Herpetologica, 57(3), 2001, 353–364

HISTORICAL EXPLANATIONS OF PHENOTYPIC VARIATION IN THE PLETHODONTID SALAMANDER *GYRINOPHILUS PORPHYRITICUS*

DEAN C. ADAMS[1] AND CHRISTOPHER K. BEACHY[2]

[1]*Department of Zoology and Genetics, Iowa State University, Ames, IA 50011, USA*
[2]*Department of Biology, Minot State University, 500 University Avenue West, Minot, ND 58707, USA*

ABSTRACT: We examined morphological variation in *Gyrinophilus porphyriticus* in six geographic regions of the southern Appalachian Mountains and tested competing evolutionary hypotheses concerning body size and life history variation in this species. Morphology was quantified with a set of linear distance measures, and three size-adjustment methods were used to generate shape variables from these values. Phenotypic variation was assessed using each set of shape variables, and phenotypic differentiation was represented as a matrix of generalized Mahalanobis distances among populations. Morphological variation was also examined in the context of three historical hypotheses of the evolution of body size. The relationships among the populations under each hypothesis were quantified and represented by a design matrix. The association between each evolutionary hypothesis and the observed phenotypic variation was then determined using Mantel tests.

We found significant phenotypic differences among populations, regardless of which size-adjustment method was used. Populations from the Blue Ridge, Piedmont, and Nantahalas were morphologically similar, while populations from the Balsams, North Cowees, and South Cowees were each unique and distinct from other groups. None of the evolutionary hypotheses were associated with phenotypic variation, but when the effect of geography was taken into account, the river vicariance model was significantly associated with morphology. Thus, there was morphological support for the river vicariance model. These results, coupled with previous results concerning sexual isolation between parapatric populations in *G. porphyriticus*, suggest that morphometric variation may be a useful diagnostic character in this species complex.

Key words: *Gyrinophilus*; Morphometrics; Morphological variability; Design matrices

IDENTIFYING the causes of morphological variation is an important component of evolutionary biology. Genealogical history, the ability to respond to environmental changes, selection for adaptive phenotypes, and other forces all contribute to morphological variation in natural populations. It is well known, however, that assessments of phenotypic variation must take historical factors into account, because closely related populations are typically more similar to one another than would otherwise be expected (Felsenstein, 1985). Therefore, each population cannot be considered an independent entity, and the identification of biological trends (such as the association of morphology and ecology) using traditional statistical analyses becomes difficult.

Modern comparative methods (Garland et al., 1992; Harvey and Pagel, 1991; Martins and Hansen, 1996) are designed to circumvent this difficulty. These procedures use the phylogenetic relationships among taxa to adjust phenotypic variation prior to the assessment of biological trends. Briefly, a phylogenetic tree is used to determine the component of phenotypic variation explained by shared evolutionary descent, which is then removed from the data through some procedure (e.g., Felsenstein, 1985; Grafen, 1989; Harvey and Pagel, 1991). The variation that remains is thus "corrected" with respect to phylogeny, and the association between phenotype and other traits, such as environmental variables, can be accurately assessed.

In recent years, comparative biology has enjoyed a renaissance, and quantitative methods that use phylogenies to account for historical events have become widely used. An alternative approach is not to control for historical events, but rather to test explicitly their effects on phenotypic diversity. With this approach, historical hypotheses describing particular events, (such as migration patterns) are formulated, and used to define the relationships among populations. These relationships are then converted to a design matrix (Sokal et al., 1997), which is a quantitative representation of the evolutionary hypothesis. Phenotypic differences among populations are determined, and they are represented as a morphological distance matrix. Mantel correlation tests (Mantel, 1967; Sokal, 1979) are then used to evaluate the statistical association between each design matrix and the morphological distance matrix, to determine which historical hypothesis is most consistent with the observed phenotypic variation.

The Mantel procedure is commonly used in ecology (e.g., Hudon and Lamarche, 1989) and anthropology (e.g., Sokal et al., 1997; Waddle, 1994), but it has been underutilized in evolutionary studies (but see Malhotra and Thorpe, 2000; Tilley et al., 1990). Recently, Douglas et al. (1999) examined phenotypic variation among populations of Gila River cyprinids in western North America. They constructed design matrices representing several alternative evolutionary scenarios, including ecophenotypy, hybridization, and several vicariance models based on river drainage systems during the Oligocene, Miocene, Pliocene, and Pleistocene. They found that a vicariance model consistent with the river drainages during the Pliocene best explained current phenotypic variation among populations. Their work demonstrates the utility of the Mantel procedure and of design matrices for statistically comparing competing evolutionary hypotheses.

Morphological variation in the plethodontid salamander *Gyrinophilus porphyriticus* is well suited for such investigations. This salamander is found throughout the Appalachian Mountains and adjacent areas of eastern North America (Brandon, 1966). Within the southwestern-most part of the range of *G. porphyriticus* there exists considerable geographic variation in body size that has been suggested to have taxonomic implications (Bruce, 1972, 1978). Populations found in the southernmost-interior Appalachians (the Nantahala Mountains, Balsam Mountains, and Cowee Mountains northwest of Watauga Gap) attain larger sizes because they reach sexual maturity later than adjacent populations (the Blue Ridge Embayment, the Piedmont, and the Cowee Mountains southeast of Watauga Gap) (Fig. 1; Bruce, 1972, 1978). Because the size (=life history) variants in the Cowee Mountains are parapatric and there is no obvious barrier preventing migration between the populations, Bruce (1978) suggested that these size variants might be reproductively isolated. In a laboratory courtship study of the size variants found in the Cowee Mountains, insemination resulted in many within-population crosses and in none of the between-population crosses (Beachy, 1996), suggesting that Bruce's (1978) contention of reproductive isolation is tenable. However, due to the difficulty of collecting *G. porphyriticus* in the southern part of its range, little additional work has been conducted.

