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Genetic Relationships Between A Southern Population, Formerly *Plethodon Longicrus*, and Three Northern Populations of *Plethodon Yonahlossee*

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ABSTRACT

Polyacrylamide gel electrophoresis was used to study enzyme variation at 13 presumed genetic loci between one population (type locality) of the former *Plethodon longicrus* and three populations of *P. yonahlossee* from the northern half of its range. Nine of 13 loci examined were found to be polymorphic. Estimates of genetic similarity indicate that there are two distinct groups, the former *P. longicrus* population, and the three northern *P. yonahlossee* populations. As other southern or intermediate populations were not sampled, the taxonomy of *P. yonahlossee* cannot be thoroughly analyzed. Electrophoretic data as well as other evidence suggest that the genetic divergence observed is not clinal and thus there probably are at least two groups undergoing speciation and these should be classified as two subspecies, perhaps even as two distinct species.

INTRODUCTION

The taxonomic status of *Plethodon longicrus*, the crevice salamander from Bluerock Mountain, North Carolina has not yet been clearly established. Alder and Dennis (1962) described *P. longicrus* as a species separate from *P. yonahlossee* on the basis of morphological and ecological criteria. Pope (1965) suggested that *P. longicrus* and *P. yonahlossee* may have a subspecific relationship. Later Highton (1972) and Guttman et al. (1978) concluded that these salamanders were conspecific. Guttman et al. (1978) based their conclusions on both biochemical and morphological observations; however, all populations studied were from the southern half of the range of *P. yonahlossee* (southwestern North Carolina and southern Tennessee). Populations of the former *P. longicrus* are known from three

localities (Guttman et al., 1978), all of which occur at the southern end, and are an extension of, the range of *P. yonahlossee*. The two morphs were found to be sympatric at the Bearwallow Mountain, North Carolina, locality by Guttman et al. (1978). Duncan and Highton (1979) studying *Plethodon* of the Ouachita Mountains also examined two northern populations of *P. yonahlossee* using electrophoresis and found virtually no difference between the two populations. At the present time there has been no comparison of the relationship of the former *P. longicrus* to northern *P. yonahlossee*. The purpose of this study is to biochemically analyze the genetic relationships between populations of *P. yonahlossee* from the northern half of its range and the type locality population of the former *P. longicrus* (Bluerock Mountain). Because of limited collecting time, other southern or intermediate populations were not able to be sampled.

Guttman et al. (1978) used primarily starch gel electrophoresis in the biochemical analysis of genetic differences, while we employed polyacrylamide disc gel electrophoresis in the present study. Polyacrylamide gel electrophoresis has been shown to have greater potential for resolving enzyme variations (Hunter et al., 1964; Frederick, 1964; Raymond and Wang, 1960; Ornstein, 1964).

MATERIALS AND METHODS

Salamanders were collected from the following four localities during May, 1979: Iron Mountain, Tennessee; Comers Rock, Virginia (near the northern tip of its range); Mount Jefferson, North Carolina; and Bluerock Mountain, North Carolina. Localities for this study and the study by Guttman et al. (1978) are shown in Fig. 1. Specimens from each locality were collected within a few ha and they were kept alive until their return to the laboratory. Homogenates were prepared from decapitated salamanders, minus stomach contents, and the homogenates were stored below −70 C until analysis. Aliquots of each homogenate were centrifuged at 2000 g for 30 min before electrophoresis. The gels were prepared according to the method described by Hagen et al. (1978) except for some enzymes which required a greater percentage of acrylamide in the gel in order to obtain better resolution. The gels, buffers and stains used are described in Table 1. Staining methods were followed exactly except that PMS was omitted from the ADA stain and α-naphthyl acetate was the only substrate used to detect esterases. Proteins that are known to be controlled by more than one locus are designated with integers ("1" being assigned to the farthest band or set of bands from the origin). Polymorphic proteins controlled by a single locus are designated alphabetically with "a" being assigned to the band migrating farthest from the origin.

All phenotypes were analyzed as if they were the result of genotypes expressed as codominant Mendalian alleles, despite the lack of breeding studies. This assumption has been commonly accepted in other biochemical studies, including those on *Plethodon* by Highton and Webster (1976), Duncan and Highton (1979) and Guttman et al. (1978). Genetic similarities between populations were estimated by computing the genetic similarity, "S" (Rogers, 1972) and the genetic identity for small samples, "I" (Nei, 1978). Estimates of heterozygosity, the mean proportion of loci heterozygous per individual (H) and the mean number of alleles per locus (A) were calculated using direct counts.

