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Body Size Variation in Two Adjacent Populations of Black Kingsnakes, *Lampropeltis nigra*, in East Tennessee

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Abstract

Intraspecific phenotypic variation of body size is often an observable phenomenon when comparing populations of snakes, but this type of divergence is typically associated with physical or environmental barriers separating the populations. However, even proximal populations separated by reasonably surmountable barriers have been shown to exhibit phenotypic variation, but such cases are rarely reported and often under-studied. This study reveals phenotypic variation in body size of black kingsnakes (*Lampropeltis nigra*) on a geographic scale smaller than previously reported for the species. We identify populations of *L. nigra* located in two adjacent habitats in East Tennessee that show difference in length (SVL; cm), regardless of sex. *L. nigra* in one habitat ranged from 33.5 to 87.1 cm with a mean of 58.57 cm SVL (\(n = 37\)), while those in the other habitat ranged from 29.1 cm to 123.9 cm with a mean of 79.25 cm SVL (\(n = 22\)), and the results were significant (F Ratio = 16.0409, Prob>F = 0.0002). We consider possible mechanisms behind this observable divergence and propose options for further research at this site.
**Introduction**

Intraspecific phenotypic variation between populations is known to occur due to either plastic response to environmental conditions or genetic canalization over time (Ghalambor, et al. 2007). This phenomenon is often studied between distinct populations separated by geographic or environmental barriers that greatly impede mixing of gene pools, or between distinct habitats across an environmental gradient (Bronikowski and Arnold 1999; Weatherhead, et al. 2011). However, phenotypic variation may also be observed among populations or subpopulations with seemingly trivial environmental differences separating them (e.g. Bronikowski 2000; Shine, et al. 2012). This is especially true in the case of some snake species, individuals of which may remain confined to relatively small ranges and rarely move between proximal populations (e.g. Russell and Hanlin 1999; Shine, et al. 2012), though these populations may exhibit divergence in one or more phenotypic traits. One particular trait that often varies between populations is body size (Madsen and Shine 1993). Although observable differences in body size can be attributable to age structure and survival rates (i.e. snakes in one population tend to live longer and therefore grow larger than another population) (King 1989), phenotypic divergence can also occur because of either genetic variation or phenotypically plastic responses to food availability and other environmental factors (Madsen and Shine 1993, Queral-Regil and King 1998).

The most common measurement of snake body size is snout-to-vent length (SVL), favored because it increases continuously throughout a snake’s lifetime and is not prone to fluctuations based on season, reproductive status, or feeding conditions, as is measurement of mass (Feldman and Meiri 2013). For these very reasons, however, mass can be regarded as an indicator of physiological condition when standardized with SVL. Winne, et al. 2007 proposed a body condition index (BCI) for kingsnakes (Lampropeltis getula) that may be an indicator of habitat suitability and overall population health. For this study, we consider SVL as a standard measurement of body size, but we also consider BCI in order to infer possible environmental influence on any observable divergence in body size. To consider difference in age structure, we also calculate growth rates based on SVL measurements of recaptured individuals.

We conducted a long-term mark-recapture study on two adjacent populations of black kingsnakes (Lampropeltis nigra Yarrow) located on parallel ridges in East Tennessee. By surveying snakes over a 6-year period, we identify difference in body size of L. nigra between the two habitats.

**Methods**

**Study Area and Sampling**

Research was conducted at The University of Tennessee Forestry Experimentation Station (FES), a 915 ha site in Anderson County, Tennessee (35°60’ N, 84°13’ W). The forest is primarily mixed pine-hardwood, fragmented by mowed fields, logged areas, and utility right-of-ways. The study area spans two distinct ridges that run southwest to northeast. These are Pine Ridge to the north and Chestnut Ridge to the south. They are located less than 0.5 km apart and are both approximately 355 m maximum elevation. A two-lane paved road, Union Valley Road, runs the length of the valley between the ridges. This road receives relatively low traffic flows, primarily daytime travel of dump trucks to and from a quarry located in the valley, east of the study site. Based on researcher observation, no other physical barriers exist on either ridge that impedes snake movement between habitats.

Snake populations were surveyed using coverboards placed on woodland-field
ecotones throughout the site. Coverboards were either wood or metal, organized in stations consisting of one board of each material placed less than 5m apart. Coverboard arrays were established in 11 fields or right-of-ways. The number of stations in each array varied based on the length of the ecotone surveyed. A total of 137 stations were used, each containing one wood and one metal object. Two arrays were located on Pine Ridge and 9 on Chestnut Ridge.

