
	Clearcut	0.10 ± 0.05	5.76 ± 4.78	3.15 ± 1.41	0.90 ± 0.50	0.92 ± 0.46	72.87 ± 54.90
MS							
	Road	0.16 ± 0.14	11.00 ± 10.57	3.45 ± 1.36	0.87 ± 0.49	0.99 ± 0.46	107.48 ± 41.44
LLP							
	Road	0.15 ± 0.12	11.97 ± 15.95	2.95 ± 1.40	0.70 ± 0.47	0.84 ± 0.45	38.00 ± 35.42
	Interior	0.10 ± 0.06	19.61 ± 23.65	3.11 ± 1.32	0.67 ± 0.42	0.93 ± 0.50	28.53 ± 16.96

TABLE 7. Proportion of the top 5 prey items comprising the largest portion of the total volume (top) and number (bottom) of all prey items consumed in each habitat. Italicized values indicate that prey group was not part of the top five prey groups consumed in that habitat. ESS = Early successional sand pine scrub. MS = Mature sand pine scrub. LLP = longleaf pine. R = road habitat. I = interior habitat. B = Burned ESS. C = Clearcut ESS. M1 and M2 = Morphospecies 1 and 2. Roads and interiors are pooled in burned and clearcut ESS.

	ESS (R)	**ESS (I)**	**ESS (B)**	**ESS (C)**	**LLP (R)**	**LLP (I)**	**MS (R)**
Volume							
Araneae	*0.076*	0.102	0.100	0.075	0.149	0.041	0.173
Coleoptera larvae	0.096	0.133	*0.087*	0.133	0.122	0.082	*0.056*
Coleoptera adult	0.186	0.077	0.179	0.163	0.156	0.246	0.203
Hymenoptera formicidae	0.169	0.117	0.124	0.206	0.210	0.222	0.132
Lepidoptera larvae	0.087	*0.061*	0.105	*0.068*	0.125	*0.029*	0.062
Orthoptera	0.217	0.128	0.182	0.233	*0.108*	0.181	0.224
Count							
Araneae	0.048	0.088	0.063	0.055	0.035	*0.014*	0.066
Coleoptera larvae	*0.030*	0.088	*0.046*	*0.042*	0.024	*0.006*	*0.011*
Coleoptera adult	0.099	0.114	0.114	0.096	0.063	0.045	0.068
Hymenoptera larvae	*0.000*	*0.000*	*0.000*	*0.000*	*0.001*	0.068	*0.005*
Hymenoptera formicidae (M1)	0.637	0.386	0.549	0.595	0.739	0.776	0.648
Hymenoptera formicidae (M2)	0.054	*0.079*	0.057	0.0611	0.063	0.025	0.052
Orthoptera	0.054	0.105	0.057	0.071	0.024	0.028	0.050

TABLE 8. Total number of hypermethylated and hypomethylated loci among all habitat comparisons and between comparisons of lizards with the first and fourth quartile of ectoparasite count, dietary volume, and volumetric dietary diversity. The number of hypermethylated and hypomethylated loci describe differences in the first habitat type listed in each comparison relative to the second. All hypermethylated and hypomethylated associated with phenotype comparison describe differences in the first relative to the fourth quartile. LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub. R= Road habitat. I = Forest interior habitat

	Number of hypermethylated loci	**Number of hypomethylated loci**	**Total**
Habitat comparisons			
ESS (R) vs. ESS (I)	103	12	115
ESS (R) vs. LLP (R)	92	3	95
LLP (R) vs. LLP (I)	78	2	80
ESS (I) vs. LLP (I)	296	0	296
MS (R) vs. ESS (R)	12	311	315
MS (R) vs. LLP (R)	4	37	41
ESS (B) vs. ESS (C)	55	42	97
Phenotype comparisons			
Ectoparasite count	7	26	33
Dietary volume	10	28	38
Volumetric diversity	2	10	12

