8-2009

Morphological convergence and character displacement in two species of polymorphic salamanders (genus Plethodon) in eastern Tennessee

Jason R. Jones
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Jason R. Jones entitled "Morphological convergence and character displacement in two species of polymorphic salamanders (genus Plethodon) in eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Benjamin M. Fitzpatrick, Major Professor

We have read this thesis and recommend its acceptance:

James Fordyce, Daniel Simberloff

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Jason R. Jones entitled "Morphological convergence and character displacement in two species of polymorphic salamanders (genus *Plethodon*) in eastern Tennessee." I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

<u>Benjamin M. Fitzpatrick</u>, Major Professor

We have read this dissertation
and recommend its acceptance:

<u>James Fordyce</u>

<u>Daniel Simberloff</u>

Acceptance for the Council:

<u>Carolyn R. Hodges</u>
Vice Provost and Dean of the Graduate
School

(Original signatures are on file with official student records.)

Morphological convergence and character displacement in two species of polymorphic salamanders (genus *Plethodon*) in eastern Tennessee

A Thesis
Presented For the
Master of Science
Degree
The University of Tennessee, Knoxville

Jason R. Jones
August 2009

Dedication

This manuscript is dedicated to Dr. Tom Haggerty, Dr. Terry Richardson, and Dr. Paul Kittle at the University of North Alabama, all true naturalists and professionals that further ignited my passion for biology and inspired me to set my sights for higher professional goals, to my uncle and friend Pat Jones, and my best friend, Robin Dunmire Long, both fellow admirers and eternal students of nature, and my two favorite people with whom to walk in the woods.

Acknowledgements

Abstract

In situations involving the co-occurrence ecologically similar species, one of two different evolutionary responses is often expected. If sympatry results in competition over a shared resource, character displacement would be a favored outcome, while morphological convergence is an alternative outcome if the species have similar responses to the shared environment. In this study, I examine cranial morphology and dorsal coloration of two ecologically similar salamander species (*Plethodon serratus*, the Southern Red-backed Salamander, and *P. ventralis*, the Southern Zig-zag Salamander) to evaluate the alternative hypotheses of character displacement or convergence. Results from linear morphometrics analyses showed no significant shifts in morphology of either species suggestive of either character displacement or convergence in any of the measured traits. However, geometric morphometric analyses showed significant morphological convergence of the two species in sympatry. In contrast, the presence or absence of a dorsal stripe showed evidence of character displacement, corroborating an earlier claim made by Highton. These results are unexpected in that features associated with feeding (cranial morphology) are expected to often exhibit character displacement if dietary resource partitioning is an important mechanism of coexistence, whereas coloration might be expected to show local convergence if its primary function is camouflage or aposematism. Convergence might reflect the overwhelming influence of developmental responses to shared environments or convergent adaptation to local prey communities. Displacement with respect to color polymorphism might be consistent with frequency-dependent selection maintaining alternative ways of being cryptic.

Table of Contents

List of Tables

List of Figures

Morphological convergence and character displacement in sympatric populations of two species of polymorphic salamanders (genus *Plethodon*) in eastern Tennessee

A slight revision of this thesis will be submitted for publication under a similar title in one of various journals in the field of ecology or evolutionary biology in the near future.

I. Introduction

Perhaps one of the best kinds of evidence for adaptation is that of the often-cited phenomenon of morphological convergence. Some classic examples include pollination syndromes (Macior 1974; Rourke & Wiens 1977), cave-associated morphologies (Christiansen 1961; Culver & Fong 1986), and the biogeographic "rules" of Bergmann (1847) and Allen (1877). Species pairs that are more similar in regions of sympatry provide more fine-grained examples of convergence. Such occurrences might be indicative of local adaptation to a shared physical environment (Emberton 1995), interference competition between species (Cody and Brown 1970), or the local predator-prey community, as in the case of aposematic mimicry rings (Mallet & Gilbert 1995).

On the other hand, competition might limit the abilities of ecologically similar species to coexist (Hutchinson 1957). If competition is a dominant factor in determining the fitness of co-occuring species, Brown and Wilson (1956) hypothesized that natural selection would result in character displacement - divergence of form between the species involved (Brown & Wilson 1956; Dayan & Simberloff 2005). A substantial proportion of the literature on ecological character displacement has focused on morphological structures associated with food procurement and processing (Dayan and Simberloff 2005). Several notable examples include beak size in Darwin's finches (Schluter et al. 1985; Grant & Grant 2006), dentition and tooth

1

dimensions in carnivores (Dayan et al. 1989; Dayan et al. 1990; Dayan et al. 1992; VanValkenburgh and Wayne 1994; Dayan and Simberloff 1994) and gill rakers in sticklebacks (Schluter and McPhail 1992). However, other kinds of character displacement, such as reproductive character displacement involving divergence of sexual signals in response to selection favoring reproductive isolation (Servedio and Noor 2003; Höbel and Gerhardt 2003) or to avoid interference in signal transmission (Russo et al. 2007), might also be evolutionary responses of co-occurring similar species.

The relative importance of competition versus other shared interactions with other species and the environment is expected to influence whether species with overlapping geographic ranges exhibit character convergence or displacement. The distribution of alternative resources, when a limiting resource is unique and essential, might have a substantial influence on the effects of competition, with convergence being a possible result of selection on the competing species (Abrams 1987a, 1987b; Fox and Vasseur 2008). The occurrence of displacement or convergence might also depend heavily on the trait in question. Divergence in diet (and/or trophic structure) is a likely response to competition (Dayan and Simberloff 2005), while convergence of physiological traits, reflecting overall adaptations to a shared environment, might also be observed (Bergmann 1847; Allen 1877; Gloger 1833).

Both convergence and character displacement have been described in terrestrial plethodontid salamanders. Evidence for convergence within plethodontid salamanders includes phylogenetic analyses of three different tongue morphologies (protrusible, attached projectile, and free), which suggest independent evolution of each of these types in various plethodontid clades (Larsen et al. 1989; Maglia and Pyles 1995; Deban and Marks 2002; Lockridge Mueller et al. 2004). Potential convergence in the color pattern of both adults and juveniles of several

2

plethodontids has also been described both within and between the *P. glutinosus and P. wehrlei* groups. The ubiquity of the striped/unstriped polymorphism within the Plethodontidae also presents an example of convergence, with at least 12 of the 55 currently recognized species within the genus *Plethodon*, as well as several species of other genera within the family, occurring in both striped and unstriped forms, a phenomenon that appears to have evolved independently multiple times (Figure 1; AmphibiaWeb 2009).

Though convergence involving the polymorphism may be inferred from the previously mentioned phylogenetic analyses, several cases of character displacement in dorsal coloration have also been described between pairs of similar species in sympatry. Highton collected only unstriped *P. ventralis* at a site where they co-occurred with the striped *P. serratus*, but observed *P. ventralis* being polymorphic elsewhere. A similar pattern was described between *P. dorsalis* and *P. websteri* in Alabama (Highton 1972, 1985).

The functional significance and mechanisms responsible for the maintenance of the striped/unstriped polymorphism in *Plethodon* have been the subject of much interest, although previous studies on the phenomenon have involved only *P. cinereus*, the Eastern Red-backed Salamander. Although it is unknown whether the striped/unstriped polymorphism in other species of *Plethodon* has a similar genetic basis, Highton (1975) suggested that dorsal coloration involves the epistatic interaction of two or more loci. A few studies (Lotter and Scott 1977; Moreno 1989; Gibbs & Karraker 2006) have provided evidence for an association between dorsal coloration and thermal tolerance, as well as differences in temperature-dependent foraging behavior and metabolic rates between the morphs of *P. cinereus*, suggesting that the polymorphism may be maintained indirectly via temperature-mediated selection acting directly on physiological traits that are correlated with the presence or absence of a stripe. Other studies

3

have also suggested a possible link between dorsal coloration and behavior, in both anti-predator and mating contexts (Venesky and Anthony 2007; Anthony et al. 2008). Experimental evidence from Fitzpatrick et al. (2009) suggests that a striped/unstriped polymorphism can be maintained by negative frequency-dependent selection, implying crypticity of both morphs, and that a survival advantage of the rare morph might be related to the formation of search images by ground-foraging birds for the more common form.

Within *Plethodon*, character displacement of other ecologically important traits, namely body size and cranial morphology, has also been reported. Several studies have presented evidence for character displacement of body size in a well-studied species pair (*P. cinereus* and *P. hoffmani*), as well as displacement in several cranial dimensions, which were also highly correlated with resource use (Adams 2000; Adams & Rohlf 2000; Jaeger et al. 2002). A study of another species pair showed evidence for character displacement in head shape, as well as supported the hypothesis of aggressive interference as the selective force favoring the observed shifts in head shape of each species in sympatry (Adams 2004).

The documentation of both convergence and character displacement within the genus makes *Plethodon* an ideal study system to investigate both phenomena, as well as to explore further the potential functional significance and evolutionary mechanisms responsible for the maintenance of the striped/unstriped polymorphism. *Plethodon* are good candidates for local adaptation over the spatial scale of this study because they tend to have large population sizes (Burton and Likens 1975) and low dispersal rates (Kleeberger and Warner 1982; Gergits and Jaeger 1990). For example, F_{ST} (based on allozymes) among populations within species is on the order of 0.7 (Larson and Highton 1978; Highton 1997), implying levels of gene flow easily overwhelmed by divergent selection. In this study, I examine the possibility of either character

4

displacement or convergence in cranial morphology between *P. ventralis* and *P. serratus*, in order to explore the potential importance of competition in these two markedly ecologically similar species. I also re-examine Highton's (1972) earlier claim of character displacement in dorsal coloration and pattern between the two species in sympatry to assess whether this pattern still is still observable nearly 40 years since its first documentation. Additionally, I examine the possibility of a link between dorsal coloration and morphology, in light of previous studies that have suggested indirect selection as a possible mechanism for the maintenance of the polymorphism in *P. cinereus* (Moreno 1989; Venesky & Anthony 2007; Anthony et al. 2008).

.

II. Materials and Methods

Study species

Plethodon serratus (Grobman 1944) and *P. ventralis* (Highton 1997) are both small terrestrial plethodontids and are markedly similar in terms of both morphology and ecology. Sexually mature individuals of both species range in size (snout-vent length, SVL) from about 33 to 52 mm (Camp 1988; Meshaka & Trauth 1995; pers. obs.), and similar to other congeners, both exhibit two distinct morphotypes: a "redback" phase (Figure 2), consisting of individuals possessing an easily discernible reddish stripe on the dorsum, and a "leadback," or unstriped phase, which possesses a relatively uniformly colored, brownish dorsum (Petranka 1998). The species are found in quite similar habitats, ranging from mesic hardwood forests to rocky slopes, and can typically be found under cover objects such as logs, rocks, and leaf litter during non-foraging periods throughout the day in the surface-active season (Thurow 1957; Keiser and Conzelmann 1969; Petranka 1998). Although both species can be seen moving about in daylight on rainy days (personal observation), they are thought to forage primarily at night (AmphibiaWeb 2009), and feed on a wide variety of small invertebrate prey (Camp & Bozeman 1981; Bausman & Whitaker 1987). Substantial similarity in seasonal activity is also exhibited by *P. serratus* and *P. ventralis*, with both species rarely found active on the surface during hot summer months (Highton and Grobman 1956; Camp 1988; Wilkinson et al. 1993; Meshaka and Trauth 1995) or during particularly dry periods (personal observation), with both species often seeking subsurface refugia in damp leaf packs in stump holes (Camp 1988) or in caves (Mohr 1952; Miller et al. 1998). Both species also exhibit similar reproductive timing, with courtship

occurring in winter or early spring, followed by subsurface oviposition around midsummer, and emergence in late autumn or early winter (Camp 1988; Miller et al. 1998).