Coverboard surveys were conducted regularly from May to August, with less frequent surveying in March, April, September, and October. Surveying took place from March 1997 to October 2012 on Chestnut Ridge, and June 2006 to October 2012 on Pine Ridge. Coverboards were surveyed by 30-second visual search of the substrate beneath the board.

Upon capture, *L. nigra* were collected and measured for snout-to-vent length (SVL; cm) and mass (g). Sex was determined by probing and gravid status by palpation. Individuals were identified with passive integrated transponder (PIT) tags injected beneath the dermal layers (Gibbons and Andrews 2004). Snakes longer than 30 cm SVL were generally PIT-tagged upon first capture. Smaller snakes, with the exception of one 29.1 cm individual, did not receive PIT-tags, so could not necessarily be identified as recaptures if encountered again; therefore, all smaller snakes were excluded from analyses.

Analysis

Because surveying began on Pine Ridge in June 2006, only captures from that month and later were considered from either ridge when analyzing body size, keeping time frames consistent between the two habitats. To avoid pseudo-replication, only the first capture of each individual was used in analysis of body size. All data were tested for normality using a Shapiro-Wilk Test, and the appropriate analysis was conducted.

For analysis of SVL, all individuals were included, but gravid females and any snakes showing an obvious food bulge were excluded in analysis of BCI (Winne, et al. 2007). Following Winne et al., BCI was calculated as $(\text{mass}/\text{SVL}^3) \times 10^5$. We used analysis of variances (ANOVAs) to determine the variation of SVL and BCI between populations with gender and habitat (i.e., Chestnut Ridge or Pine Ridge) as fixed effects, an interaction between gender and location, and year as a random effect.

Analysis of growth rates considered all snakes that could confidently be identified as recaptures and showed more than two months of growth. Growth rates (cm/mo SVL) were calculated based on a six-month growing season of April to September (Jenkins, et al. 2001). Some snakes in the Chestnut Ridge population were first captured before June 2006 and again after. For these individuals, only recaptures after June 2006 were considered to calculate growth rates. If more than one growth rate could be calculated for an individual (i.e. snake was caught three or more times with at least 2 months between each capture), all growth rates for the same individual were included in the analysis.

Results

*Lampropeltis nigra* in the Chestnut Ridge habitat ranged from 33.5 to 87.1 cm with a mean of 58.57 cm SVL ($n = 37$). The Pine Ridge habitat population ranged from 29.1 cm to 123.9 cm with a mean of 79.25 cm SVL ($n = 22$). When considering measurements from both habitats, SVL varied significantly based on habitat (F Ratio = 16.0409, Prob>F = 0.0002), but not based on gender (F Ratio = 0.4555, Prob>F = 0.5026) or habitat x gender (F Ratio = 0.5264, Prob>F = 0.4712). BCI in the Chestnut Ridge habitat ranged from 26.87 to 41.89 with a
mean of 33.96 (n = 35), and 27.45 to 39.37 with a mean of 32.34 (n = 18) in the Pine Ridge habitat. BCI showed no significant variation based on any effects (habitat F Ratio = 2.1683, Prob>F = 0.1473; gender F Ratio = 1.8342, Prob>F = 0.1820; habitat x gender F Ratio = 0.6076, Prob>F = 0.4395).

The sample size of growth rates was not large enough to conduct proper statistical tests, but examining average growth rates by species in each habitat reveals some trends, shown in Appendix. Snakes showed faster average growth rates in the Chestnut Ridge habitat (mean 1.80 cm/mo) than in the Pine Ridge habitat (mean 0.84 cm/mo).

**Discussion**

*Lampropeltis nigra* is known to exhibit variation in range and average body size among populations (Meade and Palmer-Ball 2003), but no study has identified differences in body size between adjacent populations of *L. nigra* on such small geographic scale. A previous study by Faust and Blomquist (2011) compared body size and growth rates of *L. nigra* from the Chestnut Ridge habitat at the FES with a population at the Anderson County Wildlife Sanctuary (ACWS), a site approximately 6 km away. Faust and Blomquist (2011) reported significantly larger snakes at the ACWS (mean 66.9 cm SVL, 162.4 g mass) than at the FES (mean 55.8 cm SVL, 80.5 g mass), though means for both populations are smaller than previous reports from other areas for the species (Faust and Blomquist 2011). Their study considered snakes captured in the Chestnut Ridge habitat from 1996 to 2009. No surveys were conducted on Pine Ridge. Our study revealed slightly higher mean SVL of *L. nigra* on Chestnut Ridge (58.57 cm) during the time frame of our survey.