RESULTS

Only four of the 13 loci analyzed were found to be monomorphic in all specimens; allelic frequencies for the nine polymorphic loci studied are shown in Table 2. The Bluerock Mountain population (formerly *P. longicrus*) exhibits polymorphic variants that are not expressed in the three northern *P. yonahlosse* populations at the *MR-2* and *Hp* loci, and a polymorphic variant at the *Est-1* locus which is only present in the Comers Rock population at a very low gene frequency (.03). The polymorphic Bluerock Mountain population illustrates complete divergence from the three monomorphic northern populations at the *Hp* locus. Genetic similarity estimates between the populations are shown in Table 3. "I" values between the 3 northern populations range from .96-.97 ($\ddot{I}$ = .96), and "S" values are .87 or greater ($\ddot{S}$ = .88) showing a high degree of similarity. In contrast, "I" values between the Bluerock Mountain population and the three northern populations range from .82-.86 ($\ddot{I}$ = .84) and "S" values range from .74-.77 ($\ddot{S}$ = .76), indicating considerably less genetic similarity.

Estimates of genetic heterozygosity (H) and the mean number of alleles per locus (A) are given in Table 4. The Bluerock Mountain population has a greater genetic heterozygosity (H = .358) than the three northern populations (range of H = .132-.177, $\ddot{H}$ = .149). The mean number of alleles per locus is greatest in the Bluerock Mountain population (A = 1.77).

DISCUSSION

The polyacrylamide gel electrophoretic data present evidence that significant genetic divergence has occurred between the Bluerock Mountain population and the three northern populations ($\ddot{I}$ = .84), while little divergence has occurred among the three monotypic northern populations ($\ddot{I}$ = .96). We were not able to test directly, whether the observed genetic divergence is clinal, as other southern or intermediate populations were not able to be sampled. However, several observations indicate that the observed divergence is probably not clinal. Several topographical features which impose habitat and altitudinal restrictions on *P. yonahlossee* supplemented by direct observation, indicate that the Bluerock Mountain population seems to be genetically isolated from the three northern populations. The Bluerock Mountain population is restricted to an area of approximately 4.1 ha (Alder and Dennis, 1962) and is approximately 4 km from the nearest known population at Grant Mountain. By our own observations we have also observed the Grant Mountain population to be restricted in its range. The Bluerock Mountain and Grant Mountain populations are separated by a valley (about 475 m above sea level) and Reedypatch Creek which may serve as barriers. The habitat restrictions on this salamander along with the distance between known populations would probably not allow significant migration between these isolated populations. Pope (1950) reported that *P. yonahlossee* was not abundant in the southern part of its range, while Hairston (1949) stated that it was rare in the Black Mountains, also near the southern tip of the range. Pope (1950) and Hairston (1949) reported distinct localities in the southern section which were well removed from one another, and gave no indication of a continuous range. Hairston (1949) found 80 percent of his records of *P. yonahlossee* in the Black Mountains from two virgin forest areas, and stated that this species was not distributed throughout the forest but was restricted to an area near streams (within 100 feet). The isolation of the

Bluerock Mountain population from the 3 northern populations and probably most pure *P. yonahlossee* morph populations is enhanced by the Broad River. Finally our field work suggests that *P. yonahlossee* in the southern part of its range exists as a discontinuous distribution of genetic isolate populations.

Electrophoretic data also support the theory that the genetic divergence observed in this study is not clinal. Three loci mentioned earlier, MR-2, Hp and Est-1 possess variants either not present in the Bluerock Mountain population, or not present in the three northern populations. Guttman et al. (1978) found three variants (Idh-1c, Pgm-3a, and Pgm-3b) present in one or more populations of *P. yonahlossee*, from areas farther south than those of this study, which were not present in the three former *P. longicrus* populations (including the Bearwallow Mountain population where both morphs are present). Conversely, Guttman et al. (1978) found two variants (Alb-1c and Alb-2d) which were present in the former *P. longicrus* populations but not in the *P. yonahlossee* populations.

At the present time, assigning taxonomic status to these two distinct groups, based on the observed level of genetic divergence, is speculative. Genetic identities as great as .8-.9 have been observed between sympatric, nonhybridizing species *P. glutinosus* and *P. jordani* (Duncan and Highton, 1979). In contrast Duncan and Highton (1979) described a new species, *P. fourchensis*, having a genetic identity of .75 to its closest relative, *P. ouachitae*, despite the fact these two salamanders hybridized in a narrow area where their ranges came in contact. Highton and Webster (1976), comparing populations of *P. cinereus* from non-glaciated areas, observed genetic identities frequently below .9 ($\bar{I}$ = .87), and suggested these populations might be a group of "semispecies" whose future evolution would be independent from one another.