The geographic variation in body size and life history in *G. porphyriticus* is important because it is typical of life history variation in plethodontids. In general, those populations that delay maturation achieve large sizes while accelerated maturation leads to miniaturization (Beachy, 1995; Bruce, 1972, 1978, 1988, 1989; Ryan and Bruce, 2000; Tilley, 1973, 1980; Tilley and Bernardo, 1993). However, the hypothesis that evolution in maturation age has effects on morphological and genetic differentiation has not been explored (Beachy, 1995). Our intention was to determine whether population divergence in life history and body size has been accompanied by morphological divergence.

Evaluating the extent to which morphological divergence is coupled to life history and body size differences requires explicit formulation of the historical hypotheses that have shaped life history evolution. Several mechanisms explaining the observed life history variation in plethodontids in general and *G. porphyriticus* in particular have been proposed. The first model is a vicariance-based model. Tilley (1973, 1980) documented elevational gradients in *Desmognathus ocoee* and *D. carolinensis*, and he suggested an evolutionary

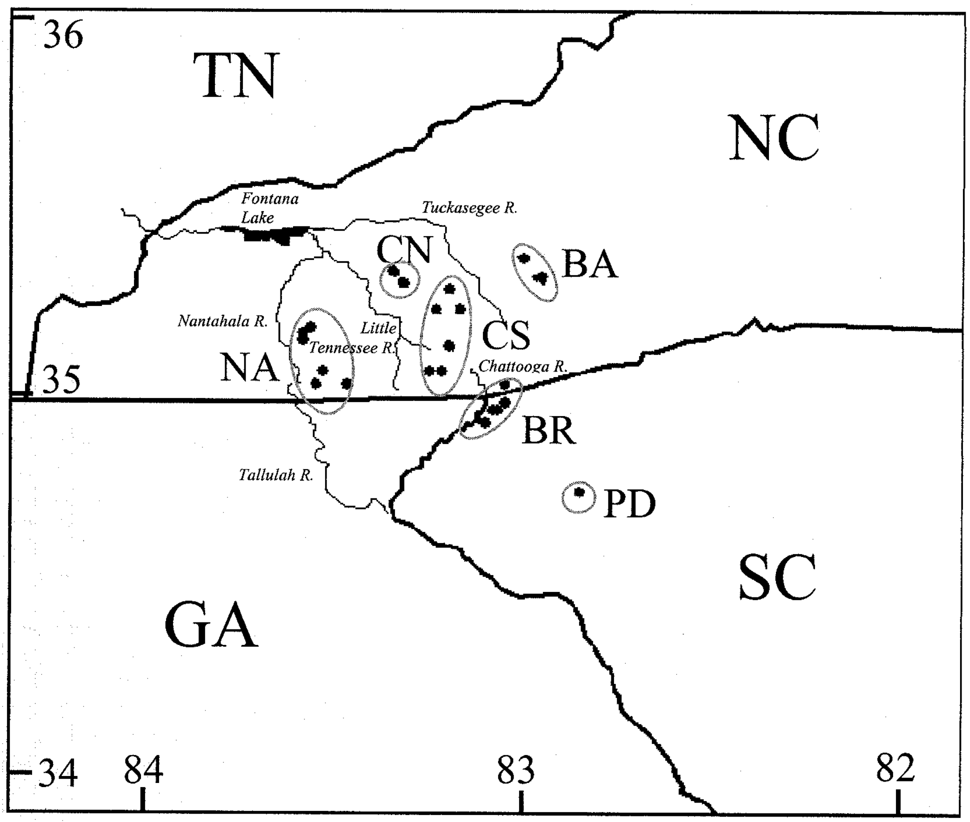

FIG. 1.—Range of *Gyrinophilus porphyriticus* examined from six populations in the southern Appalachian Mountains. The map shows the populations studied (with range in elevation): PD = Piedmont (244 m), BR = Blue Ridge Embayment (732–1068 m), NA = Nantahala Mountains (1098–1586 m), BA = Balsam Mountains (1525–1769 m), CS = southeast Cowee Mountains (732–1281 m), and CN = northwest Cowee Mountains (1342 m). TN = Tennessee, NC = North Carolina, GA = Georgia, SC = South Carolina.

hypothesis wherein higher survivorship at higher elevations resulted in selection for delayed maturation. The consequence is larger adult body size at higher elevations. This can be used as an explanatory hypothesis for *G. porphyriticus* as well. We refer to this model as the "Elevation" hypothesis.

The second model is also a vicariance-based model. Migration and gene flow in plethodontids can be influenced by rivers (e.g., Voss et al., 1995); for *G. porphyriticus,* large rivers (e.g., Little Tennessee River) serve as effective barriers to migration. Thus we hypothesized that populations that are adjacent and not separated by rivers should share more common ancestry than non-adjacent and/or river-separated populations. For example, Cowee South and Blue Ridge populations would be hypothesized to exhibit greater similarity than the Cowee South–Balsam comparison. We refer to this as the "River Vicariance" hypothesis.