The geographic range of *P. serratus* consists of several distinct isolates: one major group of populations in the Salem Plateau of southeastern Missouri, extending partially into southeastern Illinois, another in the Ouachita Mountains of Arkansas and Oklahoma, a "Piedmont and Blue Ridge group" extending from southwestern North Carolina and partially into southeast Tennessee, and southward into northwest Georgia and northeast Alabama, and a small region of isolated populations in the Red River Valley and Upper Delta regions of central Louisiana (Petranka 1998). *P. ventralis*, formerly considered to be conspecific with *P. dorsalis* until its formal description by Highton (1997), ranges southward from south-central Kentucky and southwestern Virginia southward into eastern Tennessee, northwestern Georgia, northern Alabama, and into northeastern Mississippi (Highton 1997; Dodd 2004). *Plethodon serratus* and *P. ventralis* have a narrow overlap of their geographic ranges restricted to a small portion of the southern Appalachians, including the Whiteoak Sink and Bull Cave regions of Great Smoky Mountains National Park (Huheey and Stupka 1967; Dodd 2004).

Sampling

I collected data from a total of 193 individuals from six sites: two where *P. ventralis* and *P. serratus* co-occur in Great Smoky Mountains National Park (sympatric sites), two where only *P. ventralis* occurs (allopatric *P. ventralis* sites), and two allopatric *P. serratus* sites (Figure 3). All individuals were located during daytime searches by looking under cover objects such as rocks and logs, as well as sifting through leaf litter. Upon capture, each individual was measured for snout-vent length (SVL, measured as the linear distance from the tip of the snout to the

7

posterior angle of the vent), as well as several cranial morphological traits for use in linear and geometric morphometric methods as described below.

Evaluation of dorsal coloration as either continuous or polymorphic in *Plethodon ventralis*

All 193 individuals in this study were scored as either the striped or unstriped form, and abundance of each morph recorded at each site. Although all individuals of *P. serratus* were easily distinguishable as either the striped or unstriped form, variation in dorsal pattern in *P. ventralis* appeared to be potentially continuous, rather than representing a distinct dimorphic state. In "unstriped" individuals, there is often still a faint indication of a stripe, which varies in terms of the degree of pigmentation, as well as the length of the region that is in conspicuous contrast to the overall ground color of the dorsum (Petranka 1997; Dodd 2004). To examine this trait more clearly in individuals of *P. ventralis*, I measured the length of the reddish dorsal pigmentation from the base of the head to the point of termination of the contiguous black outline of the "stripe" (Figure 4). Because of the possible confounding effect of salamander size, stripe lengths were converted to a value representing the proportional length of the stripe, relative to total SVL. In cases where the stripe length was longer than SVL (because the stripe extended beyond the vent and onto an individual's tail), proportional stripe length was recorded as being equal to 1.0, because individuals' tail lengths could vary owing to previous tail autotomy, and damaged tails could be in differing stages of regrowth, thus leaving a degree of uncertainty about how far the stripe would have extended on an intact tail.

Traditional (linear) morphometrics

All individuals were placed in a standardized position onto a gridline-marked shelf in a photography box, in which was mounted a 5MP Sony Cybershot DSC-F707 digital camera on a

8

macro focusing rail, and digital photographs of the left lateral aspect of the head were obtained

for each individual. Ten external landmarks (Figure 5) were digitized from photographs of each

individual (from Maerz et al. 2006) using the program tpsDig (Rohlf 2006), incorporating

scaling effects through the use of the marked gridlines referenced above, and tpsDig's "Set

Scale" feature. Only 150 of the total 193 individuals were used for analysis of morphometric

data, owing to the inferior image quality of photographs of 43 individuals, which made location

of one or more landmarks difficult. Using the aforementioned external landmarks, I measured

several potentially biologically relevant linear dimensions, such as jaw length (JL), posterior

head length (PL), cranial depth (CD), parietal depth (PD), and the lengths of the opening (OL)

and closing (CL) in-levers of the jaw mechanism (See Table 1, which can be found with all other

tables, in Appendix A), using the scaling information obtained from tpsDig (Rohlf 2006) as

described above.

Adams & Rohlf (2000) suggested that the ratio of the length of the dentary to that of the

squamosal might be associated with the feeding performance and dietary habits of terrestrial

salamanders. In a biomechanical model based on simple lever mechanics, increases in the value

of this ratio would result in a less forceful, but faster closure of the jaw, and decreases in this

ratio would result in slower, but more forceful jaw closure, with suggestions that differences in

speed of jaw closure might be related to differences in effectiveness at dispatching different size

classes of prey. This was supported by a later study by Maerz et al. (2006), who found

significant correlations between a similar external measure (external length of the jaw relative to

the length of the back of the head) and relative abundances of consumed prey items of different

size classes. I calculated this ratio (JR), as well as the ratios of the lengths of opening (OR) and

closing (CR) in-levers of the jaw mechanism to the length of the jaw, which might also be of biomechanical significance with regards to prey capture.

Geometric morphometrics

Landmark-based geometric morphometrics, in which the overall shapes of biological entities can be quantified and compared as functional wholes, in lieu of multivariate consideration of several linear measurements, were also used to address the questions presented in this study. In studies utilizing geometric morphometrics, the "shape" of organisms simply refers to Kendall's (1977) definition of "all the geometric information that remains when location, scale, and rotational effects are filtered out from an object." Geometric morphometric analyses were conducted using the landmarks digitized on the same 150 individuals from the linear morphometric analysis. Because the jaw and the skull are articulated structures, differences in position of the jaw among individuals could have led to improper conclusions from analyses of shape simply using the raw coordinates of the digitized landmarks. To circumvent this problem, I used the separate subset method outlined by Adams (1999) to subject the landmark coordinates from the skull and the jaw to separate generalized Procrustes alignments (GPA), in MorphoJ (Klingenberg 2008), with each subset containing landmark 1 (Figure 5) as the articulation point. Through the use of Generalized Procrustes alignment, the non-shape information of location, scale, and position are removed from a configuration of landmarks, thus allowing analysis of overall "shape" as defined by Kendall. Another variable, the ratio of centroid sizes of the skull and jaw, was calculated for each individual, and then used to recombine the subsets for an analysis of overall head shape as a single dataset, resulting in the removal of positional effects (due to rotation about the articulation point by the jaw) in

10

individual photographs, as well as ensuring, upon recombination of the subsets, that the jaws

depicted by the landmarks were scaled properly in relation to the rest of the head.

Statistical Methods

Using the data on stripe length (as a proportion of SVL) from *P. ventralis* pooled across

all sites, I evaluated the hypothesis of a normal distribution of the arcsin-transformed values of

stripe proportion using the Shapiro-Wilk test for normality in SAS/STAT® software version

9.1.3 (SAS Institute Inc., Cary, NC, USA, copyright 2000-2004). I also conducted a dip test

(Hartigan & Hartigan 1985), which assesses the hypothesis of multimodality versus the null

hypothesis of a uniform distribution, using the "diptest" package (Maechler 2009) in R software,

version 2.8.1 (R Core Development Team 2008), followed by visual inspection of a frequency

histogram of the proportional stripe length data (Figure 6).

Because there existed a strong possibility of an effect of size, as well as allometric scaling

of each linear measurement during development, I first corrected for the effect of salamander

size by conducting separate regressions of log-transformed values of each trait on log-

transformed SVL for each species, retaining the obtained residuals for further analysis of all

linear data. Two limitations affect statistical analyses of these data. First, the nature of the data

precluded a single analysis of both species because sites could not be analyzed simultaneously as

nested (within species and geography) and shared (the sympatric sites were the same for each

species). A more desirable design would have been two or more independent transects across

contact zones between species, allowing transect to be treated as a random block. However, this

was not biologically possible for this study system because their range overlap is restricted to the

small part of the Southern Appalachians where this study was performed (Highton 1997;

11

Petranka 1998). Second, random effects are not correctly accounted for in multivariate analyses (R. Muenchen, personal communication, 19 March 2009).

To evaluate the alternative hypotheses of character displacement or convergence relative to the null hypothesis that variation in each species is independent of the presence or absence of the other for the linear dataset, separate analyses were performed for *P. ventralis* and *P. serratus*, which at least allowed for the evaluation of whether either species exhibited significant shifts in morphology in sympatry relative to allopatric populations. To correctly incorporate the random effect of site into each of these analyses, I examined all linear measurements for each species via separate univariate mixed-model ANOVAs using Proc Mixed in SAS, using geography (sympatry versus allopatry) as a fixed effect, and site as a random effect. Because body size alone might also be an important, biologically relevant character, I also tested the alternative hypothesis of character displacement or convergence between the two species in terms of body size (logSVL), using separate mixed-model ANOVAs as described above.

Because salamander size might be an important factor in determining head shape, and because allometric changes in shape might vary among sites, between species, or in sympatry versus allopatry, it was important to consider correction for these factors where necessary. I performed a MANCOVA analysis of the GPA-aligned coordinates of each specimen, to test the effects of species, geography, and site (nested within species and geography), and using log-transformed centroid size (logCS) as a covariate, using Proc GLM in SAS software. All possible interaction effects, given the nested nature of site, were also included in the model. Although this analysis did not allow site to be simultaneously treated as both nested and shared (with the model treating sympatric sites as different sites for the two species), it can be reasoned that this measure conservatively allowed for the testing for possible heterogeneity of slopes of allometric

changes in shape between species within and among sites. The GPA-aligned coordinates were then regressed on log-transformed centroid size in MorphoJ (Klingenberg 2008), a Java-based software package that allows one to conduct most major types of analyses of geometric morphometric data, and regression residuals were obtained, after accounting for potentially significant interaction effects indicating differences in allometry (discussed in further detail in the results). A canonical variate analysis (CVA) of the residuals from the regressions of the Procrustes coordinates on log-transformed centroid size was then conducted in MorphoJ, as well as a 10000-round permutation test to compare Mahalanobis and Procrustes distances between mean shapes of each group (allopatric *P. serratus*, sympatric *P. serratus*, sympatric *P. ventralis*, and allopatric *P. ventralis*).

In order to explore the possibility of a link between dorsal coloration and cranial morphology, I also conducted a canonical variate analysis of the residuals of the regressions of GPA coordinates on log-transformed centroid size in MorphoJ, using allopatric striped *P. serratus*, allopatric unstriped *P. serratus*, sympatric *P. serratus* (all of which were striped), sympatric *P. ventralis* (all of which, except for one, were of the unstriped form, which was excluded), and allopatric striped and unstriped *P. ventralis* as groups.