*L. nigra* on Pine Ridge are larger by SVL, regardless of sex, than those on Chestnut Ridge. Our data is insufficient to determine whether the mechanism for this is genetic variation, phenotypic plasticity, age structure difference, or a combination of these factors, but some speculations are possible based on evaluative observation from this and related studies.

**Possibility of Genetic Variation**

Genetic canalization is most probable under circumstances of long-term gene pool separation with extremely limited migration between populations, so it may not be expected in the relatively small geographic area of the FES study site with only a road as a physical barrier. However, evidence for genetic variation on similar geographic scale with only environmental gradient as a separating factor has been shown in *Thamnophis elegans* (garter snake) (Bronikowski 2000). Interestingly, during the course of our study, no individual of any species was ever captured in both Chestnut and Pine Ridge habitats. The only evidence suggesting that snakes move between habitats was a single observation of a copperhead (*Agkistrodon contortrix*) crossing the road from south (Chestnut Ridge) to north (Pine Ridge), but the PIT tag identity of this individual was not confirmed. Although migration of *L. nigra* between habitats in our study area has not been observed, it is reasonable to assume that migration and therefore interbreeding between habitats is possible. *L. nigra* from the nearby ACWS habitat are known to travel as far as 1.5 km from site of original capture (Jenkins, *et al.* 2001), a distance more than sufficient for movement between the adjacent habitats of Chestnut and Pine Ridges. Without proper testing, however, genetic variation cannot be entirely ruled out as a possibility.

**Possibility of Phenotypic Plasticity**

Other studies have suggested that variation in body size of proximal snake populations is likely due to phenotypically plastic response to environmental conditions.
or food availability (e.g. Bronikowski and Arnold 1999, Madsen and Shine 1993). We have no empirical evidence for difference in food availability at our site, but soil types on each ridge may affect habitat suitability for both the snakes and their prey. Soil in the Pine Ridge habitat is primarily Armuchee silt loam, an acidic, moderate-medium granular soil formed from weathered shale. The Chestnut Ridge habitat is primarily Fullerton cherty silt loam, a strongly acidic, fine-medium granular soil weathered from cherty limestone (NRCS 2006). The Armuchee soil on Pine Ridge has generally larger particles; therefore it is less compact than the Fullerton soil on Chestnut ridge.

Upon comparison of the ACWS and Chestnut Ridge sites, Jenkins, et al. (2001) suggested that looser soils correlate with greater abundance of small mammals, which are not only an important food source for adult *L. nigra*, but also provide habitat in the form of mammal burrows, which are occupied by snakes as shelter and avenues for movement (Steen, et al. 2010). No study has been conducted to compare small mammal populations on Chestnut and Pine Ridges, but the looser soil of the Armuchee series on Pine Ridge could allow for higher densities of small mammals and their burrows, which would alleviate some environmental stress for *L. nigra* populations. The soil on Chestnut Ridge is more compact than soil on either the ACWS site or Pine Ridge, and body size of *L. nigra* is smaller on Chestnut Ridge than at either of these nearby sites (Jenkins, et al. 2001; Faust and Blomquist 2011). Using BCI as an indicator for health, neither population appears to be more fit than the other because although SVL shows significant variation, BCI does not. Therefore, if the lesser growth of snakes on Chestnut Ridge is attributable to environmental stress, the reduced SVL reflects a trade-off between growth and survival, which is likely to be a plastic response unique to this habitat.

Possibility of Age Structure Difference

*Lampropeltis nigra* from Pine Ridge may be larger because they are, on average, older than those from Chestnut Ridge. Growth rate data proved to be inconclusive because of small sample size, but observable trends allow for speculative estimates on age structure differences between snakes in the two habitats. As with other snakes, juvenile *L. nigra* grow faster than adults (Faust and Blomquist 2011), so a population containing relatively high numbers of juveniles should show faster average growth rates than a population of older snakes. *L. nigra* reaches sexual maturity at approximately 60 cm SVL (Jenkins et al. 2001, Mitchell 1994), so growth rates can be expected to slow around this length. Twenty-five percent of growth rate records from *L. nigra* on Pine Ridge (one individual) showed an SVL of less than 60 cm upon initial capture (see Appendix). This individual also exhibited the highest growth rate out of all *L. nigra* from Pine Ridge. In contrast, 50% of *L. nigra* growth rate records (5 individuals) from Chestnut Ridge were less than 60 cm SVL upon at least one capture. Growth rates for these individuals averaged 0.53 cm/mo faster than the average for the remaining 50% of records from larger snakes. This is nearly consistent with Faust and Blomquist’s (2011) finding that juvenile *L. nigra* grow an average of 1.1 cm/mo faster than mature snakes.