The third model is a dispersal-based model. Bruce (1972, 1978) suggested that the more peripheral populations (Cowee South, Blue Ridge, and Piedmont) experienced phases of local extinction and recolonization. In his model, Bruce (1972) envisioned the promotion of r-selection during recolonization of these subpopulations, resulting in selection for earlier maturation (and, hence, smaller body size). These recolonizations occurred from adjacent subpopulations/populations (i.e., the nearest neighbor of a local extinction event provides the founder for the new population). Under this model, one expects that the peripheral populations (=small size morphs) should have similar phenotypes and that the interior populations (=large size morphs) should have similar phenotypes. Thus, when considering interior versus peripheral population comparisons, there should be greater phenotypic similarity among adjacent and similar-sized morphs than among non-adjacent and/or non-similar size morphs. We refer to this disperal explanation as the "Peripheral Isolate" hypothesis.

Because the relationship between life history variation and morphological differentiation had not previously been explored in *G. porphyriticus,* we conducted a morphometric analysis using many of the specimens collected from these populations. Recent application of morphometric methods have shown potential for discovering patterns of morphological differentiation and specialization not previously appreciated (e.g., Adams, 1999, 2000; Adams and Rohlf, 2000; Caldecutt and Adams, 1998; Carr, 1996). In the interest of exploring the history of phenotypic differentiation in *G. porphyriticus,* we developed a design matrix (Sokal et al., 1997) for each of the three hypotheses described above to determine whether any of these hypotheses are significantly associated with variation in body shape in these six populations. Our null hypothesis was that there were no differences in size-adjusted body shape among these populations of *G. porphyriticus,* and that there was no association between phenotypic variation and the three evolutionary scenarios.

Materials and Methods

Data

We measured a total of 437 specimens from the six geographically-defined populations (hereafter called "populations") of *Gyrinophilus porphyriticus* in the southern Appalachian Mountains to quantify morphological variability (specimens from the North Carolina State Museum: NCSM). The localities used were the Balsam Mountains (BA; n = 24), the Blue Ridge Embayment (BR; n = 147), the Northwest Cowee Mountains (CN; n = 39), the Southeast Cowee Mountains (CS; n = 63), the Nantahala Mountains (NA; n = 145), and the Piedmont (PD; n = 19) (Fig. 1; Appendix I). From each specimen, we recorded length from the snout to the posterior margin of the cloaca (SVL) and eight other morphometric characters (linear distance measurements) to the nearest 0.01 mm using digital calipers (Fig. 2). The other measurements recorded were head length to the middle of the gular fold (HL), maximal head width posterior to the orbit (HW), maximal head depth posterior to the orbit (HD), distance between the anterior portion of the orbit and the nostril (EN), gape width at widest span of the jaw (GW), trunk length between the axilla and groin (TL), trunk width posterior to the forelimbs (TW), and length of the third left-hind toe (3T). These measurements were chosen as general descriptors of head and body morphology because of their repeatability (Adams, 2000; Carr, 1996; Good and Wake, 1992). All measurements were log-transformed for statistical analyses.

Linear measurements of morphological characters can be used to compare morphological variability within and among populations. However, they are usually highly correlated with body size, so any statistical differences found among populations using unadjusted measurements may simply reflect body size differences, and not other aspects of phenotypic variation. To avoid this difficulty, we generated size-adjusted shape variables, and used these in all statistical analyses. Many methods of size-correction exist for sets of linear distance measurements (Bookstein, 1989; Jungers et al., 1995); we chose to compare results from three commonly-used methods: (1) the set of residuals from regressions of the morphometric characters on SVL; (2) the set of principal component (PC) scores from a PCA of the

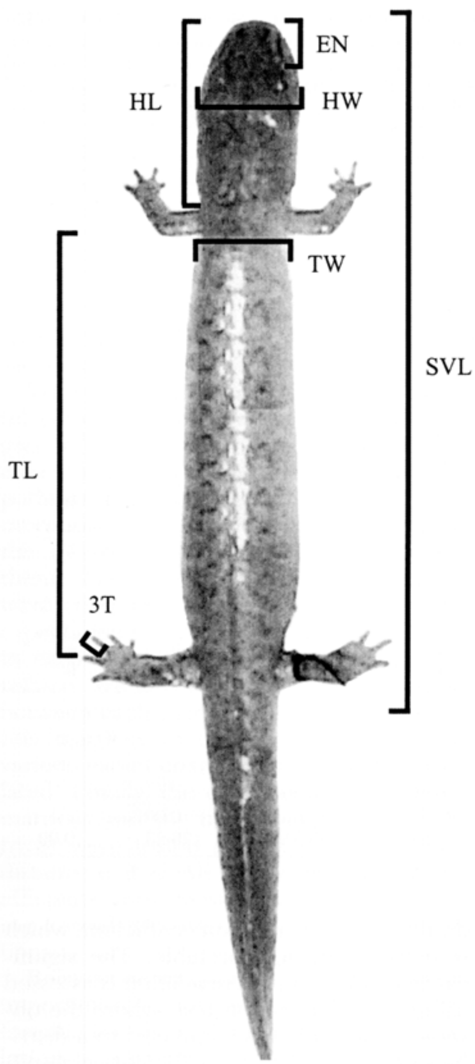

FIG. 2.–Linear measurements of body dimensions used in morphometric analysis of *Gyrinophilus porphyriticus*. Not pictured are head depth, measured on the left-lateral side of the head, and gape width, measured on the ventral side of the head.

morphometric characters *and* SVL (this set of variables excludes scores on PC1, which is generally considered to be generalized size); and (3) the set of Burnaby's (1966) size-independent shape variables using an isometric size vector (Rohlf and Bookstein, 1987). For each set of shape variables, phenotypic differentiation among populations was calculated as generalized Mahalanobis distance (D^2) among population means. All three sets of shape variables were used in all subsequent analyses.