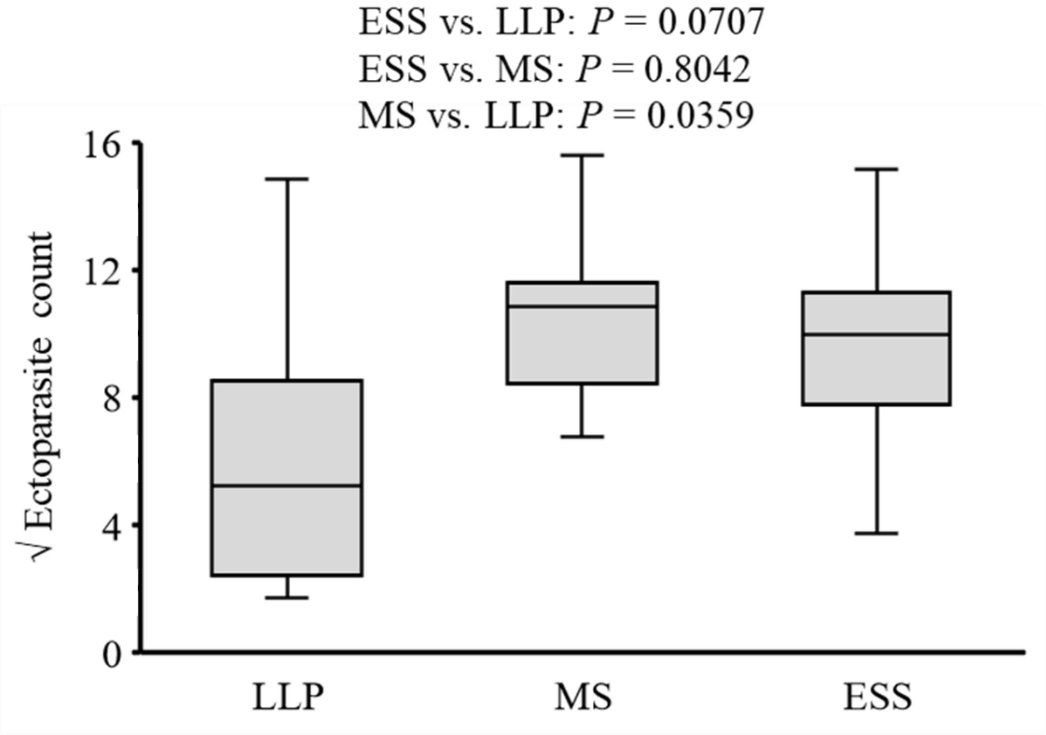

FIGURE 5. Comparison of square root transformed ectoparasite count between all road habitats. Boxes indicate interquartile range, central lines indicate median value, and whiskers indicate maximum and minimum values. ESS = Early successional sand pine scrub. MS = Mature sand pine scrub. LLP = longleaf pine.