Finally, to evaluate Highton's claim of character displacement in morph frequency between the two species in sympatry, I compared the number of striped and unstriped individuals of each species in each sympatric site to the numbers of each morph at each allopatric site, as well as the morph frequencies between species within each sympatric site using Fisher's exact tests. As the Bonferroni method is highly (and potentially overly) conservative, I also compared the morph frequencies of each species at each sympatric site to each allopatric site for that species, as well as the morph frequencies between species within sympatric sites using several

13

alternative methods allowing the combination of inferences from independent tests, including

Fisher's combined method, as well as the z-transform method (both unweighted and weighted)

outlined by Whitlock (2005). In determining the combined p-values, I partitioned tests into sets

within which each test is independent and addresses the same hypothesis. For example, SC vs

WO and SC vs RM are independent tests of the null hypothesis that morph frequencies of *P.*

ventralis from Second Creek (an allopatric site) are the same as for sympatric sites. SI vs WO

and SI vs RM are also independent tests of allopatric vs. sympatric *P. ventralis*, but the two sets

are not independent because WO and RM are used in both. Therefore p-values of the tests of

morph frequencies of each allopatric site within a species compared to each of the morph

frequencies of that species at each sympatric site were combined, as well as the p-values for tests

of morph frequencies between species for both sympatric sites. Because counts of zero would

result in standard error estimates of zero, I added one to the count of each morph for each species

before estimating standard errors for the weighted z-transform method.

III. Results

The distribution of proportional stripe length in *P. ventralis* deviated significantly from normality (W=0.842878, P<0.0001). Additionally, Hartigan's dip test resulted in rejection of the null hypothesis of unimodality (D=0.0570, P≈0.02), and the frequency histogram had a distinctly bimodal appearance (Figure 6), so I felt comfortable continuing to refer to dorsal coloration as a polymorphism within this species. Individuals with a proportional stripe length less than 0.5 were scored as "unstriped", and those with a proportional stripe length greater than 0.5 scored as "striped".

In the linear dataset, there were no significant differences of any of the size-corrected linear measurements or ratios for either species in sympatry versus allopatry (Table 2). There were also no significant effects of geography on body size (logSVL) for either *P. serratus* ($F_{1,2}$=0.86, P=0.4509) or *P. ventralis* ($F_{1,2}$=0.58, P=0.5255).

The MANCOVA of the GPA-aligned coordinates (Table 3) revealed significant main effects of site, as well as a significant interaction effect between site and log-transformed centroid size (logCS). Because there was a significant interaction effect between logCS and site, I used separate regressions of the GPA-aligned coordinates on logCS for each site to reduce the effects of site-specific allometry on the ability to detect differences between groups. A permutation test of the null hypothesis of independence between shape coordinates and log-transformed centroid size was highly significant (P<0.001), but logCS only explained 4.1758% of the variance in GPA coordinates. In a CVA of the resulting residuals in MorphoJ (Figure 7), the first two canonical axes cumulatively explained 96.96% of the variation among groups. A 10,000 round permutation test of Mahalanobis distances among groups showed significant

15

differences between all groups (allopatric *P. serratus*, sympatric *P. serratus*, sympatric *P. ventralis*, and allopatric *P. ventralis*), although the Mahalanobis distance between *P. serratus* and *P. ventralis* in sympatry were smaller than those for all other comparisons. The permutation test of Procrustes distances among groups also revealed significant differences among all group pairs, with the exception of the comparison of sympatric *P. serratus* and sympatric *P. ventralis*, which was also the comparison displaying the smallest Procrustes distances in all groupwise comparisons (Table 4). Additional tests for a potential link between cranial morphology and dorsal coloration showed no significant differences between the striped and unstriped forms of either species (Table 5).

For *P. serratus*, there were no significant differences in morph frequencies between allopatric and sympatric sites using either the separate site-wise comparisons or the combined tests, with the exception of near-significant differences in both separate site-wise and combined comparisons involving the allopatric John Oliver Cabin site (which had a significantly higher proportion of unstriped individuals in the combined test using the weighted z-transform method). However, there were significant differences in the frequency of the striped morph for *P. ventralis* in sympatry versus allopatry for all of the combined tests, as well as for both of the comparisons of each of the allopatric sites to the sympatric site at Rich Mountain Gap. Additionally, tests for differences in the frequencies of the striped morph between the two species in sympatry were all highly significant (Table 6).

IV. Discussion

Overall, the results of this study suggest a high degree of convergence of cranial morphology, with head shapes of *Plethodon serratus* and *P. ventralis* being more similar in sympatry (Figures 6 and 7). However, dorsal color pattern was more divergent in sympatric sites than between allopatric sites. This suggests that some traits might be responding to similar selection pressures or display similar plastic responses generated by general physical and climatic factors, whereas other traits might be responding to divergent selection imposed by interactions between the species. However, these results are unexpected in that features associated with feeding (cranial morphology) are expected to often exhibit character displacement if dietary resource partitioning is an important mechanism of coexistence, whereas coloration might be expected to show local convergence if its primary function is camouflage or aposematism.

Convergence in overall head shape between the two species in sympatry could be the product of local adaptation associated with the capture and processing of different prey types across sites, although dietary studies would be required to address this possibility. Both species in this study are generalists with regards to overall diet and feed on a wide variety of invertebrate taxa (Camp & Bozeman 1981; Bausman & Whitaker 1987). Prey size distributions and community compositions might vary substantially throughout the range of these species, and it could be that both are locally adapted to the prey community. Abrams (1987b) suggested that convergence is a likely response to competition for a shared resource when a resource is nutritionally nonsubstitutable. However, given the broadly generalist diets of each of these species, this seems to be an unlikely explanation for the observed convergence in this system.

17

Assessment of both taxonomic and size variation within prey communities in sympatric sites could further resolve this matter.

The lack of support for character displacement (and strong signature of convergence) in overall shape in sympatry might instead reflect a lack of competition for prey items in sympatry, concordant with several studies that have suggested that food is not limiting for salamanders, and that space (such as refugia beneath cover objects) is likely a more contested resource (Dumas 1956; Jaeger 1970; Jaeger 1972; Fraser 1976a; 1976b; Maiorana 1978; Wrobel et al. 1980; Hairston 1981). Cranial structures in terrestrial plethodontids play important roles not only in feeding, but in intra- and interspecific interactions, such as territorial defense and antipredator and mating/courtship behavior, which may impose differing selective pressures in differing environments, where density of conspecifics and congeners, density and composition of predator communities, and mating opportunities, as well as many other factors may vary widely. One interpretation of the results depicted in Figure 7 is that sympatric *P. ventralis* and *P. serratus* both show more robust jaws and posterior regions of the head compared to their respective mean shapes in allopatry. A possible mechanism for explaining such convergence in sympatry with regards to increased jaw and posterior head robustness could be that this might reflect the potential effects of increased competitive ability of both species in sympatry (Gill 1974). Adams (2004) found a significant correlation between head shape and aggression in two congeners (*P. jordani* and *P. teyahalee*), with individuals with more robust heads displaying more aggressive behavior. Mathis et al. (2000) also stated that both male and female individuals of *P. angusticlavius*, whose status as a sister species to *P. ventralis* is well supported (Wiens et al. 2006), actively defend feeding territories. In a discussion of character convergence, Cody (1973) mentions that in situations where interspecific territoriality is favored (in which both species

18

benefit from increased spatial separation between individuals), character convergence is a potential outcome. The overall convergence in shape could reflect the effects of both a shared resource base (including suitable cover objects used as refugia), as well as selection for greater territorial aggression in the two species. However, it is not clear that interspecific competition for territories should be more intense than intraspecific competition in allopatry, as there is no evidence that the overall density of salamanders is higher at sympatric sites.

Emberton (1995) suggested that sympatric convergence in the shell characters of two species of Appalachian snails could be a result of similar correlations between climate and shell shape. The elevations of sympatric sites in the present study were somewhat higher than those of the allopatric sites, implying a systematic association between sympatry and climate. Though it is unlikely that head shape directly reflects adaptation to climate, observed convergence of head shape in sympatry could result from similar genetic correlations or plasticity in developmental responses to local climate. Effects of seasonal temperatures on relative growth rates of different dimensions could result in purely phenotypic convergence. Further studies of allometry in development, differences in arthropod community composition and salamander diets between sites, as well as of potential differences in aggressive behavior between sympatric and allopatric individuals of each species, would be useful in further elucidating the mechanism(s) responsible for the overall convergence in head shape in these species.

Also quite interesting is the apparent character displacement in dorsal coloration in sympatry. This pattern was noticed by Highton, both between the focal species of this study, and in sympatric populations of *P. websteri* and *P. ventralis* in Alabama (Highton 1972, 1985). My data show strong evidence in support of Highton's original claim. Possible explanations of such

a pattern in *Plethodon* depend on the functional significance of the stripe, as well as the

mechanism responsible for maintenance of the polymorphism in allopatric populations.

Almost all previous work has been directed at the similarly polymorphic *P. cinereus*.

Moreno (1989) documented differences in *P. cinereus* morph frequency distributions at different

temperatures, as well as differences in standard metabolic rates between morphs, and suggested

that differences in temperature-dependent foraging behavior and mortality rates might be

responsible for the maintenance of the polymorphism. Although Petruzzi et al. (2006) suggested

that the relationship between metabolism and dorsal coloration is neither as simple nor as

consistent as that originally described by Moreno, Gibbs and Karraker (2006) present highly

convincing evidence of a relationship between frequencies of the striped/unstriped morphotypes

and climate in *P. cinereus* (also see Lotter and Scott 1977). In a meta-analysis of over 50,000

individuals, Gibbs and Karraker (2006) confirmed that frequencies of striped individuals were

higher at higher latitudes and altitudes. In addition, they showed a marked decrease in the

frequency of striped individuals over a 96-year period, which they interpreted as a result of

global warming and the association of striped individuals with cooler microclimates.

Though these results are quite interesting, there is no reason to assume that a similar

relationship between temperature and dorsal coloration also holds true for the focal species of

this study. Although a general relationship between dorsal coloration and thermal tolerances

might exist, and would be of interest in terms of selection, none of the aforementioned studies

truly address how such a mechanism could be responsible for the maintenance of the

polymorphism within local populations. Variation within populations might be maintained by a

balance between immigration and selection, if different morphs are selectively favored in

different environments. However, immigration is unlikely to be capable of maintaining high

frequencies of potentially maladapted phenotypes in local populations, particularly in light of the low estimates of gene flow in small eastern *Plethodon* (Larson and Highton 1978). The results of a study by Venesky and Anthony (2007) suggest a link between dorsal coloration and anti-predator behavior in *P. cinereus*, with the two morphs differing significantly in their responses to snake predators. In their study, they found that striped individuals were more likely to remain immobile and spent significantly more time in threat postures, while the unstriped morph relied significantly more often on mobility for escape. Whether color pattern differences actually function to enhance the effectiveness of alternative behaviors (as, for example in gartersnakes: Brodie 1992) is unknown in this case. In any case, association between the dorsal stripe and behavior or physiology begs the question of what might maintain polymorphism or promote character displacement.

Fitzpatrick et al. (2009) argued that the most reasonable hypothesis to explain the existence of a visual polymorphism across such a wide geographic and phylogenetic range is that selection acts directly on appearance. Several studies (Brodie and Brodie 1980; Tilley et al. 1982; Cassell and Jones 2005) have suggested that the extreme red coloration in the erythristic morph of *P. cinereus* is aposematic, possibly representing mimicry of the toxic red eft stage of the Eastern Newt (*Notophthalmus viridescens*). Though this seems to be a logical explanation for the occurrence of the erythristic morph, it is a relatively unsatisfactory at explaining the much more common occurrence of the less red striped morph, as Brodie and Brodie (1980) observed equal rates of predation on striped *P. cinereus* and "non-aposematic", palatable individuals of *Desmognathus ochrophaeus* not possessing red dorsal coloration.