We represented each of the three evolutionary hypotheses as a quantitative design matrix (Sokal et al., 1997). These matrices summarized the relationships among the six populations under each evolutionary scenario (Table 1). For the "Elevation" design matrix, we determined the mean elevation for each population (calculated as the weighted average elevation of all collection localities for that population), and for every pair of populations calculated the difference in mean elevation. The "River Vicariance" hypothesis was based on relative geography (i.e., populations must be adjacent to have the potential for gene flow), and included the Little Tennessee and Tuckasegee rivers as vicariant mechanisms. Thus populations that were separated by rivers, or were not adjacent to one another, were assigned a 1 in the design matrix. Only those populations that were adjacent and not separated by rivers received a zero in the design matrix. The "Peripheral Isolate" hypothesis proposes that the more peripheral populations (Cowee South, Blue Ridge, and Piedmont; i.e., those with small body size) are phylogenetically distinct from the interior populations (i.e., those with large body size). Thus, populations that are adjacent, not separated by rivers, and similarly sized were assigned a zero in the design matrix; populations that are not adjacent, separated by rivers, and/or have different adult body size were assigned a 1. Finally, a geographic distance matrix was generated, using pairwise geographic distances among populations based on the average latitude and longitude for each population.

Statistical Analyses

We assessed morphometric variation among populations using multivariate analysis of variance (MANOVA), and performed multiple comparison tests using generalized Mahalanobis distance (D^2) from a canonical variates analysis (CVA).

TABLE 1.—Design matrices representing the evolutionary hypotheses tested in this study. (A) Elevation hypothesis matrix, represented as the difference in elevation in meters. (B) River Vicariance hypothesis matrix. Populations separated by rivers, and populations that are not adjacent are assigned a 1; all others are assigned a zero. (C) Peripheral Isolate hypothesis matrix. Populations that are adjacent, not separated by rivers, and are similarly sized are assigned a zero; all others assigned a 1. (D) Geographic distance matrix, represented as geodesic distance (in km) calculated from latitude and longitude coordinates.

Population	Balsam	Blue Ridge	Cowee North	Cowee South	Nantahala	Piedmont
A.						
Balsam	0.00					
Blue Ridge	660.15	0.00				
Cowee North	296.59	363.56	0.00			
Cowee South	557.59	102.56	261.00	0.00		
Nantahala	164.25	495.90	132.34	393.34	0.00	
Piedmont	1386.84	726.69	1090.25	829.25	1222.59	0.00
B.						
Balsam	0					
Blue Ridge	1	0				
Cowee North	1	1	0			
Cowee South	1	0	0	0		
Nantahala	1	0	1	1	0	
Piedmont	1	0	1	1	1	0
C.						
Balsam	0					
Blue Ridge	0	0				
Cowee North	1	1	0			
Cowee South	1	0	1	0		
Nantahala	1	1	1	1	0	
Piedmont	1	0	1	1	1	0
D.						
Balsam	0.00					
Blue Ridge	36.21	0.00				
Cowee North	34.89	45.33	0.00			
Cowee South	23.22	26.55	19.59	0.00		
Nantahala	57.36	49.15	27.71	34.87	0.00	
Piedmont	64.18	33.88	79.21	60.31	79.23	0.00

The critical α for the multiple comparison tests was adjusted using the Bonferroni procedure, by dividing the standard critical α of 0.05 by the number of unplanned comparisons (Sokal and Rohlf, 1995). Phenotypic differences among populations were described using character loadings on the major CV axes. Analyses were performed on all sets of size-adjusted shape variables for comparison.

We determined the association between phenotypic variation and the three evolutionary hypotheses using Mantel tests (Mantel, 1967; Sokal, 1979). A Mantel test calculates the association between two data matrices using the sum of the products of their off-diagonal elements (Sokal and Rohlf, 1995). This sum of products (the Mantel Z-coefficient) is typically standardized to a correlation coefficient, which is more easily interpretable. The significance of the Mantel correlation is assessed using a randomization test, where the observed correlation is compared to a distribution of correlation coefficients generated by randomly permuting one of the matrices many times. The strength of the Mantel test is that it can be performed on any pair of matrices, regardless of the type of data from which they were derived. Therefore, it can be used to evaluate the association between morphology and a quantitative hypothesis matrix (e.g., elevation), as well as the association between morphology and a categorical design matrix (e.g., the Peripheral Isolate model). As long as the hypothesis can be represented as a matrix, even complicated evolutionary

TABLE 2.—Back-transformed means of nine morphological characters listed by population. All measurements are in millimeters.

Population	SVL	Head length	Head width	Head depth	Eye to nostril	Gape width	Tail length	Tail width	3rd Toe length
Balsam	77.7	16.1	10.2	5.3	4.1	9.5	43.1	8.4	3.2
Blue Ridge	71.5	14.7	9.6	4.5	3.5	9.1	40.5	7.4	2.4
Cowee North	81.1	16.0	10.8	5.5	3.9	10.3	46.6	8.3	2.7
Cowee South	73.1	14.3	9.3	4.9	3.5	8.7	41.5	7.6	2.6
Nantahala	79.9	16.5	10.8	5.3	4.0	10.2	45.2	8.4	2.9
Piedmont	75.8	15.2	9.9	4.8	3.7	9.7	43.5	8.0	2.9

scenarios can be tested (Douglas et al., 1999).