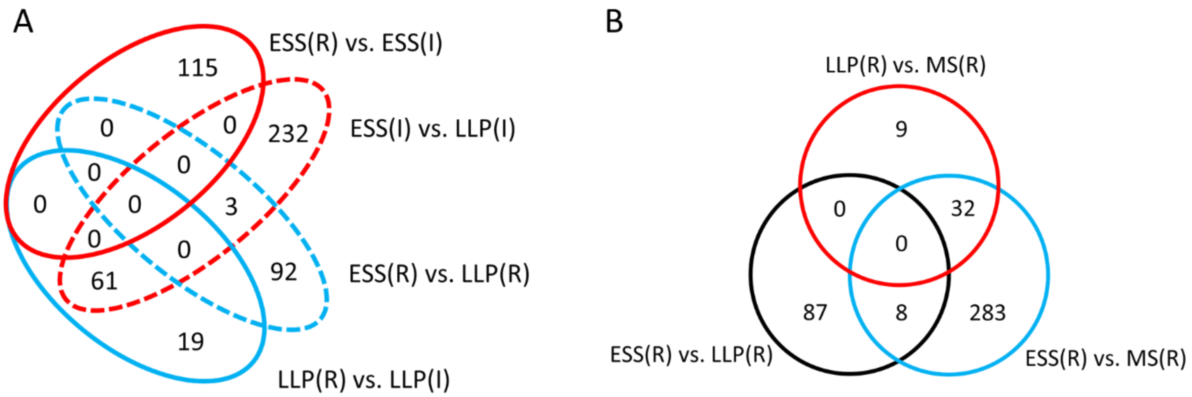

FIGURE 6. Shared and unique differentially methylated loci between A) all possible comparison of ESS roads, ESS Interiors, LLP roads, and LLP interiors and B) All three roads habitat types. Overlapping sections indicate the number of differentially methylated loci that were shared between a set of comparisons. ESS = Early successional sand pine scrub. MS = Mature sand pine scrub. LLP = longleaf pine. R = road habitat. I = interior habitat.

Discussion

Variation in lizard diets between roads and interiors and between clearcut and burned ESS anthropogenic habitat modifications (i.e., unpaved roads) can alter *S. woodi* diet. Lizards along unpaved roads did not differ in any dietary metrics (Table 6 and Appendix D), possibly due to the lack of vegetative cover or debris to increase the conspicuousness of small road traversing invertebrates. This increased exposure results in nearly double the consumption of small terrestrial hymenopterans along ESS roads compared to interiors. Whereas lizards within stand interiors consume a greater number of larger, less conspicuous prey (e.g., Aranids and larval Coleopterans; Table 2). By reducing the conspicuousness of small prey items, unpaved roads enable *S. woodi* to consume a more consistent diet regardless of the adjacent habitat (Table 1). Lizards in ESS and LLP interiors also appear to differ in prey size, as indicated by increased number of prey items per individual in LLP compared to ESS, yet similar total prey volume between habitats. This suggests, lizards in LLP interiors may expend more energy towards foraging a greater number of smaller prey items than their ESS interior counterparts. Tradeoffs associated with prey size and number can select for different predator phenotypes (Dodrill et al., 2016, Thoral et al., 2021), suggesting unpaved roads and anthropogenic disturbance patterns may affect selective pressures acting on *S. woodi*.

Unpaved roads drive more uniform ectoparasite infection rates in *S. woodi*. Given that mite abundance increases with time since disturbance (Goud, 2017), lizards within MS were expected to harbor more ectoparasites than those in ESS, yet ectoparasite count did not differ between ESS and MS roads (Figure 5). Previous reports that failed to account for variation between roads and stand interiors also indicated increased ectoparasite counts in ESS compared to LLP (Williams and McBrayer, 2015; Orton et al., 2019). However, when accounting for unpaved roads, ectoparasite counts did not differ between these two habitats (Figure 5). While ectoparasite count between roads and interiors in either habitat did not differ, these effects may be confounded by significant variation due sampling location. Future works exploring the effects of unpaved roadways on ectoparasite counts may therefore benefit from sampling more locations.

Differences in ectoparasite counts between ESS and LLP have been attributed to the presence of arboreal perches in LLP allowing lizards to reduce potential contact with ectoparasites residing in leaf litter (Orton et al., 2019). Mature trees are also present in MS, suggesting these habitats would offer similar refuge from parasites. Yet lizards in MS harbored more ectoparasites than those in LLP. While *S. woodi* substrate associations in MS were not quantitatively assessed, lizards in these habitats appear to use arboreal perches less than those in LLP (Tevs and Brennan, personal observation), suggesting other threats exist in MS that discourage arboreal perching. Records exist of spider predation on juvenile *S. woodi* (Nyffeler et al., 2021), suggesting the positive relationship between spider abundance and time since disturbance (Goud, 2017) may discourage arboreal perching in MS, thereby increasing ectoparasite encounter rates along the forest floor. Similar results have been observed in green frog (*Lithobates clamitans)* tadpoles showing increased predator exposure coincides with more time spent in parasite dense refugia (Thiemman et al., 2000), suggesting *S. woodi* in MS may experience similar trade-offs between parasite infection on the forest floor and predation on exposed tree trunks.