There also exists the possibility that striped and unstriped patterns reflect different ways of being cryptic (Venesky and Anthony 2007; Fitzpatrick et al. 2009). If this is the case, habitat

21

heterogeneity with regards to substrate composition and/or color might promote polymorphism if distinct "crypticity niches" have independent density regulation (Levene 1953). There is no evidence that striped and unstriped *Plethodon* are found on different substrates within a site and it might be that the grain of heterogeneity on the forest floor is such that the background is more or less uniformly complex rather than composed of distinct color patches that can be partitioned between forms. Nevertheless, habitat heterogeneity remains an interesting possibility for explaining dorsal color pattern in salamanders.

Another, perhaps simpler, and empirically supported mechanism for the maintenance of polymorphism within populations involves negative frequency-dependent, or apostatic, selection. Whether predation pressure results in maintenance of polymorphism within a population of a polymorphic species, or the elimination of all but one morphotype, depends on many factors, related to the characteristics of both predators and prey. For example, optimal predator behavior, including search rate, decision to attack a potential prey item post-detection, etc. is dependent on properties of the morphs of the prey, such as their detectability, food values, handling times, and risks to the predator. Polymorphism within a prey species, or fixation of a prey population to monomorphism thus depends on an interaction of many factors, as well as the relative functional significance of the polymorphism in the "detection-identification-subjugation-consumption" sequence of events in a predator-prey encounter (Endler 1986, 1988). Bond (2007) supplies an excellent review of both theoretical and experimental examples that suggest the importance of apostatic selection in maintaining polymorphism within populations. In a recent study by Fitzpatrick et al. (2009), clay models (with attached food rewards) of both striped and unstriped forms of *Plethodon* were used to address the hypothesis of apostatic selection by avian visual predators. After separate initial learning periods in which each particular morph was nine times

22

more abundant than the other, the initially most common morph was attacked disproportionately more frequently than the other when they were presented in equal frequencies.

A logical parallel can be drawn between the maintenance of polymorphism within a species and coexistence of similar species (Clarke 1962). Either apostatic selection or a Levene-type model can explain species coexistence just as easily as polymorphism within species. In the context of this study, however, an interesting question combining aspects of both maintenance of polymorphism within populations, as well as species coexistence, arises: what are some possible mechanisms that could result in the observed partitioning of morph frequencies between the focal species in sympatry? In a simple Leslie/Gower model extended by Fitzpatrick for two polymorphic species (unpublished; see Figure 9 and Appendix C), character displacement arises as a consequence of negative frequency-dependent predation under several initial conditions, namely when intraspecific competition is more intense than interspecific competition, and when the initial morph frequencies are disparate. This second condition is highly likely, given that the unstriped form of *P. serratus* is quite rare in most populations, with the exception of several populations in Alabama and Georgia, and in the Methodist Church Pond and John Oliver cabin areas in the Great Smoky Mountains (Highton 1962; Highton and Webster 1976; Petranka 1998; Dodd 2004; this study). In the model, when one form of one species is initially common, rare form advantage owing to frequency-dependent predation facilitates an increase in density of the rare form, but intraspecific competition tends to counteract the rare form advantage. As a result, the rare form of the opposite species has the highest fitness. Given these results, frequency-dependent predation could explain both the maintenance of polymorphism within populations of *P. ventralis*, as well as the partitioning of morph frequencies between the focal species in sympatry.

Though the results of this study may be interesting, both on their own, and in light of previous work on *P. cinereus*, they perhaps raise many more questions regarding the focal species involved. To date, the genetic bases of both the polymorphism and cranial morphology of both *P. serratus* and *P. ventralis* are unknown. In addition, associations of dorsal coloration with other traits, such as climatic variables, anti-predator behavior, etc. have not been explored for these species. More detailed studies of diet, as well as of predation rates by both visual and non-visual predators in these species, both in sympatry and allopatry, also offer interesting opportunities for further studies. Overall, it is in the opinion of the author that the phenomena of both ubiquitous polymorphism, as well as coexistence of ecologically similar species, within the genus *Plethodon* will continue to offer fascinating avenues for future research for years to come.

References

Abrams, P. A. 1987. Alternative models of character displacement and niche shift. I. Adaptive shifts in resource use when there is competition for nutritionally nonsubstitutable resources. *Evolution* 41:651-666.

Abrams, P.A. 1987. Alternative models of character displacement and niche shift: II. Displacement when there is competition for a single resource. *The American Naturalist* 130:271-282.

Adams, D.C. 1999. Methods for shape analysis of landmark data from articulated structures. *Evolutionary Ecology Research*. 1:959-970.

Adams, D.C. 2000. Divergence of trophic morphology and resource use among populations of *Plethodon cinereus* and *P. hoffmani* in Pennsylvania: a possible case of character displacement. Pp. 383-394 in *TheBiology of Plethodontid Salamanders.* (R.C. Bruce, R.J. Jaeger, and L.D. Houck, eds.). Klewer Academic/Plenum. New York.

Adams, D.C. 2004. Character displacement via aggressive interference in Appalachian salamanders. *Ecology*. 85:2664-2670.

Adams, D.C., and F.J. Rohlf. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences* 97(8):4106-4111.

Allen, J.A. 1877. The influence of physical conditions in the genesis of species. *Radical Review* 1:108-140.

AmphibiaWeb: Information on amphibian biology and conservation. [web application]. 2009. Berkeley, California: AmphibiaWeb. Available: http://amphibiaweb.org/. (Accessed: May 20, 2009).

Anthony, C.D., M.D. Venesky, and C.M. Hickerson. 2008. Ecological separation in a

   polymorphic terrestrial salamander. *Journal of Animal Ecology* 77(4):646-653.

Bausmann, G.A., and J.O. Whitaker Jr. 1987. Studies of the habitat and food of sympatric

   populations of *Plethodon cinereus* (Green) and *Plethodon dorsalis* (Cope) in south central

   Parke County, Indiana. *Proceedings of the Indiana Academy of Science* 97:513–523.

Bergmann, C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse.

   Göttinger Studien 3(1), 595-708.

Bond, A.B. 2007. The evolution of color polymorphism: crypticity, searching images, and

   apostatic selection. *Annual Review of Ecology, Evolution, and Systematics* 38:489-514.

Bonsall, M.B., and M.P. Hassell. 1997. Apparent competition structures ecological assemblages.

   Nature, 388:371-373.

Brodie, E.D., III. 1992. Correlational selection for color pattern and antipredator behavior in the

   garter snake *Thamnophis ordinoides*. *Evolution* 46:1284-1298.

Brodie, E.D., Jr. and E.D. Brodie, III. 1980. Differential avoidance of mimetic salamanders by

   free-ranging birds. *Science* 208:181-182.

Brown, W.L., and E.O. Wilson. 1956. Character displacement. *Systematic Zoology* 5:49-65.

Burton, T.M., and G.E. Likens. 1975. Salamander populations and biomass in the Hubbard

   Brook Experimental Forest, New Hampshire. *Copeia* 1975:541–546.

Camp, C.D. 1986. Distribution and habitat of the southern red-back salamander, *Plethodon

   serratus* Grobman (Amphibia: Plethodontidae), in Georgia. *Georgia Journal of Science*

   44:136–146.

Camp, C.D. 1988. Aspects of the life history of the southern red-back salamander *Plethodon serratus* Grobman in the southeastern United States. *American Midland Naturalist* 119:93–100.

Camp, C.D., and L.L. Bozeman. 1981. Foods of two species of *Plethodon* (Caudata: Plethodontidae) from Georgia and Alabama. *Brimleyana* 6:163–166.

Cassell, R.W., and M.P. Jones. 2005. Syntopic occurrence of the erythristic morph of *Plethodon cinereus* and *Notophthalmus viridescens* in Pennsylvania. *Northeastern Naturalist* 12:169-172.

Christiansen, K.A. 1961. Convergence and parallelism in cave Entomobryinae. *Evolution* 19:529-537.

Clarke, B. 1962. Natural selection in mixed populations of two polymorphic snails. *Heredity* 17:319–45.

Cody, M.L. 1973. Character convergence. *Annual Review of Ecology and Systematics* 4:189-211.

Cody, M.L., and J.H. Brown. 1970. Character convergence in Mexican finches. *Evolution*, 24:304-310.

Culver, D.C., and D.W. Fong. 1986. Why cave animals all look alike. *Stygologia* 2:208-216.

Cushing, J.M., S. Levarge, N. Chitnis, and S.M. Henson. 2004. Some discrete competition models and the competitive exclusion principle. *Journal of Difference Equations and Applications* 10:1139-1151.

Dayan, T., and D. Simberloff. 1994. Character displacement, sexual size dimorphism, and morphological variation among the mustelids of the British Isles. *Ecology* 75:1063-1073.

Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. *Ecology Letters* 8:875-894.

Dayan, T., D. Simberloff, E. Tchernov, and Y.Yom-Tov. 1989. Inter- and intraspecific character displacement in mustelids. *Ecology* 70:1526-1539.

Dayan, T., D. Simberloff, E. Tchernov, and Y.Yom-Tov. 1990. Feline canines: community-wide character displacement in the small cats of Israel. *The American Naturalist* 136:39-60.

Dayan, T., D. Simberloff, E. Tchernov, and Y.Yom-Tov. 1992. Canine carnassials: character displacement among the wolves, jackals, and foxes of Israel. *Biological Journal of the Linnean Society* 45:315-331.

Deban, S.M., and S.B. Marks. 2002. Metamorphosis and evolution of feeding behaviour in salamanders of the family Plethodontidae. *Journal of Biomechanics* 35:1007-1009.

Dodd, C. K., Jr. 2004. The amphibians of Great Smoky Mountains National Park. University of Tennessee Press, Knoxville. xvii + 283 pp.

Dumas, P.C. 1956. The ecological relations of sympatry in *Plethodon dunni* and *Plethodon vehiculum*. *Ecology* 37:484–495.

Emberton, K.C. 1995. Sympatric convergence and environmental correlation between two land-snail species. *Evolution* 49:469–475.

Endler, J.A. 1986. Defense against predation. In *Predator-prey relationships, perspectives and approaches from the study of lower vertebrates* (ed. M.E. Feder & G.V. Lauder), pp.109-134. University of Chicago Press.

Endler, J.A., and J.J.D. Greenwood. 1988. Frequency-dependent predation, crypsis, and aposematic coloration [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 319:505-523.

Fitzpatrick, B.M., K. Shook, and R. Izally. 2009. Frequency-dependent selection by wild birds promotes polymorphism in model salamanders. *BMC Ecology* 9:12.

Fox, J.W., and D.A. Vasseur. 2008. Character convergence under competition for nutritionally-essential resources. *The American Naturalist* 172(5):667-680.

Fraser, D.F. 1976a. Coexistence of salamanders in the genus *Plethodon*: a variation of the Santa Rosalia theme. *Ecology* 57:238–251.

Fraser, D.F. 1976b. Empirical evaluation of the hypothesis of food competition in salamanders of the genus *Plethodon*. *Ecology* 57:459–471.

Gergits, W.F., and R.G. Jaeger. 1990. Site attachment by the red-backed salamander *Plethodon cinereus*. *Journal of Herpetology* 24:91-93.

Gibbs, J.P., and N.E. Karraker. Effects of warming conditions in eastern North American forests on red-backed salamander morphology. *Conservation Biology* 20:913-917.