Based on the level of genetic identity between the Bluerock Mountain population and the three northern populations, the most accurate classification of these two groups is probably to assign each as a different subspecies or race of *P. yonahlossee*. However, there is also the possibility that these two groups are distinct species. What we may be observing at the present time is at least two distinct groups undergoing speciation. Guttman et al. (1978), comparing the three former *P. longicrus* populations to three *P. yonahlossee* populations from the southern part of the range, reported a greater genetic identity, $\bar{I}$ = .95 (Bluerock Mountain population to three southern populations $\bar{I}$ = .92) and a greater genetic similarity, $\bar{S}$ = .93 (Bluerock Mountain population to three southern populations $\bar{S}$ = .91) than those observed in this study ($\bar{I}$ = .84, $\bar{S}$ = .76), and they placed *P. longicrus* in the synonomy of *P. yonahlossee*. The observed differences between these two studies may be the result of a significant genetic divergence of the southern *P. yonahlossee* populations as a whole (including the former *P. longicrus* populations) from the northern populations. Alternatively, the increased resolution of variant proteins by the polyacrylamide gel electrophoresis methods used in the present study may also contribute to our inability to confirm the work of Guttman et al. (1978). Evidence of the increased resolving power of polyacrylamide gel electrophoresis is illustrated by the much greater heterozygosity (H = .358) and mean number of alleles per locus (A = 1.77) for the Bluerock Mountain population in our study than was reported in the study of Guttman et al. (1978) (H = .017 and A = 1.10).

Our estimates of heterozygosity for the three northern populations ($\bar{H}$ = .149) is only slightly greater than the mean for amphibians of .079 (Nevo, 1978). The

much greater heterozygosity and number of alleles per locus of the Bluerock Mountain population as compared to the three northern populations might be explained by the older southern population (assuming the three northern populations migrated from the south after the glacial periods) having acquired more polymorphic variants (alleles). Highton and Webster (1976) reported heterozygosity and the total number of alleles to be less in populations of *P. cinereus* from formerly glaciated areas than populations from unglaciated areas, and suggested this was the result of relatively younger northern populations acquiring fewer new traits. The populations from the three northern localities in this study, although from unglaciated areas, may have been influenced more by the glaciers than the more southerly Bluerock Mountain population, as the northern regions may have been uninhabitable during the glacial periods.

The possibility exists that the type locality of *P. yonahlossee* (Grandfather Mountain) is more closely related to the former *P. longicrus* populations, than the three northern populations examined in this study, and therefore the task of assigning nomenclature for these two distinct groups is difficult at the present time. Further studies over the entire range of *P. yonahlossee* are necessary to resolve these important questions about the taxonomy and evolution of these salamanders.

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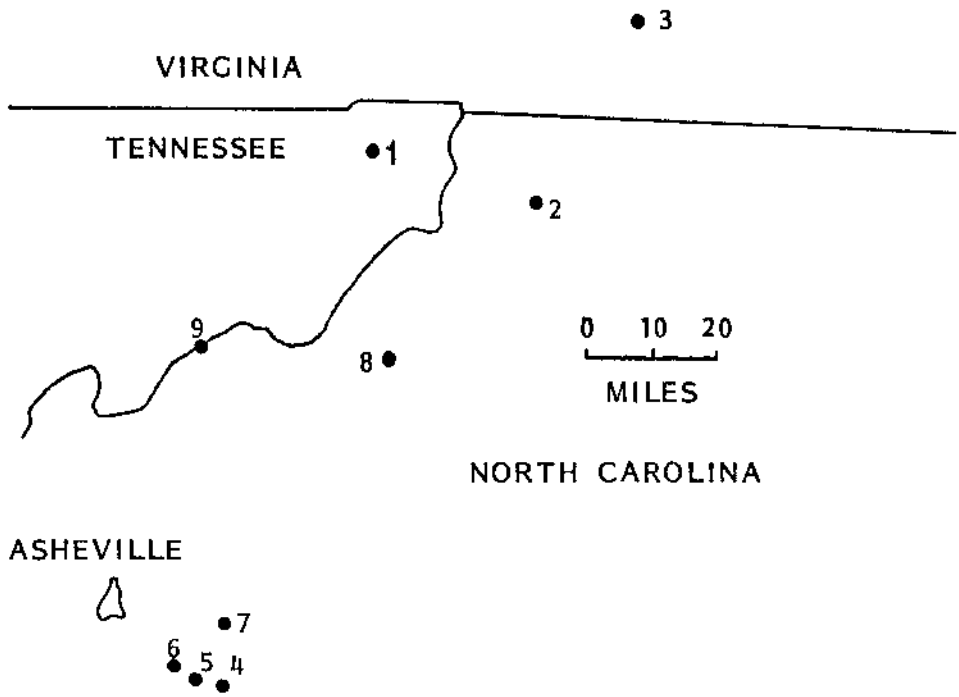

