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VARIATION IN CRANIAL MORPHOLOGY OF POLYMORPHIC POPULATIONS OF THE EASTERN RED-BACKED SALAMANDER, PLETHODON CINEREUS

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VARIATION IN CRANIAL MORPHOLOGY OF POLYMORPHIC POPULATIONS OF THE EASTERN RED-BACKED SALAMANDER, *PLETHODON CINEREUS*

A Thesis Submitted to the Office of Graduate Studies College of Arts & Sciences of John Carroll University in Partial Fulfillment of the Requirements for the Degree of Master of Science

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2017
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Variation in Cranial Morphology of Polymorphic Populations of the Eastern Red-Backed Salamander, *Plethodon cinereus*

**Abstract**

Differences in cranial morphology among species of plethodontid salamanders have been linked to variations in diet and behavior. Individuals that consume smaller prey have larger jaw to posterior-cranium ratios. Elongation and expansion of the jaw, coupled with expansion of the posterior of the cranium (defined as robustness) are positively associated with individuals that exhibit increased levels of aggression. The bulk of previous research has focused on the effects of interspecific competition on cranial morphology; however, polymorphic populations of the Eastern Red-backed Salamander, *Plethodon cinereus*, represent a unique opportunity to examine the role of cranial morphology in ecological divergence among individuals of the same species. I tested for differences in cranial morphology between the striped and unstriped morphs of *P. cinereus*. Previous research suggests that diet and aggression differ between color morphs of this species. At the Summit Co. field site in Northeastern Ohio, the striped morph is more aggressive and maintains consistent access to high-quality territories. These territories contain numerous small-bodied prey items that are commonly consumed by the striped morph. Therefore, I hypothesized that striped salamanders would either exhibit a cranial shape consistent with increased aggression, or a morphology consistent with a diet of small prey items. We examined head shape differences between morphs (n = 1146 specimens) from 9 polymorphic sites using geometric morphometrics. Differences in head shape were significant among localities. At the Summit Co. site (i.e., the PA clade), the cranial morphology of striped morphs was significantly more robust, which is consistent with elevated aggressive and territorial behavior by this morph.
Within the OH clade, unstriped individuals from the Ottawa Co. locality showed an increase in the jaw:posterior-cranium ratio. Although not statistically significant, four of five remaining populations mirrored this result. I suggest that differences in head shape in the PA clade result from interference competition for territories, and those differences within the Ohio clade derive from exploitative competition for limited prey resources.
Introduction

To understand evolutionary processes that maintain diversity and promote speciation, one must first identify the causes of phenotypic variation within a species (McLean et al. 2015). Identifying causes of variation is a fundamental goal for evolutionary biologists because of the role that variation plays in the capacity for adaptive change. Polymorphic species are defined by the simultaneous occurrence of two or more genetically discrete phenotypes within a population (Huxley 1955, Highton 1975, Moore & Ouellet 2014). The presence of easily observed phenotypic variation provides a unique opportunity to study how different morphs respond to the environment. Selective pressures, such as predation, competition for prey, and intraspecific aggression, may drive genetic and phenotypic variation. This is especially true in polymorphic species, where different morphs may respond differently to selection. Such forces could also act as a precursor to divergence, reproductive isolation, and perhaps ultimately result in incipient speciation. Understanding why some species exhibit polymorphism, whereas others do not, might help researchers identify processes that maintain genetic diversity within polymorphic populations, and perhaps contribute to speciation (McLean & Stuart-Fox 2014).

Numerous studies have found that competition for prey is an important selective pressure acting on individual predators within populations. Competition for particular prey types or sizes can be an influential force for adaptive radiation driving speciation (McGee et al. 2013). As divergence progresses, morphological changes can occur in response to different morphs using different resources (Adams 2004). Stickleback fish (*Gasterosteus*) provide an excellent example of trophic adaptation resulting from utilization of prey in limnetic and benthic habitats (Pritchard & Schluter 2001, McGee et al. 2013). Morphological changes in feeding structures have been linked to rapid ecological divergence within sticklebacks. For example, Canadian populations of
closely-related, sympatric *Gasterosteus* spp. have undergone niche separation, and exhibit enhanced feeding performance in their respective microhabitats (McGee et al. 2013).