Patterns of differentially methylated loci between unpaved roads and stand interiors suggest environmental pressures along roads in ESS and LLP are more similar than those in the interiors of these same stands (Table 8). The nearly 3x increase in differentially methylated loci associated with ESS and LLP interiors compared to roads indicates regulation of more genomic regions is necessary to mediate the different environmental pressures associated with the interior of each habitat. These trends are likely a consequence of near uniform habitat conditions along the unpaved road surface and on the habitat immediately adjacent to the road (Tevs et al., unpublished data). Unpaved roads are regularly cleared of debris and vegetation to facilitate safe vehicular passage (Gesford and Anderson, 2007), which creates large expanses of open sand along the road surface. In doing so, unpaved roads appear to create more ideal habitat conditions for *S. woodi* than forest interiors. This is supported by increased *S. woodi* occurrence rates along unpaved roads in ESS and LLP relative to stand interiors (Kaunert and McBrayer, 2015) and by equal relative abundance along roads bordered by all possible combinations of ESS, MS, and LLP (Tevs et al., unpublished data). However, multiple differentially methylated loci were detected between all road habitat types, suggesting these features do not provide perfectly uniform habitat.

In addition to the effects of diet and ectoparasites, variation in the number of differentially methylated loci between road habitat types, stand interiors, and ESS management styles may also be attributable to differences in thermal environment. The relationship between altered thermal environment and DNA methylation in ectotherms is well documented (Metzger and Schlute, 2017; Ragsdale et al., 2020; Liu et al., 2022). Longleaf pine stands offer cooler temperatures and greater thermal opportunity than ESS (Neel and McBrayer 2018; Anderson, 2021), likely due to the intact canopy cover and availability of arboreal perches that elevate lizards off the warm forest floor. While no study has explored *S. woodi* thermal opportunity in MS, thermal opportunity along road surfaces in MS may be comparable to LLP due to intact canopy in these habitats. This would explain the smaller number of differentially methylated loci between MS and LLP roads compared to any other road habitat combination (Table 3).

Multiple differentially methylated loci shared between several habitat comparisons (Figure 6a, b) suggests responding to environmental pressures associated with different habitats in ONF requires regulation of many of the same genomic regions. Nearly 75% of the differentially methylated loci detected between LLP roads and interiors were also shared with the comparison between LLP and ESS interiors. Canopy gaps created by unpaved roads can increase the temperature along the road relative to stand interiors (Delgado et al, 2007b), possibly resulting in a more similar thermal environment for lizards occupying LLP roads and ESS interiors. The comparison of LLP roads to MS roads also shared approximately 75% differentially methylated loci with the comparison between ESS roads and MS roads. Aside from altered thermal environment, these shared loci associated with MS roads may be a product of altered predation rates. Mature sand pine scrub offers increased vegetative cover (Greenberg et al., 1994,1995), which can reduce predation in risk in lizards (Pietrek et al., 2009). Differences in predation rates may also contribute to the 97 differentially methylated loci detected between clearcut and burned ESS. Increased availability of woody debris in burned ESS (Greenberg et al., 1994,1995) may increase the availability of adjacent cover needed to survive potential predation events. In cane toads (*Rhinella marina*), transgenerational effects of altered predator cues on the epigenetic characteristics of parents can result in different offspring DNA methylation signatures and phenotypes (Sarma et al., 2021), possibly suggesting similar effects among *S. woodi* populations with different predation rates (e.g., ESS and LLP; Orton et al., 2018).