Gill, D.E. 1974. Intrinsic rate of increase, saturation density, and competitive ability. II. The evolution of competitive ability. *The American Naturalist* 108:103-116.

Gloger, C.L. 1833. Das Abändern der Vögel durch Einfluss des Klimas. Breslau, Germany.

Gould, S.J. 1969. An evolutionary microcosm: Pleistocene and recent history of the land snail *P.* (*Poecilozonites*) in Bermuda. *Bulletin of the Museum of Comparative Zoology* 138:407-532.

Grant, P.R., and R. Grant. 2006. Evolution of character displacement in Darwin's finches. *Science* 313:224-226.

Grobman, A.B. 1944. The distribution of the salamanders of the genus *Plethodon* in the eastern United States and Canada. *Annals of the New York Academy of Science* 45:261-316.

Hairston, N.G. 1981. An experimental test of a guild: salamander competition. *Ecology* 62:65–72.

Hartigan, J.A., and P.M. Hartigan. 1985. The Dip Test of Unimodality. *Annals of Statistics* 13:70-84.

Highton, R. 1962. Revision of North American salamanders of the genus *Plethodon*. *Bulletin of the Florida State Museum* 6:235–367.

Highton, R. 1972. Distributional interactions among eastern North American salamanders of the genus *Plethodon*. Pp. 139–188. In Holt, P.C. (Ed.), *The Distributional History of the Biota of the Southern Appalachians. Part III: Vertebrates*. Research Division Monograph, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Highton, R. 1985. The width of the contact zone between *Plethodon dorsalis* and *P. websteri* in Jefferson County, Alabama. *Journal of Herpetology* 19:544–546.

Highton, R. 1995. Speciation in eastern North American salamanders of the genus *Plethodon*. *Annual Review of Ecology and Systematics* 26:579-600.

Highton, R. 1997. Geographic protein variation and speciation in the *Plethodon dorsalis* complex. *Herpetologica* 53:345–356.

Highton, R., and A.B. Grobman. 1956. Two new salamanders of the genus *Plethodon* from the southeastern United States. *Herpetologica* 12:185–188.

Highton, R., and T.P. Webster. 1976. Geographic protein variation and divergence in populations of the salamander *Plethodon cinereus*. *Evolution* 30:33–45.

Höbel, G., and H.C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of Green Tree Frogs (*Hyla cinerea*). *Evolution* 57(4):894-904.

Holt, R.D. 1977. Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology* 12:197-229.

Huheey, J.E., and A. Stupka. 1967. *Amphibians and Reptiles of the Great Smoky Mountains National Park*. University of Tennessee Press, Knoxville, Tennessee.

Hutchinson, G.E. 1957. Concluding remarks. *Cold Spring Harbor Symposium on Quantitative Biology* 22:415-427.

Jaeger, R.G. 1970. Potential extinction through competition between two species of terrestrial salamanders. *Evolution* 24:632–642.

Jaeger, R.G. 1972. Food as a limited resource in competition between two species of terrestrial salamanders. *Ecology* 53:535–546.

Jaeger, R.G., E.D. Prosen, and D.C. Adams. 2002. Character displacement and aggression in two species of terrestrial salamanders. *Copeia* 2:391-401.

Joron, M., and J. Mallet. 1998. Diversity in mimicry: paradox or paradigm? *Trends in Ecology and Evolution* 13: 461-466.

Keiser, E.D., and P.J. Conzelmann. 1969. The red-backed salamander, *Plethodon cinereus* (Green) in Louisiana. *Journal of Herpetology* 3:189–191.

Kendall, D.G. 1977. The diffusion of shape. *Advances in Applied Probability* 9:428-430.

Kleeberger, S.R., and J.K. Werner. 1982. Home range and homing behavior of *Plethodon cinereus* in Northern Michigan. *Copeia* 1982:409-415.

Klingenberg, C.P. 2008. MorphoJ. Faculty of Life Sciences, University of Manchester, UK. http://www.flywings.org.uk/MorphoJ_page.htm

Larsen, J.H., Jr., J.T. Beneski, Jr., and D.B. Wake. 1989. Hyolingual feeding systems of the Plethodontidae: Comparative kinematics of prey capture by salamanders with free and attached tongues. *The Journal of Experimental Zoology* 252:25-33.

Larson, A. and R. Highton. 1978. Geographic protein variation and divergence in the salamanders of the *Plethodon welleri* group (Amphibia: Plethodontidae). *Systematic Zoology* 27:431–448.

Leslie, P.H., and J.C. Gower. 1958. The properties of a stochastic model for two competing species. *Biometrika* 45:316-330.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. *The American Naturalist* 87:331-333.

Lockridge Mueller, R., J.R. Macey, M. Jaekel, D.B. Wake, and J.L. Boore. 2004. Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proceedings of the National Academy of Sciences* 101(38):13820-13825.

Lotter, F., and N.J. Scott. 1977. Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. *Copeia* 1977:681–690.

Macior, L.W. 1974. Behavioral aspects of coadaptations between flowers and insect pollinators. *Annals of the Missouri Botanical Garden* 61(3):760-769.

Maechler, M., based on Fortran and S-plus from Dario Ringach (NYU.edu). 2009. diptest: Hartigan's dip test statistic for unimodality – corrected code. R package version 0.25-2.

Maerz, J.C., E.M. Myers, and D.C. Adams. 2006. Trophic polymorphism in a terrestrial salamander. *Evolutionary Ecology Research* 8:23-35.

Maglia, A.M., and R.A. Pyles. 1995. Modulation of prey-capture behavior in *Plethodon cinereus* (Green) (Amphibia: Caudata). *The Journal of Experimental Zoology* 272:167-183.

Maiorana, V.C. 1978. Differences in diet as an epiphenomenon: space regulates salamanders. *Canadian Journal of Zoology* 56(5): 1017–1025

Maerz, J.C., E.M. Myers, and D.C. Adams. 2006. Trophic polymorphism in a terrestrial salamander. *Evolutionary Ecology Research* 8:23-35.

Mallet, J., and L.E. Gilbert. 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biological Journal of the Linnean Society*, 55: 159-180.

Marvin, G.A. 1998. Territorial behavior of the plethodontid salamander *Plethodon kentucki*: influence of habitat structure and population density. *Oecologia* 114:133-144.

Mathis, A., D.W. Schmidt, and K.A. Medley. 2000. The influence of residency status on agonistic behavior of male and female Ozark zigzag salamanders, *Plethodon angusticlavius*. *The American Midland Naturalist* 143:245-249.

Meshaka, W.E., Jr., and S.E. Trauth. 1995. Reproductive cycle of the Ozark zigzag salamander, *Plethodon dorsalis angusticlavius* (Caudata, Plethodontidae), in north central Arkansas. *Alytes* 12:175–182.

Miller, B.T., J.L. Miller, D.L. Campbell, and P. Wyatt. 1998. Natural history notes: *Plethodon dorsalis* (zigzag salamander). Reproduction. *Herpetological Review* 29:38–39.

Mohr, C.E. 1952. The eggs of the zig-zag salamander, *Plethodon cinereus dorsalis*. *National Speleological Association Bulletin* 14:59–60.

Moreno, G. 1989. Behavioral and physiological differentiation between the color morphs of the salamander, *Plethodon cinereus*. *Journal of Herpetology* 23:335–341.

Petranka, J.W. 1998. *Salamanders of the United States and Canada.* Smithsonian Institution Press, Washington and London.

Petruzzi, E.E., P.H. Niewiarowski, and F.B. Moore. 2006. The role of thermal niche selection in maintenance of a colour polymorphism in redback salamanders (*Plethodon cinereus*). *Frontiers in Zoology* 3:10.

R Development Core Team. 2008. R: A language and environment for statistical computing. R

Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL
http://www.R-project.org.

Rohlf, F.J. 2006. tpsDig, digitize landmarks and outlines, version 2.10. Department of Ecology
and Evolution, State University of New York at Stony Brook.

Rourke, J., and D.Wiens. 1977. Convergent floral evolution in South African and Australian
Proteaceae and its possible bearing on pollination by nonflying mammals. *Annals of the
Missouri Botanical Garden* 64(1):1-17.

Russo, D., M. Mucedda, M. Bello, S. Biscardi, E. Pidinchedda, and G. Jones. 2007. Divergent
echolocation call frequencies in insular rhinolophids (Chiroptera): a case of character
displacement? *Journal of Biogeography* 34:2129-2138.

SAS Institute Inc., SAS 9.1.3, Cary, NC: SAS Institute Inc., 2000-2004.

Schluter, D., and J.D. McPhail. 1992. Character displacement and speciation in sticklebacks. *The
American Naturalist* 140(1):85-108.

Schluter, D., T.D. Price, and P.R. Grant. 1985. Ecological character displacement in Darwin's
finches. *Science* 227:1056-1059.

Servedio, M.R., and M.A.F. Noor. 2003. The role of reinforcement in speciation: theory and
data. *Annual Review of Ecology and Systematics* 34:339–364.

Swart, C.C., and D.C. Adams. 2004. The role of muscle mass and tooth number in ecological
character displacement between *Plethodon cinereus* and *P. hoffmani* (Caudata:
Plethodontidae). *Herpetologica*. 60:408-413.

Thurow, G.R. 1957. A new *Plethodon* from Virginia. *Herpetologica* 13:59–66.

Tilley, S.G., B.L. Lundrigan, and L.P. Brower. 1982. Erythrism and mimicry in the salamander
Plethodon cinereus. *Herpetologica* 38(3):409-417.

VanValkenburgh, B., and R.K. Wayne. 1994. Shape divergence associated with size
   convergence in sympatric East-African jackals. *Ecology* 75:1567-1581.

Venesky, M.D., and C.D. Anthony. 2007. Antipredator adaptations and predator avoidance for
   two color morphs of the Eastern Red-backed Salamander, *Plethodon cinereus. Herpetologica*
   63:450-458.

Whitlock, M.C. 2005. Combining probability from independent tests: the weighted Z-method is
   superior to Fisher's approach. *Journal of Evolutionary Biology* 18:1368-1373.

Wiens, J.J., T.N. Engstrom, and P.T. Chippindale. 2006. Rapid diversification, incomplete
   isolation, and the "speciation clock" in North American salamanders (genus *Plethodon*):
   testing the hybrid swarm hypothesis of rapid radiation. *Evolution* 60:2585–2603.

Wilkinson, R.F., C.L. Peterson, D. Moll, and T. Holder. 1993. Reproductive biology of
   Plethodon dorsalis in northwestern Arkansas. *Journal of Herpetology* 27:85–87.

Wrobel, D.J., W.F. Gergits, and R.G. Jaeger. 1980. An experimental study of interference
   competition among terrestrial salamanders. *Ecology* 61:1034–1039.

Appendices

Appendix A: Tables

Table 1. List of measurements and corresponding landmarks for endpoints of distances used in

linear morphometric analyses.

Measurement	Description
Jaw length (JL)	Distance between LM1 and LM5
Posterior head length (PHL)	Distance between LM1 and LM11
Cranial depth (CD)	Perpendicular distance from jaw length to LM 9
Parietal depth (PD)	Perpendicular distance from posterior head length to LM10.
Opening in-lever (OL)	Distance between LMs 1 and 2
Closing in-lever (CL)	Distance between LMs 1 and 3
Jaw to head ratio (JHR)	Calculated from above measurements (JL/PHL)
Opening in-lever to jaw ratio (OJR)	Calculated from above measurements (OL/JL)
Closing in-lever to jaw ratio (CJR)	Calculated from above measurements (CL/JL)

Table 2. Tests of linear measurements and ratios in sympatry versus allopatry. Abbreviations are

as listed in Table 1.