Behavior, such as aggression, can also be a factor contributing to ecological divergence. For example, differently-colored male cichlids are more likely to occupy territories in close proximity, compared to males of similar colors (Seehausen & Schluter 2004). Male-male aggression is directed toward individuals of the same color, whereas the rare color morphs enjoy an advantage while mating, as they elicit less aggressive behavior from males of the abundant color morph. Seehausen and Schluter (2004) suggest that male competition could be a factor in diversifying color and territorial behavior. Lattanzio and Miles (2014) found that male tree lizards (*Urosaurus ornatus*) also exhibit multiple color morphs, and these phenotypes may be maintained by differences in diet and aggression. When both habitat and prey were limited, there was evidence of a shift in aggressive behavior exhibited by male tree lizards toward other males (Lattanzio & Miles 2014). When resources were abundant, males with blue dewlaps occupied trees regularly, whereas males with yellow and orange dewlaps showed little evidence of habitat selection. However, when sites were burned, and fewer trees were available, males with yellow dewlaps exhibited aggressive behavior to compete for tree space, outcompeting blue and orange males. Individuals with yellow dewlaps are more successful in resource-limited environments and gain access to higher-quality prey available in the trees. This trophic niche differentiation in habitat and aggression could be linked to eventual speciation (Lattanzio & Miles 2014). Habitat segregation in sympatric species due to aggressive interactions has been linked with character displacement in body size and head shape (Melville 2002, Adams 2004). Species that are more aggressive may gain access to higher quality habitat, protein rich food, and access to higher quality mates (Melville 2002, Seehausen & Schluter 2004, Lattanzio & Miles 2014). In these
systems, interspecific and intraspecific behavioral interactions may generate or maintain morphological shifts (Adams 2004).

*Plethodon cinereus* is a polymorphic woodland salamander found throughout Eastern North America and Southeastern Canada, and it is the most abundant amphibian in the Eastern United States (Anthony & Pfingsten 2013). This species has served as a model organism for addressing many key questions in evolutionary biology, behavioral ecology, and conservation biology (Jaeger & Forester 1993, Mathis et al. 1995, Anthony & Pfingsten 2013, Jaeger et al. 2016). *Plethodon cinereus* has been used as a model species because of their abundance and ease in experimental field and laboratory studies. As a result, there is a vast literature base from which to draw when posing hypotheses regarding the roles of territoriality, diet, and aggression in evolutionary change. *Plethodon cinereus* has two common color morphs: the striped (i.e., red-backed morph); and the unstriped (i.e., lead-backed morph) (Stejneger & Barbour 1927, Highton 1962, Anthony & Pfingsten 2013). The frequency at which each morph is found within a population is variable (Highton 1962, Pfingsten & Walker 1978) but has been relatively stable over time (Test 1952, Brown 1965, but see Gibbs & Karraker 2006).

*Plethodon cinereus* is particularly suitable for studies of ecological divergence because differing phenotypes in a single population (striped and unstriped) may respond differently to variable ecological pressures (Anthony et al. 2008). The two most common color morphs vary along numerous niche axes (Table 1). Many of these differences could potentially be important drivers of ecological divergence. For example, the morphs differ in mate choice (Acord et al. 2013, Jaworski 2014), disease resistance (Venesky et al. 2015), aggression (Reiter et al. 2014), diet (Anthony et al. 2008, Paluh et al. 2015, Stuczka et al. 2016), temperature and moisture preference (Burger 1935, Test 1952, Lotter & Scott 1977), and fossoriality (Highton 1977,
Fisher-Reid & Wiens 2015; Table 1). These differences (specifically aggression and diet) warrant further study because of the potential impact that they might have on morphological shifts, which, in turn, may be linked to divergence in some populations. Behavioral and dietary differences found in *Plethodon* have also been shown to drive morphological changes (Adams 2000, Adams & Rohlf 2000, Adams 2004, Adams 2011). Adams (2004) found that when comparing *P. teyahalee* (an aggressive species) and *P. jordani* (a less aggressive species), there was a significant difference in head shape between the two species, but only when found in sympatry. *Plethodon teyahalee* exhibited aggressive behaviors and had a more robust head shape, with an elongation of the lower jaw, coupled with expansion of the posterior region of the jaw and head, compared to more submissive individuals of *P. jordani* (Adams 2004). In addition, differences in the morphology of head shape have been linked to diet (Adams 2000, Maerz et al. 2006). In a study comparing upland and lowland populations of *P. cinereus* in New York and Pennsylvania, Maerz et al. (2006) found that individuals from populations that consumed mostly small prey had relatively longer jaws. They arrived at this conclusion by comparing size ratios (jaw:posterior part of the cranium) between populations. They found that in upland populations, where mostly small prey such as Collembola and mites were consumed, the jaw:cranial ratios were larger. In lowland habitats, where larger prey such as insect larvae and earthworms were common, jaw:cranial ratios were smaller.

For my study, I was interested in using known differences in aggression and diet of *Plethodon cinereus* to investigate the potential for cranial shape differences between the different color morphs. Previous research has revealed behavioral differences in aggression and territoriality between morphs (Reiter et al. 2014). For example, striped salamanders are significantly more aggressive toward intruders, as measured by an aggression index, whereas
unstriped salamanders are more submissive in territorial contests. In the field, striped salamanders hold their territories longer and are found more often under cover objects. These differences in territoriality and aggressive behavior may result in striped males gaining access to larger females with higher fecundity, and could contribute to assortative mating by morph (Anthony et al. 2008, Acord et al. 2013).