We performed a series of pairwise Mantel correlation tests to determine the degree of association between the various data matrices. First, each evolutionary hypothesis matrix was compared to the three morphological data matrices to determine the degree of correspondence between them. The morphological data matrices were compared to one another, with the expectation that the information expressed in each was similar and thus highly correlated. We also calculated the association between each pair of evolutionary hypothesis matrices to determine whether the various evolutionary scenarios were correlated. Finally, the evolutionary hypothesis matrices and the morphological data matrices were tested against the geographic distance matrix. All Mantel correlation coefficients were assessed for significance using a randomization test with 9999 iterations.

Because geography is known to have an important effect on patterns of genetic differentiation in plethodontids (i.e., an isolation-by-distance model; Tilley et al., 1990), it was desirable to account for the geographic relationships among populations when testing the association between the evolutionary hypotheses and the observed phenotypic variation. To do this, we performed a series of three-way Mantel tests (Legendre and Legendre, 1998; Smouse et al., 1986). A three-way Mantel test calculates the partial Mantel correlation between two variables while holding the effect of a third variable constant (Sokal and Rohlf, 1995). The partial Mantel correlation coefficient is calculated as:

$$r_{XY \cdot Z} = \frac{r_{XY} - r_{XZ}r_{YZ}}{\sqrt{(1 - r_{XZ}^2)(1 - r_{YZ}^2)}}$$

where r_{XY}, r_{XZ}, and r_{YZ} are the pairwise Mantel correlation coefficients between the three data matrices. Like the pairwise Mantel correlation coefficient, the significance of the partial Mantel correlation coefficient is determined using a randomization test. We performed three-way Mantel tests between each of the evolutionary hypothesis matrices and the three morphological data matrices while holding the effects of geographic distance constant.

RESULTS

Morphometric Shape Variation

Mean values for each of the log-transformed characters are found in Table 2. Using MANOVA, we found significant differences among populations, regardless of which method of size-adjustment was used (Table 3). Multiple comparison tests among populations for each set of size-adjusted shape variables revealed that all populations were significantly different from one another in body shape except for the Balsams and the Piedmont, even at the Bonferroni-adjusted $\alpha = 0.00333$ (Blue Ridge and Piedmont were also not statistically different for PCA characters). CVA revealed that populations from the Blue Ridge, Piedmont, and Nantahalas were morphologically similar, while populations from the Balsams, North Cowees, and South Cowees were more distinct. To describe the phenotypic differences among populations in more detail, we inspected the character loadings on the first CV axis. For size-adjusted shape data generated from the Burnaby and PCA methods, we

TABLE 3.—Summary of multivariate analysis of *G. porphyriticus*. (A) Results from MANOVA comparisons of the six geographic populations of *Gyrinophilus* using each of the size-adjusted shape measures, and (B) matrix of generalized Mahalanobis distance (D^2) among populations based on residual data.

A.

Shape variables	Wilks' Λ	F	df 1	df 2	P-value
Burnaby	0.489	8.231	40	1851	1.03×10^{-42}
PCA	0.457	9.087	40	1851	2.75×10^{-48}
Residuals	0.426	9.981	40	1851	4.30×10^{-54}

B.

Population	Balsam	Blue Ridge	Cowee North	Cowee South	Nantahala	Piedmont
Balsam	0					
Blue Ridge	2.088	0				
Cowee North	2.860	1.462	0			
Cowee South	2.466	1.776	1.966	0		
Nantahala	1.649	0.837	1.560	2.198	0	
Piedmont	2.214	1.271	2.066	2.169	1.488	0

found high positive loadings for head length and gape width on the first CV axis, and high negative loadings for trunk length and width, and head width on the first CV axis. Thus the first CV axis describes the contrast between salamanders with *relatively* larger head length and gape width and smaller trunks (e.g., Nantahalas), versus salamanders with *relatively* smaller head length and gape and larger trunks (e.g., Cowee South). For data generated from residuals the same contrast is present, but the positive and negative loadings for the individual characters are reversed.

Association of Shape with Evolutionary Hypotheses

Results from pairwise Mantel correlation tests are found in Table 4A. Only five of the 21 pairwise matrix comparisons were significant at $\alpha = 0.05$. All three morphometric data matrices were highly correlated. The pairwise Mantel correlation coefficients for these comparisons were 0.92, 0.96, and 0.98, implying that the three methods for generating size-adjusted shape variables characterized phenotypic variability in a nearly identically manner. None of the evolutionary hypothesis ma-

TABLE 4.—(A) Pairwise Mantel correlation coefficients between each of the seven data matrices (Mantel correlation coefficients above the diagonal; significance levels below the diagonal). Codes for the various data matrices are: EL = elevation hypothesis, PI = peripheral isolate hypothesis, RV = river vicariance hypothesis, GD = geographic distance, SH1 = Burnaby shape variables, SH2 = PCA shape variables, SH3 = residual shape variables. Significant matrix correlations are represented in bold-face. (B) Association of phenotypic variation and the three evolutionary hypothesis matrices when holding geography constant in a three-way Mantel test. Only results from the residual shape variables are shown, because of the high correlation between the three sets of shape variables. Significance of Mantel correlation coefficients for all analyses was evaluated using randomization tests based on 9999 iterations.

A.

	EL	PI	RV	GD	SH1	SH2	SH3
EL	—	0.1055	0.2347	**0.7348**	0.0695	0.0612	0.0697
PI	0.4097	—	0.7071	0.3349	0.1016	0.1786	0.1619
RV	0.2143	0.0563	—	**0.4410**	0.4179	0.4591	0.4073
GD	0.0190	0.1505	0.0308	—	−0.0337	−0.0823	−0.1390
SH1	0.5104	0.3476	0.1334	0.4554	—	**0.9617**	**0.9208**
SH2	0.5251	0.2965	0.1185	0.3897	0.0013	—	**0.9847**
SH3	0.4870	0.3405	0.1141	0.3598	0.0017	0.0013	—

B.