Measurement	$F_{1,2}$	P
P. serratus		
JL	0.07	0.8139
PHL	1.71	0.3206
CD	0.44	0.5746
PD	2.38	0.2630
OIL	6.04	0.1332
CIL	0.32	0.6290
JHR	0.21	0.6945
OJR	3.10	0.2205
CJR	1.19	0.3892
P. ventralis		
JL	1.35	0.3655
PHL	0.69	0.4932
CD	0.20	0.6973
PD	12.20	0.0731
OIL	0.52	0.5454
CIL	7.94	0.1063
JHR	0.03	0.8823
OJR	1.12	0.4016
CJR	5.97	0.1344

Table 3. MANCOVA of GPA aligned coordinates.

Effect	Wilks' λ	F	P-Value
Species	0.9117	$F_{18,117} = 0.63$	0.8698
Geography	0.9178	$F_{18,117} = 0.58$	0.9061
Species × Geography	0.8206	$F_{18,117} = 1.81$	0.1334
logCS	0.6328	$F_{18,117} = 3.77$	< 0.0001
Species × logCS	0.9202	$F_{18,117} = 0.56$	0.9185
Geography × logCS	0.8998	$F_{18,117} = 0.72$	0.7812
Species × Geography × logCS	0.8284	$F_{18,117} = 1.35$	0.1722
Site	0.4396	$F_{72,462.43} = 1.27$	0.0085
Site × logCS	0.4334	$F_{72,462.43} = 1.52$	0.0061

Table 4. Mahalanobis (first value, above diagonal) and Procrustes (second value, above diagonal) distances and associated P-values (below diagonal) of mean shapes between sympatric and allopatric populations of each species, after correcting for effects of site-specific allometry.

	AS	SS	SV	AV
AS	–	4.6234	4.3018	5.5118
		0.1080	0.1008	0.1200
SS	0.0000	–	2.1155	5.2877
	0.0000		0.0439	0.0937
SV	0.0000	0.0410	–	4.7823
	0.0000	0.3488		0.0897
AV	0.0000	0.0000	0.0000	–
	0.0000	0.0000	0.0000	

Table 5. Mahalanobis (first value, above diagonal) and Procrustes (second value, above diagonal) distances and associated P-values (below diagonal) of mean shapes between striped and unstriped forms in sympatric and allopatric populations of each species, after correcting for effects of site-specific allometry.

	AS	AUS	SS	SV	ASV	AUV
AS	–	2.7843	4.5712	4.2015	5.3639	5.4185
		0.0429	0.1057	0.0983	0.1221	0.1162
AUS	0.0051	–	5.3915	5.3572	6.5095	6.6059
	0.3021		0.1227	0.1165	0.1376	0.1325
SS	0.0000	0.0001	–	2.1144	5.1369	5.3974
	0.0000	0.0027		0.0439	0.0910	0.0955
SV	0.0000	0.0000	0.0452	–	4.6188	4.8804
	0.0000	0.0013	0.3518		0.0881	0.0911
ASV	0.0000	0.0000	0.0000	0.0000	–	0.8612
	0.0000	0.0000	0.0000	0.0000		0.0181
AUV	0.0000	0.0000	0.0000	0.0000	0.6503	–
	0.0000	0.0000	0.0000	0.0000	0.5984	

Table 6. Comparisons of frequencies of striped versus unstriped individuals. Two-letter codes within comparisons designate sites (SC=Second Creek, SI=Seven Islands, RM=Rich Mountain Gap, WO=White Oak Sink, JO = John Oliver cabin, TS=Turkeypen-Schoolhouse Gap trail junction). Fisher's exact p-values with asterisks were those that were significant after Bonferroni correction.

| Comparison	P-value (Fisher's exact)	P-value (Fisher's combined)	P-value (unweighted z-transform)	P-value (weighted z-transform)
Allopatric versus sympatric *P. ventralis*				
SC vs RM	0.0167*	0.0189	0.0141	0.0018
SC vs WO	0.1641			
SI vs RM	0.0070*	0.0029	0.0029	<0.0001
SI vs WO	0.0742			
Allopatric versus sympatric *P. serratus*				
JO vs RM	0.0806	0.0773	0.0518	0.0155
JO vs WO	0.1839			
TS vs RM	1.0000	1.0000	1.0000	1.0000
TS vs WO	1.0000			
Sympatric *P. serratus* versus sympatric *P. ventralis*				
RMS vs RMV	< 0.0001*	< 0.0001	< 0.0001	<0.0001
WOS vs WOV	0.0020*			

Appendix B: Figures
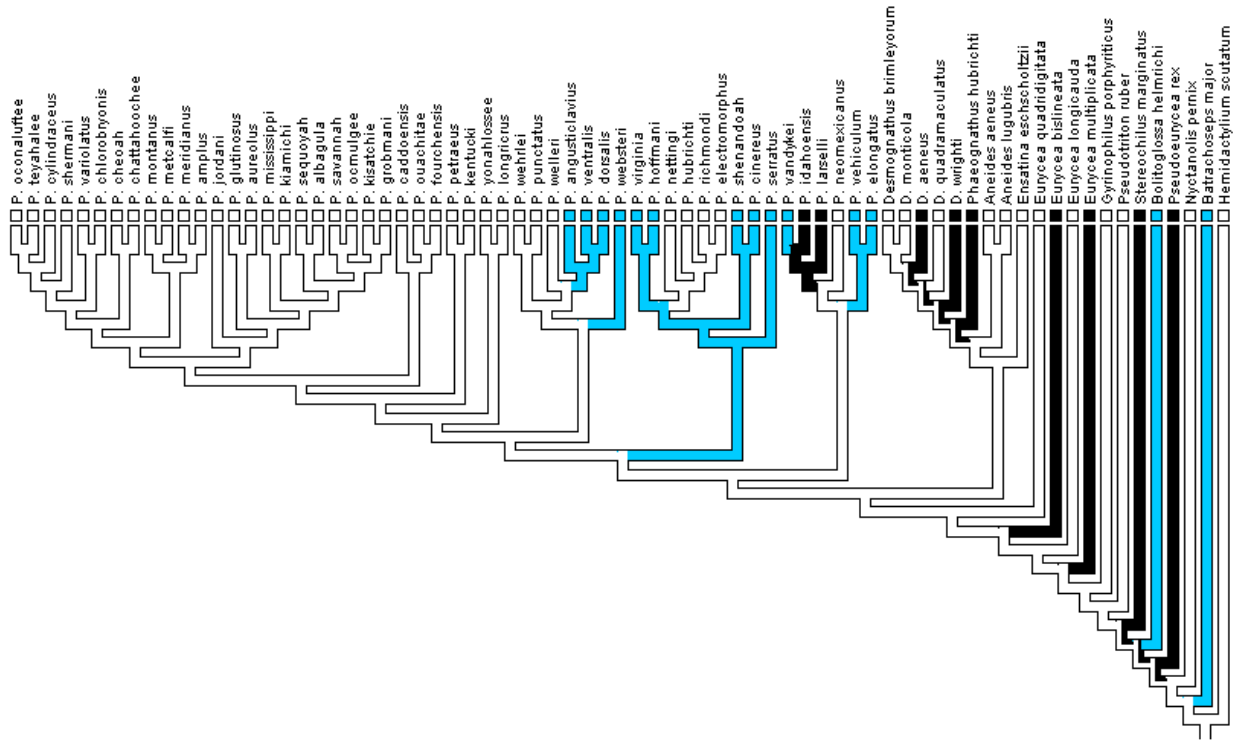
Figure 1. Phylogeny of *Plethodon* and other plethodontids (redrawn from Wiens et al. 2006)

showing character states of extant taxa, and ancestral character state reconstructions, using

maximum parsimony. White=unstriped, black=striped, blue=polymorphic.