Color morphs of *P. cinereus* vary in their aggressive behavior, territoriality behavior, and diet. Anthony et al. (2008) reported differences in gut contents between the different color morphs. Striped individuals had more prey in their guts than did unstriped individuals, and the diet of the striped morph was composed of more easily digested prey. Additionally, striped individuals had a more diverse diet overall, whereas compared to unstriped salamanders. Paluh et al. (2015) examined diet and territory quality of the morphs and found that the diets differed between morphs and that striped morphs were found in more prey-rich territories when compared to unstriped morphs. These studies suggest that the two morphs may enter into competition for prey. Furthermore, ecological theory predicts that competition should be most intense during periods of increased surface activity when morphs are in most contact with one another. Stuczka et al. (2016) tested this hypothesis by examining the diets of striped and unstriped morphs during 21 sampling days throughout the year, and as predicted, diet differences between the morphs were greatest during periods of increased surface activity and, presumably, when limited prey resources were in demand.

I used landmarked-based geometric morphometrics, a powerful approach to the analysis of biological shape variation (Rohlf & Marcus 1993, Adams et al. 2004, Webster & Sheets 2010, Adams et al. 2013), to compare cranial shape among populations and between color morphs of *P. cinereus*. The approach provides visualizations (thin-plate spline deformation grids) with
extraordinary explanatory power, allowing researchers to compare shape in a phylogenetic or biogeographic context. Under a scenario of intense interference competition, I hypothesized that the striped morph of *P. cinereus* would exhibit cranial shape consistent with increased aggression and territoriality, and would have a more robust jaw compared to the unstriped morph. Specifically, I predicted that the striped morph would have larger posterior-cranial and dentary elements relative to the unstriped morph. Alternatively, exploitative competition for prey might be more important in determining head shape. Striped morphs eat smaller prey, such as Collembola and Acari (Anthony et al. 2008); therefore I hypothesized that they would have a larger jaw:posterior-cranium ratio (Maerz et al. 2006). Isopods and ants (relatively large prey) are an important component of the diet of the unstriped morph (Anthony et al. 2008); therefore, I predicted that the unstriped morph would have a smaller jaw:posterior-cranium ratio. Most studies examining differences between color morphs in Ohio have targeted a single population in the Cuyahoga Valley (Summit Co., OH). Additional polymorphic populations in northern Ohio were selected to test the hypothesis that differences between morphs are consistent across populations. Discovering the relative morphologies of the two morphs will provide a better understanding of the selective forces driving head shape differences within polymorphic populations of *P. cinereus*.

**Methods**

*Population Selection:* My sampling design targeted multiple polymorphic populations of Eastern Red-backed Salamanders, *Plethodon cinereus*. I predicted that if the two morphs interact in similar ways across populations, then I should be able to detect consistent differences in cranial morphology across populations. These populations were selected from the holdings at the Cleveland Museum of Natural History (CMNH). I examined head shape in salamanders from
nine polymorphic populations with a minimum of 10 individuals of each morph (Table 2). My final site selections were based on morph frequency estimates reported in Pfingsten and Walker (1978). The Summit County population was the focal population of my study as I had access to over 400 specimens, and this population has a relatively large proportion of unstriped salamanders (30%). This population is also well characterized with many studies on ecological separation of morphs (Venesky & Anthony 2007, Anthony et al. 2008, Acord et al. 2013, Reiter et al. 2014, Paluh et al. 2015, Stuczka et al. 2016). I utilized 1,146 salamander specimens for this study, all of which were from the CMNH. Only adult salamanders (greater than 32 mm snout-to-vent length) were used in this study. A recent phylogenetic analysis illustrates the presence of multiple genetic lineages throughout the geographic range of P. cinereus, two of which (the Ohio and Pennsylvania Clades) are utilized in the current study (Radomski et al. in review). My focal population in Summit County, Ohio is within the Pennsylvania clade, six other localities are within the Ohio clade, and two localities could not be assigned to one or the other clade (Table 2; Fig. 1).

**Specimen Processing and Head Morphology Analysis:** I used landmark-based geometric morphometric methods to quantify head shape (Rohlf & Marcus 1993, Small 1996, Dryden & Mardia 1998, Adams 2004, Zelditch et al. 2004). Digital images of the right-lateral side of the head were obtained from each specimen using a Leica M165C dissecting microscope fitted with a CoolSnap-Pro CF camera. The x, y coordinates of 11 homologous landmarks (Adams 2004, Maerz et al. 2006) from the skull and jaw were recorded for each image using TpsDig software (Fig. 2; Rohlf 2001). I used the R statistical program package ‘geomorph,’ to align specimens by rotating the mandible of all the specimens to a fixed angle relative to the skull. This approach corrects for any size variation among specimens. The alignments were treated as shape variables.
for analyses (Adams & Otarola-Castillo 2013, Rohlf & Slice 1990). A least squares rotation was performed to find the mean of each landmark on the specimens. Specimens were then aligned using a Generalized Procrustus Analysis (GPA) and an orthogonal projection was used to convert the spherical shape variable that was produced to a 2-dimensional variable. Thin Plate Spline (TPS) was used to provide a smooth map of the landmarks based on mathematics of bending a thin metal plate to create a geospatial image. Deformation grids were used to visualize shape differences.