A portion of the variation in DNA methylation between habitat types may also be a product of differences in ectoparasite exposure and dietary composition. Multiple differentially methylated loci were detected between lizards with low (first quartile) and high (fourth quartile) ectoparasite count, dietary volume, and volumetric dietary diversity (Table 3). Similar associations between dietary composition have been reported in yellow baboons (*Papio cyncocephalus*) belonging to separate populations with different forage bases. DNA methylation analyses revealed multiple differentially methylated genomic locations between these *P. cyncocephalus* populations, many of which were proximal to metabolism related genes (Lea et al., 2016). Similarly, Trinidad guppies (*Poecilia reticulata*) at different stages of parasite infection differed in DNA methylation at multiple genomic regions, many of which were proximal to immune response genes (Hu et al., 2018). By providing a mechanism of response to environmental pressures at the molecular level, DNA methylation likely facilitates occupation of different habitat types by *S. woodi*.

Mediation of environmental pressures associated with unpaved roads through altered patterns of DNA methylation likely enhances *S. woodi* connectivity in the fragmented ONF landscape. In the rosemary scrub habitats of Archbold Biological Station in Highlands County Florida, *S. woodi* hailing from long-undisturbed patches of Florida scrub show reduced genetic diversity and increased inbreeding (Schrey et al., 2011; Miller et al., in review). While no study has explored *S. woodi* genetic structure in long undisturbed stands of MS, roads bisecting stands of MS have long been speculated to enhance connectivity in the fragment ONF landscape (Greenberg et al., 1994; Tiebout and Anderson 1997, 2001). However, aside from demographic surveys (Kaunert and McBrayer, 2015; Tevs et al., unpublished data) and one study examining genetic structure (Orton et al., 2020), little evidence has been brough to bear on this speculation.

Altered patterns of DNA methylation and the presence of unpaved roadways likely contribute to *S. woodi* occupation of MS and, consequently, facilitate occupation of and/or dispersal through these otherwise inhospitable habitats. By mediating altered environmental pressures associated with MS habitat, altered DNA methylation patterns may allow *S. woodi* to use MS roads as corridors to enhance gene flow in the larger fragmented landscape.

In conclusion, these data indicate unpaved roads create consistent environmental conditions for small lizards regardless of the surrounding habitat. Lizards within interiors of ESS and LLP differed in multiple dietary metrics, whereas lizards along unpaved roads differed in none. Ectoparasite infection rates also appear to be affected by unpaved roads, but additional work including more sampling locations is needed to affirm this conclusion. Additional work is also needed to understand potential trade-offs between ectoparasite exposure and predation rates on exposed tree trunks in MS. Further differences in the number of differentially methylated loci between unpaved roads and forest interiors indicate road edge habitat can impact multiple aspects species biology between individuals separated by as little as 15m. Finally, these data also lend evidence to the previous speculation that unpaved roadways act as alternative habitat and possible dispersal corridors for early successional lizards in fragmented habitats and are, therefore, of conservation value.

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APPENDIX A

Site name, survey start/stop coordinates, and heading of each visual encounter survey. Verge habitat either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub. CS = cut scrub. BS = burned scrub.