Figure 2. Comparison of striped and unstriped forms of *P. ventralis* (left) and *P. serratus* (right). Note the wavy-edged stripe and considerable dorsal flecking of *P. ventralis* compared to the straight-edged stripe and more uniform dorsum of *P. serratus*. Photo credits: Ben Fitzpatrick.
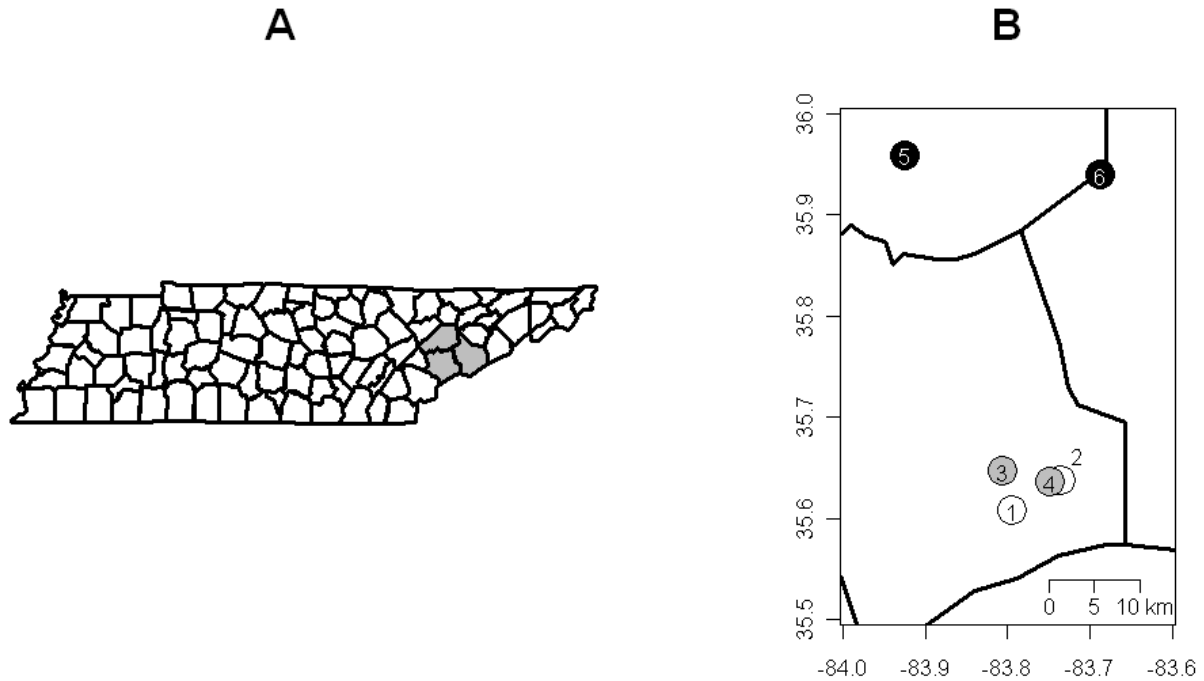
Figure 3. Locations of study sites. A) shows the general location of all study sites within the state, with a more fine-scale view in B. White circles represent allopatric *P. serratus* sites, black circles represent allopatric *P. ventralis* sites, and gray circles represent sympatric sites. 1=John Oliver Cabin, 2=Turkeypen-Schoolhouse Gap Trail Junction, 3=Rich Mountain Gap, 4=White Oak Sink, 5=Second Creek, 6=Seven Islands.
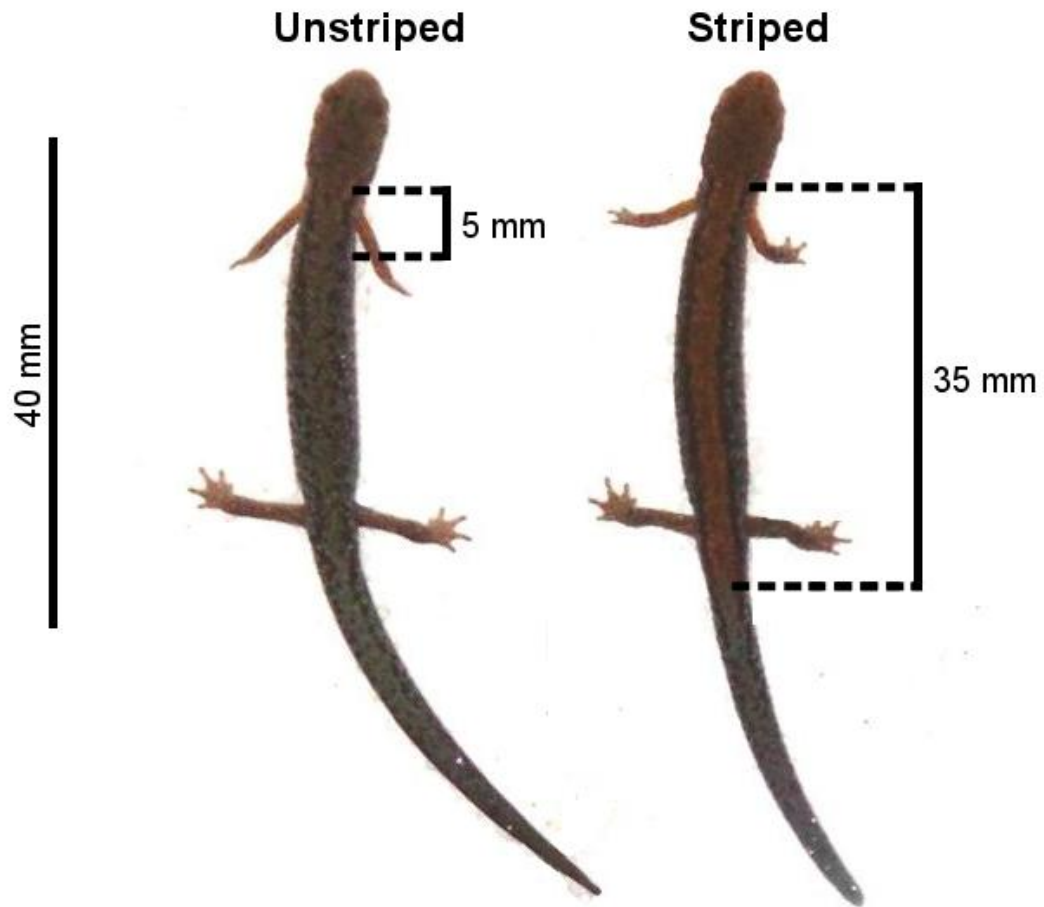
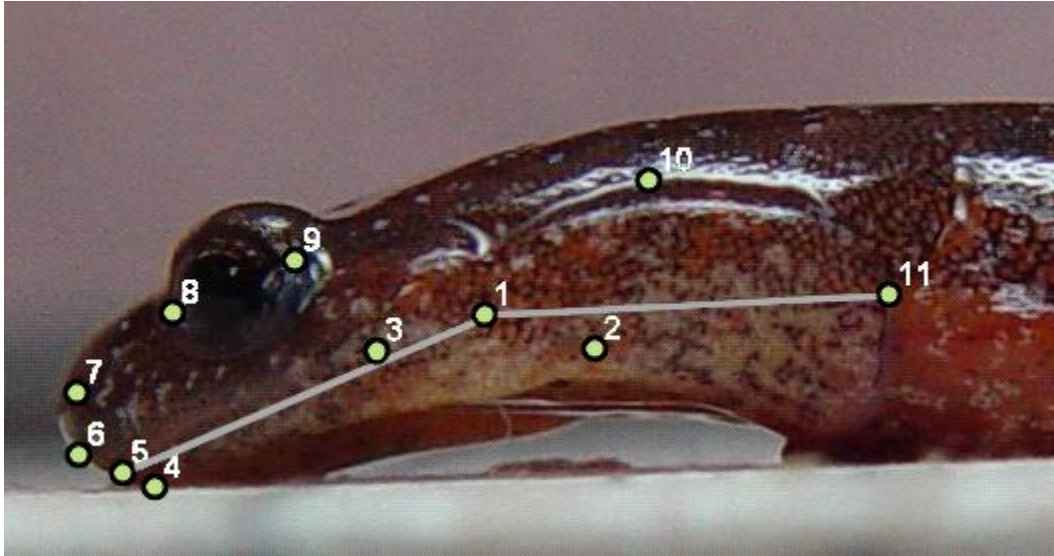**Figure 4.** Illustration of determination of stripe length in *P. ventralis*.

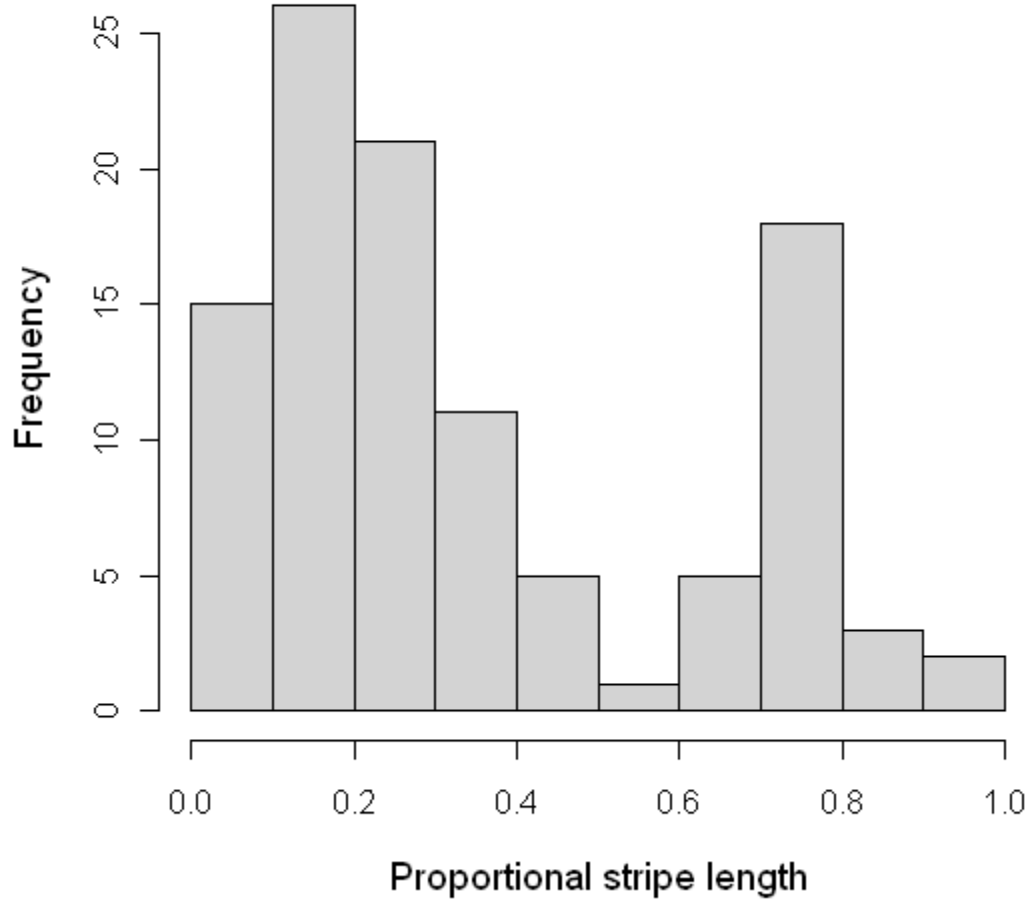**Figure 5.** Location of external cranial landmarks used for geometric morphometric analyses.