Statistical Analysis: To assess potential head shape variation between morphs and among localities, I used a nonparametric multivariate analysis of variance (np-MANOVA) with a residual randomization permutation procedure (9,999 permutations) on the landmark data. I used the model Head Shape ~ Locality * Morph. Separate analyses were run for each clade (PA and OH) and for populations that could not be definitively assigned to clade. Within the OH clade, pair-wise comparisons were run among the six localities with a sequential Bonferroni correction to account for the multiple comparisons. I used separate principle components analyses (PCA) for each locality to graphically visualize differences between individuals of each morph in shape space.

I calculated ratios of linear measurements taken between landmarks that are roughly analogous to the dentary bone (i.e., lower jaw; distance between landmarks 5 and 1) and the posterior cranium (i.e. back of the head sensu Maerz et al. 2006; distance between landmarks 1 and 11). Ratios were calculated as jaw length divided by posterior of the head length (see Maerz et al. 2006). I compared mean ratios of the two morphs of *P. cinereus* at each of the nine localities using two-tailed t-tests. Sequential Bonferroni adjustments of alpha were utilized for tests on the six localities within the OH clade. Additionally, I selected the two populations with
the largest sample sizes (Summit County, PA clade and Ottawa County, OH clade) to more closely examine potential differences in head size between morphs. I compared the mean lengths of the jaw and the posterior cranium between morphs from these populations to help me determine which elements of the ratio calculation (jaw or posterior cranium) might be driving relative increases or decreases in the ratio. These comparisons were made using two-tailed t-tests.

**Results**

**PA Clade**

Within the single locality assigned to the PA clade (Summit Co. population), I observed significant differences in head shape between striped and unstriped salamanders (F\(_{1,414}\) = 3.58; Z = 2.71; P = 0.02). To visualize the differences in head shape between morphs I used deformation grids to compare the mean head shape of striped and unstriped individuals (Fig. 3). Deformation grids show that relative to unstriped salamanders, striped salamanders have a more robust head morphology typified by increases in the lengths of the jaw and posterior-cranium. I detected no difference in jaw to posterior-cranium ratios between morphs in the PA clade (t\(_{414}\) = 0.039; P = 0.969; Fig. 4); however, there were significant differences in the sizes of these two elements among morphs. The striped morph had a significantly longer jaw (t\(_{414}\) = 2.69; P = 0.007) and posterior-cranium (t\(_{414}\) = 2.76; P = 0.006). Increases in jaw length and the posterior portion of the head correlate well to an increase in robustness, as defined by Adams (2004). Results of the PCA show considerable individual variation at this polymorphic locality (Fig. 5).

**Ohio and Unassigned Clades**

Color morph was a significant predictor of head shape in the OH clade (F\(_{1,598}\) = 23.42; Z = 8.79; P = 0.01; Fig. 6), although there was significant individual variation within each of the 6
sites (Fig. 7). Morph differences were dependent on locality (morph * locality; F_{1,588} = 1.60; Z = 1.56; P = 0.02); however, pairwise comparisons within localities failed to reveal significant differences in head shapes between morphs (Table 3). Differences between morphs at the Ottawa County locality approached significance following multiple test correction (Z = 1.271; P = 0.012; adjusted Bonferroni alpha = 0.008; Table 3; Fig. 6). Additionally, morphs differed significantly in the jaw:posterior-cranium ratio at this site (t_{310} = 3.81; P < 0.0001; adjusted Bonferroni alpha = 0.008; Fig. 4) with the unstriped morph having the higher ratio. This difference was driven by increase in jaw length (t_{310} = 3.44; P = 0.001), with no increase in the posterior-cranium length (t_{310} = 0.385; P = 0.70) in the unstriped morph. I failed to detect any other differences in jaw to posterior-cranium ratio at the other Ohio localities (Fig. 4); however in 5 of 6 localities, the ratio was higher for the unstriped morph and visual inspection of the deformation grids suggests that there may be a consistent trend of increasing jaw elongation in the unstriped morph in the Ohio clade. In the unassigned localities, I observed no significant differences in head shape between morphs (Medina: F_{1,67} = 1.63; Z = 1.28; P = 0.16; Figs. 8, 9; Wayne: F_{1,57} = 1.91; Z = 1.53; P = 0.1; Figs. 10, 11). Visual inspection of the deformation grids for both unassigned localities revealed no obvious differences between color morphs when striped and unstriped individuals were compared in morphospace.

