

**Allozyme Variation in Neighboring
Isolated Populations of the
Plethodontid Salamander
*Leurognathus marmoratus***

S. RANDAL VOSS,^{1,3} DAVID G. SMITH,^{1,4} CHRISTOPHER K. BEACHY,^{2,5} AND DAVID G. HECKEL,¹ ¹*Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634, USA, and* ²*Department of Biology, University of Southwestern Louisiana, Lafayette, Louisiana 70504, USA.*

Watersheds in the southern Appalachians have experienced a dynamic hydrological history, including episodes of stream capture—the diversion of a stream from one river drainage system to another (Ross, 1971). For obligate stream dwellers incapable of overland dispersal, stream capture isolates some populations that were previously connected and creates new connections between previously isolated populations. In many cases with geological and hydrological evidence for stream capture, patterns of distribution and diversity in several aquatic groups of organisms are concordant, suggesting that stream capture has been an important vicariant mechanism in their biogeography (Hobbs, 1971; Holsinger, 1971; Swift et al., 1986; Mayden, 1988; Lydeard et al., 1991).

The plethodontid salamander *Leurognathus marmoratus* lives in headwater streams and small rivers in the southern Appalachians. The present distribution of this obligately aquatic species over three contiguous river systems (Tennessee, Chattahoochee, and Savannah) draining this area suggests that vicariant events have influenced its distributional history (Fig. 1). Populations of *Leurognathus marmoratus* from the Tennessee drainage have presumably been isolated from populations of the Savannah and Chattahoochee drainages since the pre-Pleistocene establishment of the Eastern Continental Divide. Streams of the present-day Chattahoochee and Savannah drainages were inter-connected until the Pleistocene, flowing southwest into the Gulf of Mexico. However, during the Pleistocene a stream capture at Tallulah Falls, Georgia diverted the headwaters of the ancestral Chattahoochee River southeastward into the Tugaloo River of the Savannah Drainage and thence to the Atlantic Ocean (Campbell, 1896; Johnson, 1907a,b), isolating these streams from the Chattahoochee drainage.

This scenario predicts that gene flow between populations of obligate stream dwellers from the Savannah and Chattahoochee drainages has occurred more recently than between either drainage and the Tennessee. We tested the following predictions: (1) The

³ Present Address: Section of Evolution and Ecology, University of California, Davis, California 95616, USA.

⁴ Present Address: Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019, USA.

⁵ Present Address: Department of Biology, Clarke College, Dubuque, Iowa 52001, USA.