These data seem to suggest that age structure differences contribute to variation in body size between the populations. The larger, slower-growing snakes on Pine Ridge could reasonably be older than the smaller, faster-growing snakes on Chestnut Ridge. This is no reason to rule out prey availability and habitat suitability, however, because these factors may contribute to increased longevity in the Pine Ridge habitat when
compared to Chestnut Ridge. Improved environmental conditions due to soil types and small mammal abundance on Pine Ridge may allow snakes to live longer in this habitat.

One notable shortcoming of this study is that many juvenile snakes were excluded from analyses because small body size did not allow for PIT tag identification. Our data do not suggest that this affects the trend of results, however, because we captured juvenile snakes too small for PIT tags a total of 13 times on Chestnut Ridge, as opposed to only one on Pine Ridge over the course of the study. Snout-to-vent length and location data suggest that most, if not all, of the 13 captures on Chestnut Ridge were of unique individuals, so inclusion of these juvenile snakes' growth rates could be expected to only strengthen the trend of smaller snakes with more rapid growth in the Chestnut Ridge habitat.

Conclusion
Our study at the University of Tennessee Forestry Experimentation Station (FES) in East Tennessee reveals two populations of Lampropeltis nigra that differ in average SVL, regardless of sex, but inhabit the adjacent and nearly analogous habitats of Pine Ridge and Chestnut Ridge, separated only by a two-lane paved road. Current data and analyses leave the mechanism for this divergence somewhat enigmatic, but growth rate trends and juvenile capture rates suggest that age structure differences may be an explanation. One notable difference between habitats—soil type—may affect prey availability and habitat suitability, thereby imposing environmental stress on snakes living in more compact soils and phenotypically or genetically selecting for shorter SVL. This factor could be either causal or complementary to age structure differences.

In order to explain the observed divergence in body size, future studies should collect more data on growth rates of snakes in both habitats and attempt to determine actual age of individuals in order to analyze life span and age structure. Also, specific surveys should be conducted on small mammal populations to compare population density and borrow abundance in the two FES habitats. As it stands, this study serves to identify phenotypic variation in body size between adjacent populations on a geographic scale smaller than previously reported for Lampropeltis nigra, but further research is needed to identify the exact mechanism driving this variation.

Acknowledgements
Special thanks to A. C. Echternacht, University of Tennessee, and J. Byrd, Clinch River Environmental Studies Organization (CRESO). Additional thanks to CRESO team T. Faust, Z. Sherrod, A. Wray, and A. Burchett.


Appendix

Growth rates for *Lampropeltis nigra* individuals in Chestnut Ridge habitat:

<table>
<thead>
<tr>
<th>Individual (PIT#)</th>
<th>SVL₁ (cm)</th>
<th>SVL₂ (cm)</th>
<th>Month interval</th>
<th>Monthly growth rate (cm/mo)</th>
</tr>
</thead>
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<tr>
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<td>76.8</td>
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<td>59.7</td>
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<td>59.5</td>
<td>3.5</td>
<td>2.57</td>
</tr>
<tr>
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<td>55</td>
<td>71</td>
<td>12.5</td>
<td>1.28</td>
</tr>
<tr>
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<td>71</td>
<td>73.5</td>
<td>2</td>
<td>1.25</td>
</tr>
<tr>
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<td>19.5</td>
<td>1.95</td>
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<td>84.6</td>
<td>12.5</td>
<td>1.5</td>
</tr>
<tr>
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<td>85.2</td>
<td>4</td>
<td>2.83</td>
</tr>
<tr>
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<td>85.2</td>
<td>93</td>
<td>10</td>
<td>0.78</td>
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<tr>
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<td>50</td>
<td>61.1</td>
<td>3.5</td>
<td>3.17</td>
</tr>
</tbody>
</table>

**Mean:** 1.80

Growth rates for *Lampropeltis nigra* individuals in Pine Ridge habitat:

<table>
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<tr>
<th>Individual (PIT#)</th>
<th>SVL₁ (cm)</th>
<th>SVL₂ (cm)</th>
<th>Month interval</th>
<th>Monthly growth rate (cm/mo)</th>
</tr>
</thead>
<tbody>
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<td>0.84</td>
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<td>126.1</td>
<td>5.5</td>
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<td>126.1</td>
<td>126.1</td>
<td>3</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Mean:** 0.84

SVL₁ is the snout-to-vent length from the initial capture of an individual used to calculate growth rate. SVL₂ is the snout-to-vent length of the next capture of the same individual.

Month interval is the number of months, rounded to the nearest half month, elapsed between measurements of SVL₁ and SVL₂.