Fig. 1. Relative localities of *P. yonahlossee* for this study (1-4) and the study of Guttman et al., 1978(4-9). 1 = Iron Mountain, 2 = Mount Jefferson, 3 = Comers Rock Picnic Ground, 4 = Bluerock Mountain (type locality of former *P. longicrus*), 5 = Grant Mountain (former *P. longicrus*), 6 = Bearwallow Mountain (both morphs), 7 = Lakey Gap, 8 = Grandfather Mountain, 9 = Crest of Unaka Mountains.

Table 1. Polyacrylamide disc gel electrophoresis methods. a = Tris-glycine, pH 8.9; b = Tris-glycine, pH 8.5; c = Tris-HCL, pH 8.9 (Hagen et al., 1978); d = Barbitol-Tris, pH 7.0; e = Tris-HCL, pH 7.5 (Williams and Reisfeld, 1964); f = Tris-citrate, pH 8.8 (Schiff, 1975).

Protein Tested	Upper Buffer	Lower Buffer	Gel Buffer	Percent Acrylamide	Reference for Stain
Haptoglobin	a	d	c	7.5	Hagen et al., 1978
Esterases	d	d	e	7.5	Van Zutphen, 1974*
Adenosine Deaminase	a	b	f	8.0	Spencer et al., 1968*
Mannose-6 Phosphate Isomerase	a	b	f	10.0	Nichols et al., 1973
Malic Enzyme	a	b	c	7.5	Harris and Hopkinson, 1976
Methemoglobin Reductase	a	b	c	7.5	Niethammer and Huennekens, 1971

*Modified

Table 2. Allelic frequencies of polymorphic enzyme loci observed from populations of *P. yonahlossee* and the former *P. longicrus*.

Locality	1 Iron Mountain	2 Mount Jefferson	3 Comers Rock	4 Bluerock Mountain
Locus (N)				
MPI (5,7,19,9)	a (.60) b (.40)	a (.57) b (.43)	a	a (.67) b (.33)
MR-2 (10,7,18,9)	b	b	b	a (.11) b (.89)
Hp (10,7,19,9)	a	a	a	b (.28) c (.72)
ME-2 (10,7,19,9)	a (.65) b (.35)	a (.93) b (.07)	a (.68) b (.32)	a (.67) b (.33)
Est-1 (10,7,18,8)	b	b	a (.17) b (.81) c (.03)	b (.44) c (.56)
Est-2 (9,5,17,9)	a (.17) b (.83)	a (.70) b (.30)	a (.21) b (.79)	a (.50) b (.50)
Est-3 (10,7,19,9)	a (.80) b (.20)	a (.93) b (.07)	a (.89) b (.11)	a (.72) b (.28)
Est-4 (10,7,19,8)	b (.55) c (.45)	b (.79) c (.21)	a (.29) b (.53) c (.18)	a (.75) b (.06) c (.19)
Est-5 (7,5,6,9)	a (.50) b (.50)	a (.80) b (.20)	a (.75) b (.25)	a (.50) b (.50)

ADA*, MR-1, ME-1 and Hg were monomorphic.
*ADA for location 1 was not tested.

Table 3. Genetic identity (I) and genetic similarity (S) between populations of *P. yonahlossee* and the former *P. longicrus*. The means of group comparisons are also given.

	I				S			
Population	1	2	3	4	1	2	3	4
1 Iron Mountain		.957	.969	.816		.874	.894	.773
2 Mount Jefferson			.956	.832			.868	.740
3 Comers Rock				.863				.770
4 Bluerock								

Groups	Ï	S̈
Within Northern *P. yonahlossee* (Populations 1,2,3)	.961	.879
Between Northern *P. yonahlossee* and Former *P. longicrus* (Populations 4)	.837	.761

Table 4. Observed genetic heterozygosity (H) and mean number of alleles per locus (A) of populations of *P. yonahlossee* and the former *P. longicrus*.

Population	H Observed Mean Proportion of Heterozygous Loci per Individual	A Mean Number of Alleles per Locus
1 Iron Mountain	.177	1.50
2 Mount Jefferson	.137	1.46
3 Comers Rock	.132	1.62
4 Bluerock Mountain	.358	1.77

Ḧ = .149 for the three northern populations (1,2,3)