Figure 6. Frequency histogram of proportional stripe length in *P. ventralis*.
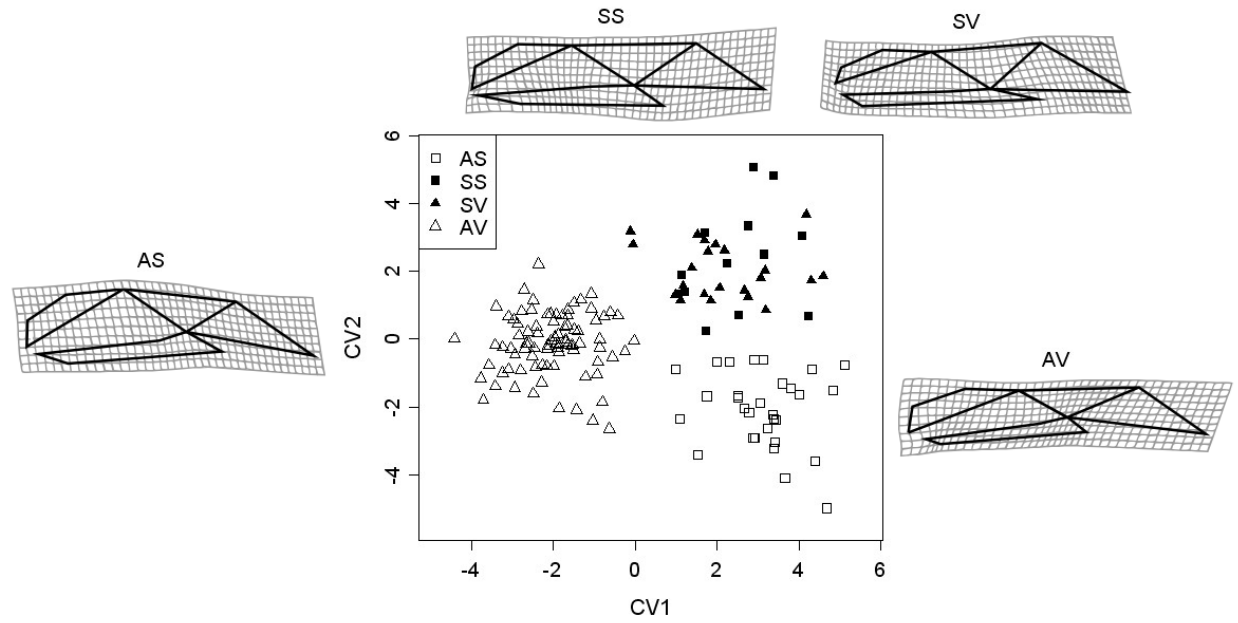
Figure 7. Plot of CV scores of residuals from regression of Procrustes coordinates on log-transformed centroid size, and mean head shapes of each species in sympatry and allopatry. Shape changes depicted in mean shapes are exaggerated by a factor of 2 to more clearly illustrate differences.
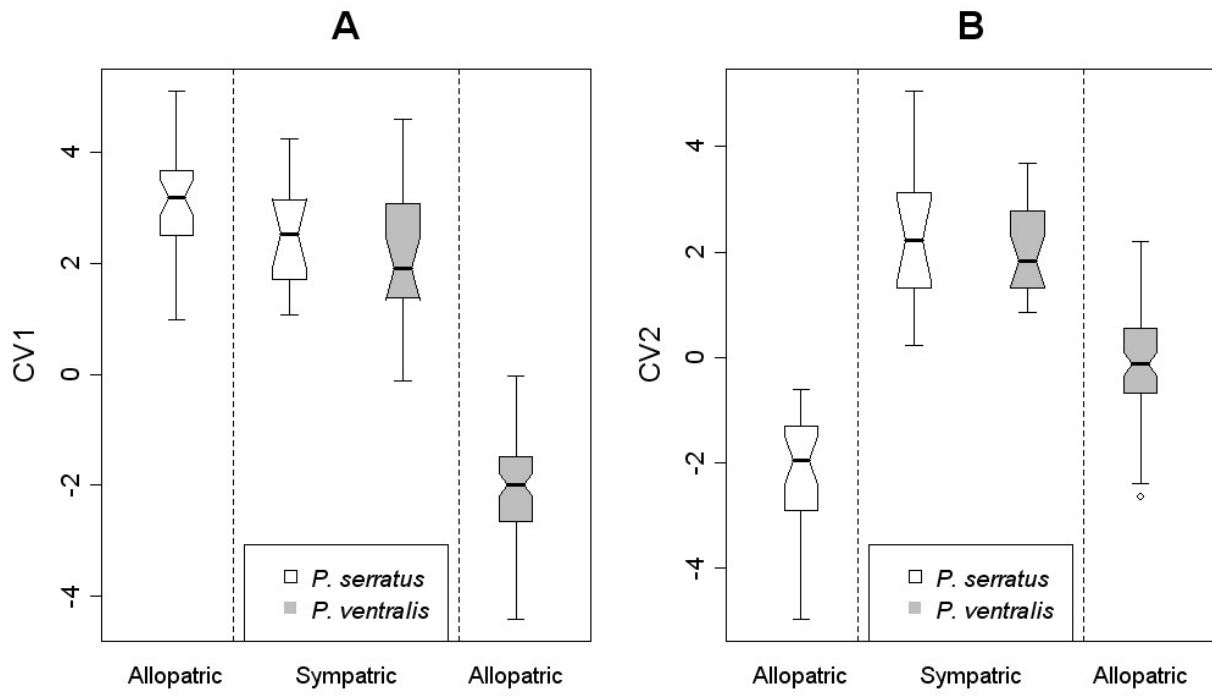
Figure 8. Boxplots of scores along the first (A) and second (B) canonical axes.
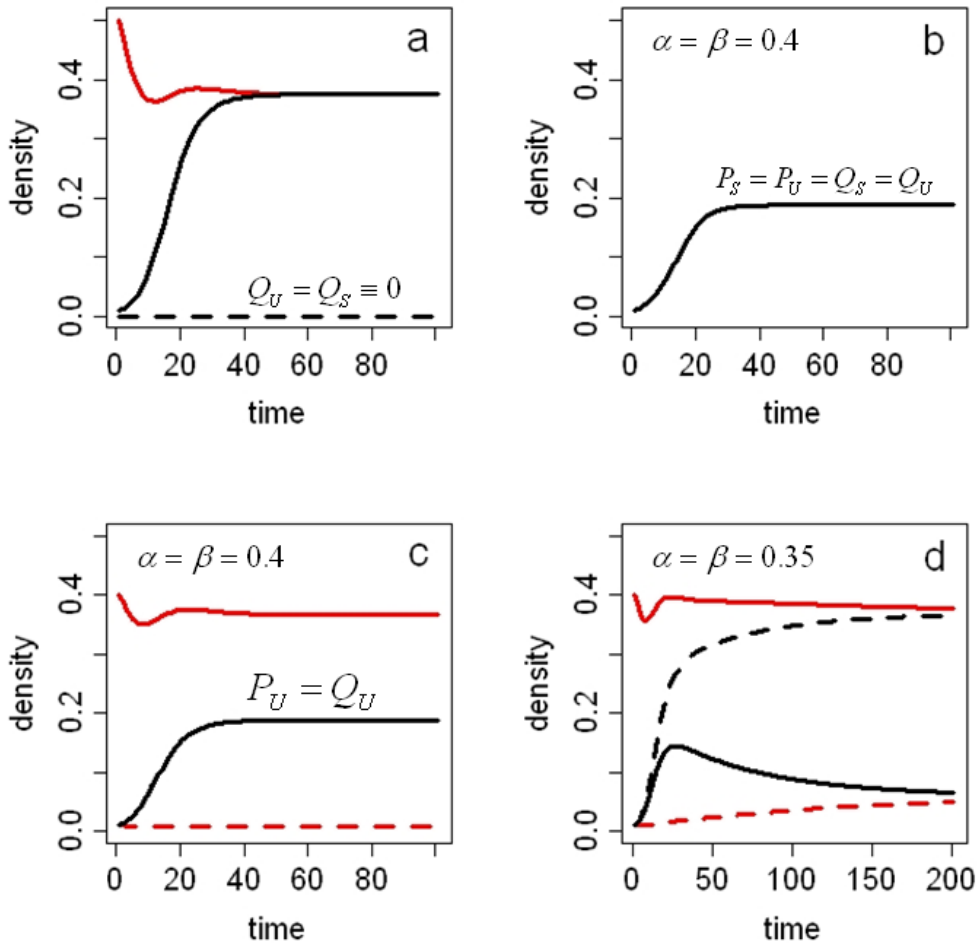
Figure 9. Changes in morph frequencies of striped and unstriped *P. serratus* and *P. ventralis* over time, using a discrete time model with negative frequency-dependent selection. Solid lines represent species P, while dashed lines represent species Q, with red lines representing the striped morph, and black lines representing the unstriped morph of each species. The graph in (a) illustrates maintenance of a stable polymorphism within a single species, as initial densities of each morph of species Q are equal to zero. In (b), each morph of each species have equal initial densities, with identical conditions (except differing initial densities) in (c). In (d), a pattern of character displacement emerges when intraspecific competition is more intense than interspecific competition. See Appendix C for further details regarding the model.

Appendix C: Supplemental Material

Below, I present a simple modification of the Leslie/Gower model (Leslie and Gower 1958), a discrete difference equation model that displays the same dynamics as the continuous Lotka/Volterra model of competition and exclusion (Cushing et al. 2004). In this model, I incorporate both the initial densities of the striped and unstriped forms of two polymorphic species, as well as the effects of negative frequency-dependent selection. The following equations model the growth of each morph of each species, where P represents the density of one species, and Q represents the density of the other species. The subscripts S and U denote the two morphs of each species (representing "striped" and "unstriped", respectively, here). For convenience, I assume perfect haploid inheritance. The traditionally used variables α and β respectively represent the effects of intra- and interspecific competition on population growth, and m_i represents the frequency of predation on morph i in a monomorphic population. The first two terms incorporating these variables in the denominator of the model are easily interpretable, in that population growth of each morph of each species is density-dependent, with increases in either species having a negative effect on the population growth of both. The final, fractional term in the denominator incorporates the effect of negative frequency-dependent selection, in that mortality of a morph is maximized (approaches m_i) when that morph is predominant (regardless of species) and approaches zero when that morph is rare.

$$P_S(t+1) = \frac{\lambda_P P_S(t)}{1 + \alpha_P[P_S(t) + P_U(t)] + \beta_P[Q_S(t) + Q_U(t)] + \dfrac{m_S[P_S(t) + Q_S(t)]}{P_S(t) + P_U(t) + Q_S(t) + Q_U(t)}}$$

$$P_U(t+1) = \frac{\lambda_P P_U(t)}{1 + \alpha_P[P_S(t) + P_U(t)] + \beta_P[Q_S(t) + Q_U(t)] + \dfrac{m_U[P_U(t) + Q_U(t)]}{P_S(t) + P_U(t) + Q_S(t) + Q_U(t)}}$$

$$Q_S(t+1) = \frac{\lambda_Q Q_S(t)}{1 + \alpha_Q[Q_S(t) + Q_U(t)] + \beta_Q[P_S(t) + P_U(t)] + \dfrac{m_S[P_S(t) + Q_S(t)]}{P_S(t) + P_U(t) + Q_S(t) + Q_U(t)}}$$

$$Q_U(t+1) = \frac{\lambda_Q Q_U(t)}{1 + \alpha_Q[Q_S(t) + Q_U(t)] + \beta_Q[P_S(t) + P_U(t)] + \dfrac{m_U[P_U(t) + Q_U(t)]}{P_S(t) + P_U(t) + Q_S(t) + Q_U(t)}}$$

Given that the two focal species of this study are morphologically convergent in sympatry, and that there is also no apparent correlation between dorsal color/pattern and morphology, it seems reasonable to parameterize the model with competition coefficients that are equal between morphs within a species, as well as between species. Also, in light of their markedly similar life histories, similar population growth rates for each species does not seem unreasonable. Since Brodie & Brodie (1980) also presented evidence that the striped form of *P. cinereus* did not exhibit a lower risk of predation than a palatable, non-striped species of similar size, predation rates on each morph of each species were also assumed to be equal in monomorphic populations. If assuming equality of these parameters between morphs within species, as well as between species, coexistence of the two species is possible so long as intraspecific competition is more intense than interspecific competition. One could then envision a scenario of a single-species population, in which one morph is very rare, as in the case of *P. serratus* throughout most of its range, which is invaded by a small number of individuals of both morphs of another polymorphic species.

To assess the potential for character displacement under the simplest scenario, the system of equations was iterated in R (R Development Core Team 2008) with $\lambda_P = \lambda_Q = \lambda$, $\alpha_P = \alpha_Q = \alpha$, $\beta_P = \beta_Q = \beta$, and $m_S = m_U = m$. A basic parameter combination that results in stable dynamics is $\lambda = 1.5$, and $\alpha = \beta = m = 0.4$. With these values, a single species system converges to equal densities of the two morphs regardless of initial densities (Figure 9a). For two species, if $\alpha = \beta$, then all forms are functionally equivalent except for the frequency-dependent component. Under these conditions, if all four forms start at the same density, they show equivalent dynamics and achieve the same equilibrium density (Figure 9b). If one morph of one species is initially at a higher density, then it tends to prevent the increase of the same morph of the other species. For example, if P_s is high (0.4) and $P_U = Q_U = Q_S = 0.01$, both P_U and Q_U increase owing to rare morph advantage, but Q_S is held down (Figure 9c). If $\alpha > \beta$, then intraspecific competition is more severe than interspecific competition and the opposite morph of the opposite species has the greatest advantage. For example, with the initial densities above, P_S and Q_U converge toward 0.35, while P_U and Q_S converge toward 0.05 (Figure 9d). Thus, in this example, negative-frequency dependent selection is capable of generating character displacement.

Vita

Jason R. Jones was born in Florence, Alabama, and spent several years of his early life in rural Waterloo, Alabama, where he attended Waterloo School through the third grade. He then moved back to Florence, Alabama, where he attended St. Joseph Regional Catholic School from fourth to eighth grades. After completing middle school at St. Joseph, he went on to Brooks High School, in Killen, Alabama, graduating in 1997. He later received a Bachelor of Arts degree in Professional Biology from the University of North Alabama in 2005, before relocating to Knoxville, Tennessee, where he earned a Master of Science degree in Ecology and Evolutionary Biology at the University of Tennessee in 2009. He currently lives in Knoxville, sharing his home with three neurotic cats, two snakes, and a friendly black widow named Elvira. As in his childhood, he currently spends most of his spare time with his nose buried in a book, or chasing bugs and various slimy or scaly creatures.