Discussion

Understanding the ecological interactions that contribute to the selective pressures experienced by individuals within a population is fundamental to understanding the evolution of species (McLean et al. 2015). Previous work has illustrated the importance of interference and exploitative competition in shaping salamander communities (Jaeger 1970, Hairston 1980, Arif et al. 2007, Bruce 2008). Among communities of terrestrial plethodontid salamanders,
competitive interactions and variation in diet promote morphological changes within conspecific populations and among heterospecific populations (Adams 2004). Data suggest that morphs of *P. cinereus* in some polymorphic populations are ecologically differentiated along the diet and territoriality niche axes (Anthony et al. 2008, Reiter et al. 2014, Paluh et al. 2015, Stuczka et al. 2016). The purpose of my study was to test for differences in cranial morphology between morphs, and among multiple polymorphic populations. I hypothesized that differences in aggression or diet would drive cranial morphology to differ between the two morphs. Geometric morphometric techniques were used to explore nine different polymorphic populations. I observed differences in cranial morphology between the striped and unstriped morphs of *P. cinereus*, but the ways in which the morphs differed were unique in the two clades. In the PA clade, striped morphs had a more robust head shape at the locality where that morph is known to be more aggressive. In contrast, unstriped individuals within the Ohio clade exhibited a larger jaw:posterior-cranium ratio, which is consistent with a morphology driven by prey selection.

Behavioral differences such as aggression and territoriality have been linked to cranial robustness in more aggressive species of *Plethodon*, which may lead to a fitness advantage for individuals in territorial contests (Nishikawa 1985a, b, Anthony et al. 1997, Adams 2004). In larger species of *Plethodon*, morphological traits such as head robustness provide an advantage in aggressive encounters between individuals, and may be linked to possible ecological character displacement (Adams 2004). Small plethodontids, such as red-backed salamanders, also defend territories under leaf litter, rocks, and logs from intruders (Jaeger & Forester 1993, Mathis et al. 1995, Hickerson et al. 2004 & 2012). Within the PA clade, recent evidence from the Summit County locality suggests that striped individuals are significantly more aggressive toward intruders when compared to unstriped salamanders (Reiter et al. 2014). My analysis of specimens
from this population in the PA clade revealed that striped individuals had head shapes that were elongated and more robust than those of unstriped individuals from this population. These differences in shape may be driven by interference competition between morphs and may result from increased levels territorial defense in striped individuals. This hypothesis corroborates findings reported by Reiter et al. (2014). With a more robust head, the striped color morph may assert competitive dominance in territorial encounters. This may allow access to high quality invertebrate prey, more mating opportunities, and subsequently may allow striped salamanders to maintain their advantage. In a model proposed by Anthony et al. (2008), females may compete for access to higher quality males (and their territories), which may result in positive assortative mating. Striped males are more aggressive at this field site, resulting in the highest quality females (mostly striped) gaining access to these territories. Co-defense of territories by males and females (Lang & Jaeger 2000) may occur among striped salamander pairs (Reiter et al. 2014), representing a critical first step in the process of genetic divergence.

from my PA clade site in Summit Co. OH have documented differences in both prey availability and in gut contents between morphs of *P. cinereus*. Striped individuals had more prey in their guts, and were often found in more prey-rich territories compared to unstriped individuals (Anthony et al. 2008, Paluh et al. 2015, Stuczka et al. 2016, Anthony et al. in press). Diet differences between morphs were greatest during periods of high surface activity in the spring and the fall (Stuczka et al. 2016) when exploitative competition for prey is expected to be most intense. Differences in diet between morphs in those seasons were driven by striped individuals consuming increased numbers of smaller prey, such as Acari and Collembola (Stuczka et al. 2016).

Comparisons of the jaw:posterior cranium ratios between morphs allows me to generate specific hypotheses relating to the ecological interactions responsible for driving differences in head shape. I observed a significant increase in jaw length of unstriped individuals in the Ottawa Co. population (OH clade). Four of five remaining populations within the OH clade mirror this pattern. Increases in jaw length are indicative of an alteration of cranial morphology that may be associated with consumption of smaller prey by the unstriped morph. At least within the five OH clade populations, diet (rather than behavior) appears to have a stronger influence on head shape. Conversely, within the PA clade we observed equal increases in jaw and cranium length in the striped morph. This increase in size or robustness may be a result of increased aggression in striped individuals. Therefore, it is presumable that interference competition in the form of territorial defense represents the selective pressure influencing cranial morphology within this population.