Habitat	Site	start coordinates	stop coordinates	Heading
ESS/ESS	CS234	29.296284, -81.824494	29.290696, -81.824264	N/S
	BS626	29.120640, -81.798046	29.117929, -81.796931	SE/NW
	BS429	29.159486, -81.756891	29.157672, -81.762557	E/W
	CS534	29.121130, -81.821460	29.126670, -81.821370	N/S
	BS513	29.175790, -81.772500	29.170570, -81.772510	N/S
	BS404	29.183240, -81.826780	29.182820, -81.821370	E/W
	CS604	29.130920, -81.756200	29.130530, -81.760990	E/W
	CS182	29.333450, -81.820760	29.328090, -81.822410	NE/SW
ESS/MS	CS394	29.209630, -81.807070	29.211152, -81.805260	E/W
	CS196	29.182871, -81.819628	29.184098, -81.815689	E/W
	BS340	29.184561, -81.725330	29.185117, -81.722166	E/W
	BS292	29.253398, -81.766849	29.251926, -81.762024	SE/NW
	CS234	29.293652, -81.830159	29.296640, -81.827391	NE/SW
	CS181	29.340343, -81.848643	29.339615, -81.843094	E/W
	CS586/588	29.091900, -81.682960	29.091760, -81.675580	E/W
	CS153	29.368120, -81.832950	29.374110, -81.832950	N/S
LLP/ESS	Hughes	29.250783, -81.759410	29.246847, -81.756995	SE/NW
	Salt Springs	29.305999, -81.772662	29.302463, -81.777397	NE/SW
	Norwalk	29.432087, -81.739103	29.431706, -81.746066	E/W
	Salt Springs	29.308180, -81.809270	29.302730, -81.809270	N/S
LLP/LLP	Hughes	29.250621, -81.757544	29.250387, -81.753043	E/W
	Kerr	29.362505, -81.822932	29.362260, -81.828922	E/W
	Kerr	29.362038, -81.832683	29.362415, -81.826567	E/W
	Norwalk	29.409296, -81.737570	29.405102, -81.742846	NE/SW
	Salt Springs (east)	29.317280, -81.749820	29.323830, -81.748650	N/S
	Syracuse Island	29.380130, -81.707470	29.380160, -81.700050	E/W
	Alexander Springs	29.055250, -81.567330	29.057140, -81.572460	E/W
	FR66	29.457650, -81.809850	29.457590, -81.815960	E/W
LLP/MS	Salt Springs	29.336718, -81.793748	29.329384, -81.799410	E/W
	Hughes	29.250900, -81.739600	29.257200, -81.740100	N/S
	Kerr	29.346268, -81.818799	29.345079, -81.825745	NE/SW
	Norwalk	29.400984, -81.742562	29.400830, -81.749156	E/W
	Alexander Springs	29.051960, -81.609730	29.057830, -81.611290	N/S
	Kerr2	29.471170, -81.828260	29.467230, -81.832150	N/S

APPENDIX A: Continued

	Syracuse Island	29.400510, -81.709490	29.397430, -81.714140	N/S
	Kerr2	29.399830, -81.811470	29.403330, -81.816670	NW/SE
MS/MS	Rt394	29.209244, -81.824051	29.209401, -81.816317	E/W
	MS626	29.121772, -81.788483	29.121839, -81.782365	E/W
	MS234	29.296800, -81.824100	29.296300, -81.817800	E/W
	MS494	29.142520, -81.713910	29.148420, -81.715840	N/S
	FR305	29.423930, -81.825810	29.418100, -81.825390	N/S
	FR65	29.076260, -81.709140	29.076020, -81.715290	E/W
	FR68	29.283110, -81.758710	29.289390, -81.758920	N/S
	MS69	29.428800, -81.880700	29.437000, -81.880550	N/S

APPENDIX B

Comparisons of survey proportions where *S. woodi* (SW) and *A. sexlinetus* (AS) were present for all pairwise verge type combinations. Bold indicates significance at α = 0.05 without Bonferroni correct, * indicates significance after adjusting α for multiple comparisons (n = 5). Verge habitat either side of the road are separated by a "/". LLP = longleaf pine. ESS = early successional scrub. MS = mature scrub.