Alternative models	Mantel r	P-randomization
Elevation model	0.2559	0.1921
Peripheral Isolate model	0.2235	0.2634
River Vicariance model	**0.5273**	0.0388

trices were significantly associated with phenotypic variation, and none of the evolutionary hypothesis matrices were significantly correlated with one another. This implied that each of the three hypothesis matrices represented distinct information concerning possible evolutionary scenarios. Two of the evolutionary hypotheses, the Elevation model and the River Vicariance model, were significantly associated with geographic distance. When the effects of geography were taken into account using a three-way Mantel test, only the River Vicariance model was significantly associated with phenotypic variation (Table 4B). Thus it appears that the observed pattern of phenotypic variation in these populations is consistent with the idea that the Little Tennessee and Tuckasegee rivers prevent substantial gene flow between otherwise adjacent populations.

Discussion

One goal of evolutionary biology is to understand the forces driving population differentiation. In this study, we investigated phenotypic variation among six populations of *G. porphyriticus* found in close geographic proximity in the southern Appalachian Mountains. Using a set of size-adjusted shape variables, we found significant phenotypic differentiation among populations. When this variation was examined in light of several evolutionary hypotheses explaining life history variation in *G. porphyriticus* (elevation, river vicariance, and peripheral isolation), we found that morphology was not associated with any of these models. However, when geographic distance (as in an isolation-by-distance model; Tilley et al., 1990) was considered, we found a significant correlation between morphology and the River Vicariance hypothesis. These findings imply that an isolation-by-distance model cannot completely explain the shape variation in *G. porphyriticus*, but it can be used to improve the explanatory power of other models. In addition, these results are consistent with the hypothesis that large rivers restrict sizable gene flow.

The significant correlation between the morphometric distance matrix and the River Vicariance model supports one of two non-mutually exclusive hypotheses. First, nearby populations, which are also separated by a major river, experience similar selection for changes in body shape, and second, these nearby populations share a recent common ancestry. Support for the hypothesis of recent common ancestry would imply that drainage history is a primary factor influencing the phylogenetic history of *G. porphyriticus.* While many elements of the southern Appalachian fauna, including plethodontid salamanders, appear to follow this pattern (e.g., Holt, 1971), an assay of genetic variation in *G. porphyriticus* would provide a direct test of this hypothesis.

Coupled with the life history differences (Bruce, 1972, 1978) and possible sexual isolation (Beachy, 1996) between Cowee North and Cowee South populations, this analysis supports the hypothesis that significant divergence exists between these two populations. Difficulties in courtship based on body size differences may be preventing significant gene flow between these populations (Beachy, 1996). While significant shape differentiation exists for almost all pairwise comparisons, this size-based behavioral-isolating mechanism does not appear to be able to explain the remainder of the phenotypic divergence. Significant shape variation exists when comparing adjacent, yet similarly sized animals. This suggests that other mechanisms reducing gene flow among populations may also exist.

In testing for sexual isolation between the Cowee North and Cowee South populations, Beachy (1996) found that 20% (nine of 45) of within population crosses resulted in insemination, compared to 0% (0 of 67) of between population crosses. This level of isolation is equivalent (and even exceeds) the level of ethological isolation between well-established species of sympatric *Desmognathus* (e.g., Maksymovitch and Verrell, 1993; Verrell, 1990), and suggests that reduced courtship success is at least correlated with the differences in body size (Bruce, 1978) and body shape (this study) in *G. porphyriticus.* While a direct assay of interrupted gene flow is not currently available, the significant variation in the life history/body size, in size-adjusted shape, and the apparent sexual isolation between the Cowee North and Cowee South populations, certainly suggest that fixed allozyme differences could be found. Given that the life history/body size and phenotypic shape variation exists among most of the pairwise comparisons, a similar hypothesis can be suggested for all the populations that we examined. Clearly, a detailed genetic study of these populations of *G. porphyriticus* would allow further examination of the phylogeographic and historical models tested here.

The evolution of accelerated maturation, and consequent smaller body size, in plethodontids has been hypothesized to be the result of local adaptation (Bernardo, 1994; Tilley, 1973, 1980). In *G. porphyriticus*, there are phenotypic differences that accompany this reduction in body size. A comparison of head shape between Nantahala and Blue Ridge populations reveals a reduction in head dimensions that are independent of body size. Animals from the Piedmont have heads that are similar to those from the Blue Ridge, suggesting common local selection regimes to the more southeasterly localities. Similar differences are observed when comparing the Cowee North and Cowee South populations. Individuals from the more interior population (Cowee North) exhibit larger values for head shape than the southern peripheral population (Cowee South). Although it is difficult to demonstrate directly the importance of among-taxa variation in head shape, such variation is often associated with selective factors. Variation in jaw length and head dimensions in some plethodontids has resulted in biomechanical variation, which can be associated with local food preferences in these populations (Adams, 1999, 2000; Adams and Rohlf, 2000).