An additional driver of differences in cranial morphology could be burrowing, which can influence head shape across many taxa. *Plethodon cinereus* is a weak burrower (Heatwole 1960).
and mostly uses existing crevices and tunnels created by other animals as refugia when avoiding harsh surface conditions (Vernberg 1953). However, cranial morphology that aids in pushing into crevices and burrowing in loose soil might be favored by selection in this species. In order to burrow, individuals use their head as a wedge, with the feet and tail supplying force to push the head into the substrate or between objects (Heatwole 1960). This type of burrowing requires great force and may account for shifts in cranial morphology. Garden eels (Heterocongrinae), for example, have a shortened snout in species that exhibit burrowing (Schepper et al. 2007).

Furthermore, Barros et al. (2011) found that the evolution of Gymnophthalmidae cranial morphology is shaped by microhabitat use rather than diet, with burrowers having shorter heads. Animals such as amphibianians, which spend a majority of their life burrowing, exhibit shovel like heads, a morphological adaptation hypothesized to aid in borrowing efficiency (Kearney 2003, Kearney & Stuart 2004, Barros et al. 2011). In addition to these taxa, the longer head of the Schistometopum homense males was found to negatively affect burrowing speed (Teodecki et al. 1998). Species that are capable of burrowing deeply and more quickly have shorter skulls (Gans 1969, Teodecki et al. 1998). At the Summit County locality (PA clade), unstriped salamanders retreat to underground refugia earlier in the fall (Anthony et al. 2008), thus they spend more time underground compared to the striped morph. The differences between morphs in surface activity may result from different moisture and temperature optima or from competitive interactions related to these optima (Lotter & Scott 1977, Moore & Ouellet 2014).

On Long Island, Fisher-Reid et al. (2013) found that the unstriped color morph of P. cinereus had more costal grooves compared to the striped morph, a condition that correlates with increased vertebral number (Highton 1957). It has been hypothesized that elongation (i.e., increased numbers of costal grooves) is an adaptation to a fossorial lifestyle in many species of
salamanders (Wake 1966, Jockusch 1997) including *P. cinereus* (Fisher Reid et al. 2013, Fisher-Reid et al. 2015). Thus, elongation in the unstriped morph may represent an adaptation to a more fossorial habit. In a mesocosm experiment, unstriped individuals were forced to use inhospitable subterranean habitat in the presence of striped individuals (Dallalio 2013). My geometric morphometric analyses seem to support the hypothesis regarding increased fossoriality in unstriped individuals, since I observed shorter heads in the unstriped individuals at the PA locality. Increased robustness of the cranium in striped individuals at the Summit Co. OH (PA clade) site may be indicative of increased interference competition by which the striped morph gains access to high quality territories (e.g., mates, prey etc.) at the expense of the unstriped morph, which may be forced in to suboptimal habitat below ground.

Within the OH clade there was a strong morph X locality interaction, suggesting that observed differences in head shape depended on location. A number of factors may contribute to the local variation observed in the OH clade populations. First, site-specific differences in prey availability and/or abiotic factors such as soil type and microclimate can drive speciation in plethodontids (Maerz et al. 2006) and could provide the selective background for divergence between morphs. Excellent examples of site-specific variation in prey availability come from studies on non-native prey species (Ivanov et al. 2011, Walton et al. 2006, Maerz et al. 2006) and the presence or absence of non-native species may play a significant role in variation of head shapes among localities. Additionally, non-native species can create novel environments with differing selection pressures (Cox 2004, Maerz, et al. 2006). For example, red-backed salamanders inhabiting urban environments specialize on non-native prey such as invasive ant species (Ivanov et al. 2011) and earthworms can serve as prey, create habitat for salamanders (Cáceres-Charneco et al. 2010), and disrupt microhabitat use and foraging by salamanders.
(Ziemba et al. 2015; Ziemba et al. 2016). Second, interactions with guild members most likely vary site to site as well. For example the Northern Ravine Salamander, *Plethodon electromorphus*, is ecologically similar to *P. cinereus* and occurs sympatrically at some but not all localities. *Plethodon electromorphus* is monomorphic for color and does not have a dorsal stripe. The species is more elongate and possesses smaller limbs than does *P. cinereus*. The two species enter into competition where they co-occur (Deitloff et al. 2013) and this may be influencing how color morphs of *P. cinereus* partition resources. For example, if *P. electromorphus* is present at a polymorphic locality, *P. cinereus* color morphs may become more similar to one another with regard to cranial morphology. When *P. electromorphus* is present at a monomorphic striped site, *P. cinereus* may look like a striped morph from the PA clade, with more aggressive morphological features. With different selective pressures influencing individual populations independently, head shape at each locality may be unique (Jastrebski & Robinson 2004, Berner at al. 2008, Deitloff et al. 2013). This may be creating an interplay between biotic and abiotic factors that differs among localities, resulting in phenotypic and behavioral variation.