Verge type	**ESS/MS**	**MS/MS**	**LLP/LLP**	**LLP/ESS**	**LLP/MS**
ESS/ESS	SW: G = 0.292, P = 0.5887 **AS: G = 4.857, P = 0.0275**	**SW: G = 4.857, P = 0.0275** AS: G = 1.453, P = 0.2280	SW: G = 0, P = 1.000 **AS: G = 4.857, P = 0.0275**	SW: G = 0.193 P = 0.6607 **AS: G = 5.268, P = 0.0217**	SW: G = 0.255, P = 0.6138 AS: G = 1.453, P = 0.2280
ESS/MS	-	SW: G = 3.059, P = 0.0803 AS: G = 1.381, P = 0.2399	SW: G = 0.292, P = 0.5887 AS: G = 0, P = 1.000	SW: G = 0, P = 1.000 AS: G = 0.170, P = 0.6797	SW: G = 1.082, P = 0.2982 AS: G = 1.381, P = 0.2399
MS/MS	-	-	**SW: G = 4.857, P = 0.0275** AS: G = 1.381, P = 0.2399	SW: G = 2.385 P = 0.1225 AS: G = 1.923, P = 0.1656	**SW: G = 6.904, P = 0.0086*** AS: G = 1.381, P = 0.2399
LLP/LLP	-	-	-	SW: G = 0.193, P = 0.6607 AS: G = 0.170, P = 0.6797	SW: G = 0.255, P = 0.6138 AS: G = 1.381, P = 0.2399

APPENDIX B: Continued

LLP/ESS	-	-	-	-	SW: $G = 0.712$, $P = 0.3989$ AS: $G = 1.381$, $P = 0.2399$

APPENDIX C

Results of Scheirer-Ray-Hare tests analyzing the effect of verge type and the random effect of sampling location (site) nested within verge type on the temperature of each substrate/light combination. We detected no significant effects for verge or site for the temperature of any substrate/light combination.

Substrate & light combination	**Effect**	**Degrees of freedom**	**H**
Sand sun	Verge	5	8.53
	Site [verge type]	23	20.59
Sand shade	Verge	5	5.44
	Site [verge type]	20	19.88
Sand dappled	Verge	5	3.68
	Site [verge type]	23	23.78
Litter sun	Verge	5	4.29
	Site [verge type]	33	31.60
Litter shade	Verge	5	7.18
	Site [verge type]	35	32.09
Litter dappled	Verge	5	6.07
	Site [verge type]	35	32.58

APPENDIX D

Results from nested ANOVAs testing the effects of road habitat types and roads vs. interiors of LLP on dietary metrics. Both models include the random effect of sampling location nested within the predictor of interest. Bold values are significant at $\alpha = 0.05$. LLP = longleaf pine.

	All road habitats		**LLP road vs. interior**	
	Habitat	**Sampling Location**	**Road vs. Interior**	**Sampling Location**
Total volume	$F_{2,45.430} = 0.3698$, $P = 0.6929$	$F_{19,131} = 1.7378$, **$P = 0.0374$**	$F_{1,18.78} = 0.0162$, $P = 0.9002$	$F_{8,70} = 2.5524$, **$P = 0.0168$**
Total number	$F_{2,49.239} = 1.9038$, $P = 0.1598$	$F_{19,131} = 1.5382$, $P = 0.0826$	$F_{1,24.296} = 0.8792$, $P = 0.3576$	$F_{8,70} = 1.7706$, $P = 0.0977$
Number of prey groups	$F_{2,68.508} = 1.6326$, $P = 0.209$	$F_{19,131} = 0.9729$, $P = 0.4967$	$F_{1,70.476} = 0.0032$, $P = 0.9551$	$F_{8,70} = 0.3607$, $P = 0.9376$
Diversity (volume)	$F_{2,87.22} = 2.1139$, $P = 0.1269$	$F_{19,131} = 0.7020$, $P = 0.8115$	$F_{1,74.818} = 0.0723$, $P = 0.7887$	$F_{8,70} = 0.2824$, $P = 0.9697$
Diversity (count)	$F_{2,57.796} = 0.3074$, $P = 0.7365$	$F_{19,131} = 1.2245$, $P = 0.2477$	$F_{1,72.748} = 0.8746$, $P = 0.3528$	$F_{8,70} = 0.3221$, $P = 0.9550$