The history of the plethodontids has been difficult to resolve because of the extreme morphological conservatism within genera and species. As a result, most recent phylogenetic analyses have focused on molecular data (e.g., Highton, 1997; Wake and Schneider, 1998). It appears, however, that shape variation may contain sufficient biological signal to allow one to test biogeographic and phylogenetic hypotheses in the absence of molecular data. This would be especially useful in taxa that are difficult to obtain and that are found in larger numbers in museum and private collections. Underlying the analyses presented here is the question "Do the statistics of shape variation represent phylogeny?" Presumptively, a component of shape variation is due to a genetic effect and may be used as an estimate of phylogenetic distance. However, morphometric phenograms do not always correspond to genetic distance phenograms (e.g., Carr, 1996; Shaffer et al., 1991), which are typically thought to be a more accurate estimate of phylogeny. At the very least, these data suggest that evolutionary hypotheses may be explicitly tested using estimates of phenotypic variation.

Acknowledgments.—We sincerely thank R. Brandon and R. C. Bruce for sharing their knowledge of these elusive animals and J. Mendelson III for suggesting a closer inspection of morphology. Special appreciation goes to R. C. Bruce for his uncanny ability to find animals that no other herpetologist will work on simply because the animals are difficult to find. J. Beane and A. Braswell (North Carolina State Museum) kindly provided the specimens and R. Jaeger, S. Tilley, and two anonymous reviewers greatly improved versions of the manuscript. This work was supported in part by National Science Foundation grants IBN-9800636 (D. C. Adams and F. J. Rohlf) and IBN-9728160 (to F. J. Rohlf), and by Institutional funds of Minot State University (C. K. Beachy).

Literature Cited

ADAMS, D. C. 1999. Ecological Character Displacement in *Plethodon* and Methods for Shape Analysis of Articulated Structures. Ph.D. Dissertation, State University of New York, Stony Brook, New York, U.S.A.

———. 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* R. C. Bruce, R. G. Jaeger, and L. D. Houck (Eds.), The Biology of Plethodontid Salamanders. Kluwer Academic/Plenum, New York, New York, U.S.A.

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 Science U.S.A. 97:4106–4111.

BEACHY, C. K. 1995. Age at maturation, body size, and life-history evolution in the salamander family

Plethodontidae. Herpetological Review 26:179–181.

———. 1996. Reduced courtship success between parapatric populations of the plethodontid salamander *Gyrinophilus porphyriticus*. Copeia 1996: 199–203.

BERNARDO, J. 1994. Experimental analysis of allocation in two divergent, natural salamander populations. American Naturalist 143:14–38.

BOOKSTEIN, F. L. 1989. "Size and shape": a comment on semantics. Systematic Zoology 38:173–180.

BRANDON, R. A. 1966. Systematics of the salamander genus *Gyrinophilus*. Illinois Biological Monographs 35:1–86.

BRUCE, R. C. 1972. Variation in the life cycle of the salamander *Gyrinophilus porphyriticus*. Herpetologica 28:230–245.

———. 1978. Life-history patterns of the salamander *Gyrinophilus porphyriticus* in the Cowee Mountains, North Carolina. Herpetologica 34:53–64.

———. 1988. Life history variation in the salamander *Desmognathus quadramaculatus*. Herpetologica 44:218–227.

———. 1989. Life history of the salamander *Desmognathus monticola*, with a comparison of the larval periods of *D. monticola* and *D. ochrophaeus*. Herpetologica 45:144–155.

BURNABY, T. P. 1966. Growth-invariant discriminant functions and generalized distances. Biometrics 22: 96–110.

CALDECUTT, W. C., AND D. C. ADAMS. 1998. Morphometrics of trophic osteology in four ecotypes of the threespine stickleback, *Gasterosteus aculeatus*. Copeia 1998:827–838.

CARR, D. E. 1996. Morphological variation among species and populations of salamanders in the *Plethodon glutinosus* complex. Herpetologica 52: 56–65.

DOUGLAS, M. E., W. L. MINCKLEY, AND B. D. DEMARAIS. 1999. Did vicariance mold phenotypes of western North American fishes? Evidence from Gila River cyprinidids. Evolution 53:238–246.

FELSENSTEIN, J. 1985. Phylogenies and the comparative method. American Naturalist 125:1–15.

GARLAND, T. J., P. H. HARVEY, AND A. R. IVES. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology 41:18–32.

GOOD, D. A., AND D. B. WAKE. 1992. Geographic variation and speciation in the torrent salamanders of the genus *Rhyacotriton* (Caudata: Rhyacotritonidae). University of California Publications, Zoology 126:1–91.

GRAFEN, A. 1989. The uniqueness of phylogenetic regression. Journal of Theoretical Biology 156:405–423.

HARVEY, P. H., AND M. D. PAGEL. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford, U.K.

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

HOLT, P. C. (Ed.). 1971. Distribution History of the Biota of the Southern Appalachians. Part III: Vertebrates. Research Division Monograph 4, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, U.S.A.

HUDON, C., AND G. LAMARCHE. 1989. Niche segregation between American lobster *Homarus americanus* and rock crab *Cancer irroratus*. Marine Ecology Progress Series 52:155–168.

JUNGERS, W. L., A. B. FALSETTI, AND C. E. WALL. 1995. Shape, relative size, and size-adjustments in morphometrics. Yearbook of Physical Anthropology 38:137–161.

LEGENDRE, P., AND L. LEGENDRE. 1998. Numerical Ecology, 2nd ed. Elsevier, Amsterdam, The Netherlands.

MAKSYMOVITCH, E., AND P. A. VERRELL. 1993. Divergence of mate recognition systems among conspecific populations of the plethodontid salamander *Desmognathus santeetlah* (Amphibia: Plethodontidae). Biological Journal of the Linnean Society 49:19–29.

MALHOTRA, A., AND R. S. THORPE. 2000. The dynamics of natural selection and vicariance in the Dominical anole: patterns of within-island molecular and morphological divergence. Evolution 54: 245–258.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:719–748.