**Conclusions**

This is the first study to investigate potential differences in cranial shape in polymorphic populations of striped and unstriped color morphs of *Plethodon cinereus*. I found that in the target population within the PA clade, striped salamanders had elongated and robust head shapes that significantly differed from those of unstriped individuals. These differences in cranial morphology may be caused by interference competition, in the form of territorial contests, between striped and unstriped salamanders at the Summit Co. locality. Within the OH clade, shape differences between morphs were characterized by larger jaw to cranium ratios in
unstriped individuals; a result more indicative of exploitative competition for limited prey resources. Although drivers such as diet, behavior, and climate could vary by location, causing inconsistent morphology (Arif et al. 2007, Deitloff et al. 2016), my data provide additional evidence of ecological divergence in polymorphic populations of the red-backed salamanders.

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Citations:


Table 1. Ecological differences between striped and unstriped morphs of *Plethodon cinereus*.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Traits of the striped morph, relative to unstriped morph</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-predator (snake)</td>
<td>more likely to exhibit immobility, less likely to flee</td>
<td>Venesky and Anthony 2007</td>
</tr>
<tr>
<td>Seasonal activity</td>
<td>more tolerant of colder conditions, less tolerant of dry conditions</td>
<td>Anthony et al. 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anthony and Pfingsten 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lotter and Scott 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Burger 1935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test 1952</td>
</tr>
<tr>
<td>Mating behavior</td>
<td>more attractive, more likely paired to larger mate</td>
<td>Acord et al. 2013</td>
</tr>
<tr>
<td>Mate choice</td>
<td>males prefer &quot;redder&quot; females</td>
<td>Jaworski 2014</td>
</tr>
<tr>
<td>Diet</td>
<td>more diverse, higher quality, found in more prey rich territories</td>
<td>Anthony et al. 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paluh et al. 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stuczka et al. 2016</td>
</tr>
<tr>
<td>Territoriality</td>
<td>more aggressive, more likely to exhibit territorial residency</td>
<td>Reiter et al. 2014</td>
</tr>
<tr>
<td>Disease</td>
<td>more resistant to Bd</td>
<td>Venesky et al. 2015</td>
</tr>
<tr>
<td>Stress</td>
<td>lower levels of circulating stress hormones</td>
<td>Davis and Milanovich 2010</td>
</tr>
<tr>
<td>Water balance</td>
<td>rehydrates more rapidly</td>
<td>Smith et al. 2015</td>
</tr>
<tr>
<td>Response to disturbance</td>
<td>less affected by human alteration of habitat</td>
<td>Fleming et al. 2011</td>
</tr>
<tr>
<td>Response to soil pH</td>
<td>occupies soils with higher pH</td>
<td>Mitchell and Woolcott 1985</td>
</tr>
<tr>
<td>Fossorality</td>
<td>Fewer costal grooves, less likely to burrow often</td>
<td>Highton 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fisher-Reid and Weins 2015</td>
</tr>
</tbody>
</table>
Table 2. Location information for the localities used in this study. The site numbers correspond to those in Figure 1. For each locality the sample size of each color morph of *Plethodon cinereus* is represented by striped and unstriped. Coordinate data are based on Cleveland Museum of Natural History records and personal collecting records.

<table>
<thead>
<tr>
<th>Site Number</th>
<th>Locality Name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Striped</th>
<th>Unstriped</th>
<th>Clade Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fulton</td>
<td>41° 33' 24&quot;N</td>
<td>84° 22' 05&quot;W</td>
<td>53</td>
<td>12</td>
<td>Ohio</td>
</tr>
<tr>
<td>2</td>
<td>Ottawa</td>
<td>41° 34' 22&quot;N</td>
<td>82° 50' 15&quot;W</td>
<td>97</td>
<td>215</td>
<td>Ohio</td>
</tr>
<tr>
<td>3</td>
<td>Erie</td>
<td>41° 21' 02&quot;N</td>
<td>82° 29' 08&quot;W</td>
<td>16</td>
<td>14</td>
<td>Ohio</td>
</tr>
<tr>
<td>4</td>
<td>Lorain</td>
<td>41° 24' 06&quot;N</td>
<td>82° 15' 08&quot;W</td>
<td>65</td>
<td>27</td>
<td>Ohio</td>
</tr>
<tr>
<td>5</td>
<td>Hancock</td>
<td>41° 00' 08&quot;N</td>
<td>83° 27' 33&quot;W</td>
<td>12</td>
<td>61</td>
<td>Ohio</td>
</tr>
<tr>
<td>6</td>
<td>Logan</td>
<td>40° 19' 21&quot;N</td>
<td>83° 36' 26&quot;W</td>
<td>18</td>
<td>10</td>
<td>Ohio</td>
</tr>
<tr>
<td>7</td>
<td>Medina</td>
<td>41° 02' 47&quot;N</td>
<td>81° 48' 10&quot;W</td>
<td>55</td>
<td>14</td>
<td>Not Assigned</td>
</tr>
<tr>
<td>8</td>
<td>Wayne</td>
<td>40° 56' 15&quot;N</td>
<td>82° 01' 09&quot;W</td>
<td>48</td>
<td>11</td>
<td>Not Assigned</td>
</tr>
<tr>
<td>9</td>
<td>Summit</td>
<td>41° 13' 33&quot;N</td>
<td>81° 31' 12&quot;W</td>
<td>251</td>
<td>165</td>
<td>Pennsylvania</td>
</tr>
</tbody>
</table>
**Table 3.** Results for pair-wise comparison among location within morph groups. Morphs at each locality were not found to be significantly different in cranial morphology after a sequential Bonferroni correction (alpha = 0.008) was applied.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erie</td>
<td>0.503</td>
<td>0.998</td>
</tr>
<tr>
<td>Fulton</td>
<td>0.965</td>
<td>0.478</td>
</tr>
<tr>
<td>Hancock</td>
<td>0.638</td>
<td>0.975</td>
</tr>
<tr>
<td>Logan</td>
<td>1.249</td>
<td>0.109</td>
</tr>
<tr>
<td>Lorain</td>
<td>0.853</td>
<td>0.767</td>
</tr>
<tr>
<td>Ottawa</td>
<td>1.271</td>
<td>0.012</td>
</tr>
</tbody>
</table>
**Figure 1.** Location of polymorphic populations that were used for this study. The color of the dots indicates with clade assignment; black, Ohio clade; white, Pennsylvania clade; and gray, not assigned a clade. Additional information on sites can be found in Table 2.
Figure 2. Positions for 11 landmarks used for this study. All landmarks were digitized from photographs of the right lateral side of heads of *Plethodon cinereus* (image modified from Adams 2004).
Figure 3. Deformation grids showing differences in head shape between color morphs at the Summit Co., Ohio locality (Pennsylvania clade) relative to the average specimen, (A) striped color morph, (B) unstriped color morph. Differences are exaggerated by a factor of four to enhance biological interpretation.
Figure 4. Jaw length (distance between landmarks 1-5) to posterior-cranium (distance between landmarks 1 and 11) ratios for striped and unstriped salamanders from 9 counties in northern Ohio. Bars below counties on the x-axis designate clade assignment. PA = Pennsylvania clade; OH = Ohio clade and Unassigned = clade assignment not known. Bars are +1 SE. Differences between morphs are represented by asterisks.
**Figure 5.** Principle components plot describing differences in head shape between color morphs in the Summit Co., Ohio locality (Pennsylvania clade). Individual striped color morphs are represented by small black squares whereas individual unstriped color morphs are represented by small gray squares. The larger squares represent morph averages. PC1 and PC2 explains 57.4% of total variation among specimens. There was no directional pattern in morphospace indicated by PCA.
Figure 6. Deformation grids showing differences in head shape between color morphs in the Ohio Clade; Erie, Fulton, Hancock, Logan, Lorain, and Ottawa county, relative to average specimen, striped color morph, unstriped color morph. Differences are exaggerated by a factor of four to enhance biological interpretation. No significant pattern was observed between morphs within populations, but head shape did vary significantly across populations.
**Figure 7.** Principle components plot describing differences in head shape between color morphs at localities in the Ohio clade. Individual striped color morphs are represented by small black squares, whereas individual unstriped color morphs are represented by small gray squares. Larger squares represent morph averages. Erie county, PC1 and PC2 explains 53.3% of total variation among specimens; Fulton county, PC1 and PC2 explains 44.7% of total variation among specimens; Hancock county, PC1 and PC2 explains 46.3% of total variation among specimens; Logan county, PC1 and PC2 explains 42.5% of total variation among specimens; Lorain county, PC1 and PC2 explains 46.8% of total variation among specimens; Ottawa county, PC1 and PC2 explains 54.4% of total variation among specimens. Head shape varied greatly with no clear directionality in morphospace as indicated by PCA.
Figure 8. Deformation grids showing differences in head shape between color morphs in Medina County (a locality that could not be assigned to either the OH or PA clades) relative to average specimen, (A) striped color morph, (B) unstriped color morph. Differences are exaggerated by a factor of four to enhance biological interpretation.
Figure 9. Principle components plot describing differences in head shape among color morphs in Medina county. Individual striped color morphs are represented by small black squares, whereas individual unstriped color morphs are represented by small gray squares. The larger squares represent morph averages. PC1 and PC2 explain 51.2% of total variation among specimens.
Figure 10. Deformation grids showing differences in head shape between color morphs in Wayne County (a locality that could not be assigned to either the OH or PA clades) relative to average specimen, (A) striped color morph, (B) unstriped color morph. Differences are exaggerated by a factor of four to enhance biological interpretation.
Figure 11. Principle components plot describing differences in head shape among color morphs in Wayne county. Individual striped color morphs are represented by small black squares, whereas individual unstriped color morphs are represented by small gray squares. The larger squares represent morph averages. PC1 and PC2 explains 50% of total variation among specimens.