MARTINS, E. P., AND T. F. HANSEN. 1996. The statistical analysis of interspecific data: a review and evaluation of phylogenetic comparative methods. Pp. 22–75. *In* E. P. Martins (Ed.), Phylogenies and the Comparative Method in Animal Behavior. Oxford University Press, Oxford, U.K.

ROHLF, F. J., AND F. L. BOOKSTEIN. 1987. A comment on shearing as a method for "size correction". Systematic Zoology 36:356–367.

RYAN, T. J., AND R. C. BRUCE. 2000. Life history evolution and adaptive radiation of hemidactyliine salamanders. Pp. 303–326. *In* R. C. Bruce, R. G. Jaeger, and L. D. Houck (Eds.), The Biology of Plethodontid Salamanders. Kluwer Academuc/Plenum, New York, New York, U.S.A.

SHAFFER, H. B., J. M. CLARK, AND F. KRAUS. 1991. When molecules and morphology clash: a phylogenetic analysis of North American ambystomatid salamanders (Caudata: Ambystomatidae). Systematic Zoology 40:284–303.

SMOUSE, P. E., J. C. LONG, AND R. R. SOKAL. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology 35:627–632.

SOKAL, R. R. 1979. Testing statistical significance of geographic variation patterns. Systematic Zoology 28:227–231.

SOKAL, R.R., N. L. ODEN, J. WALKER, AND D. M. WADDLE. 1997. Using distance matrices to choose between competing theories and an application to the origin of modern humans. Journal of Human Evolution 32:501–522.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry, 3rd ed. Freeman, San Francisco, California, U.S.A.

TILLEY, S. G. 1973. Life histories and natural selection in populations of the salamander *Desmognathus ochrophaeus.* Ecology 54:3–17.

———. 1980. Life histories and comparative demography of two salamander populations. Copeia 1980: 806–821.

TILLEY, S. G., AND J. BERNARDO. 1993. Life history evolution in plethodontid salamanders. Herpetologica 49:154–163.

TILLEY, S. G., P. A. VERRELL, AND S. J. ARNOLD. 1990. Correspondence between sexual isolation and allozyme differentiation: a test in the salamander *Desmognathus ochrophaeus.* Proceedings of the National Academy of Science U.S.A. 87:2715–2719.

VERRELL, P. A. 1990. Tests for sexual isolation among sympatric salamanders of the genus *Desmognathus.* Amphibia Reptilia 11:147–153.

VOSS, S. R., D. G. SMITH, C. K. BEACHY, AND D. G. HECKEL. 1995. Allozyme variation in neighboring populations of the plethodontid salamander *Leurognathus marmoratus.* Journal of Herpetology 29: 493–497.

WADDLE, D. M. 1994. Matrix correlation tests support a single origin for modern humans. Nature 368:452–454.

WAKE, D. B., AND C. J. SCHNEIDER. 1998. Taxonomy of the plethodontid salamander genus *Ensatina.* Herpetologica 54:279–298.

Accepted: 11 September 2000
Associate Editor: Stephen Tilley

APPENDIX I

Specimens Examined

Gyrinophilus porphyriticus BALSAM MOUNTAINS, NCSM 40779, 40780, 40789, 40790, 40963–40981, 41095; BLUE RIDGE EMBAYMENT, NCSM 41006, 41045, 41046, 41048–41051, 41056–41067, 41069–41079, 41082–41084, 41088–41091, 41093, 41167–41169, 41180, 42168, 42172, 42177–42187, 42191, 42193–42195, 42197, 42199, 42201–42203, 42205–42210, 42212, 42213, 42215, 42216, 42218, 42564–42570, 42577–42580, 42582, 42586, 42588, 42589, 42592, 42593, 42595–42597, 42599, 42601, 42602, 42617, 42626, 42628–42635, 42637–42639, 42641–42644, 42646, 42648—42652, 42656–42661, 42663–42666, 42668–42672, 42674, 42675, 42678–42685, 42687; NORTHWEST COWEE MOUNTAINS, NCSM 41256, 42464, 42465, 42467, 42468, 42470, 42472–42475, 42477–42479, 42481, 42483–42496, 42499–42503, 42505, 42506, 42508–42511; SOUTHEAST COWEE MOUNTAINS, NCSM 40844–40847, 41053, 41129, 41152–41154, 41165, 41166, 41221, 41228, 41230, 41231, 42116–42119, 42121–42133, 42138–42141, 42144, 42145, 42148, 42149, 42151, 42152, 42223, 42231–42234, 42236, 42270–42274, 42294, 42295, 41200, 41201, 41213–41216, 41219, 41220; NANTAHALA MOUNTAINS, NCSM 28164 (9 specimens with same number), 28178 (4 specimens with same number), 28181 (3 specimens with same number), 41272–41274, 42221, 42222, 42230, 42237–42242, 42245–42254, 42257–42268, 42275, 42277, 42278, 42280, 42282, 42292, 42298–42301, 42303, 42305–42313, 42315–42318, 42320, 42322–42324, 42326, 42350, 42351, 42353, 42354, 42356, 42358–42362, 42366, 42367, 42396, 42397, 42400–42402, 42406–42410, 42412, 42414–42421, 42423, 42424, 42426, 42429–42441, 42444–42450, 42453–42456, 42283–42290; PIEDMONT, NCSM 42696, 42697, 42705–42707, 42713, 42715, 42716, 42719–42721, 42723, 42725, 42727, 42737, 42738, 42741, 42742, 42746.