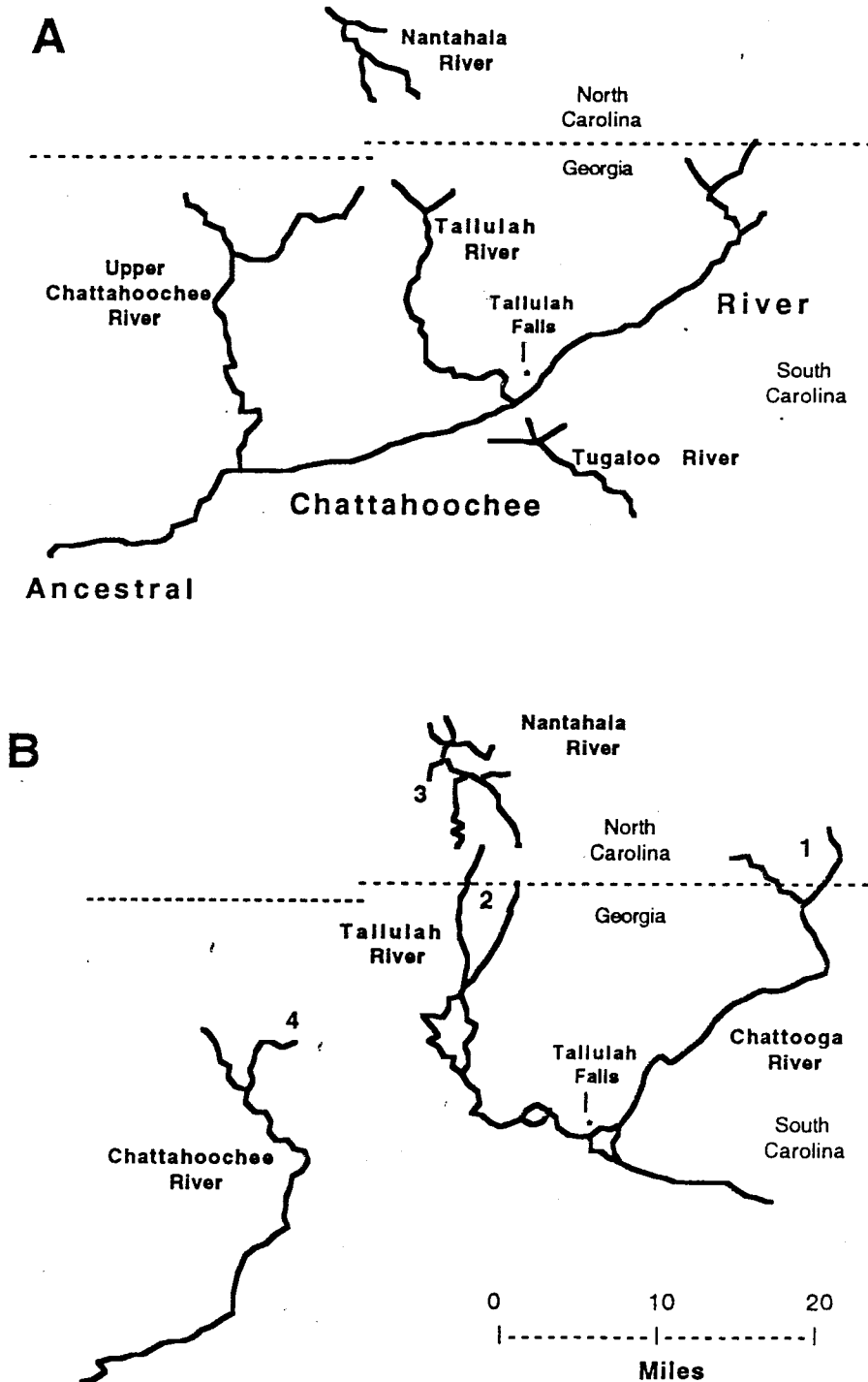


FIG. 1. Vicariant events isolating populations of *Leurognathus marmoratus* (modified after Ross 1971). The broken line in both figures is the present-day border between North Carolina and Georgia. (A) Before the Pleistocene, the Eastern Continental Divide isolated the Tennessee River drainage (Nantahala River) from the others, and the ancestral Chattahoochee drained most of the area shown. (B) During the Pleistocene, a stream capture near Tallulah Falls diverted the Tallulah and Chattooga into the Savannah River drainage (Tugaloo River), isolating the populations there from the Chattahoochee drainage. These are the present-day locations of all drainages. Numbers indicate sampling sites as described in the text.

TABLE 1. Enzymes, buffers, and tissues used in electrophoretic analysis. Buffers are TC8/d (twofold dilution of tris-citrate, pH 8.0 modified from Selander et al., 1971), phos-cit (phosphate-citrate, pH 7.0, Selander et al., 1971), and AC 5.8 (aminopropylmorpholine-citrate, pH 5.8, modified from Clayton and Tretiak, 1972). Tissues are muscle (M) or liver (L).

E.C. #	Enzyme (loci)	Buffer	Tissue
1.1.1.8	Glycerol-3-phosphate dehydrogenase (G3PDH)	TC8/d	M
1.1.1.27	Lactate dehydrogenase (LDH-1, LDH-2)	TC8/d	L
1.1.1.37	Malate dehydrogenase (MDH-1, MDH-2)	phos-cit	L
1.1.1.44	6-phosphogluconate dehydrogenase (6-PGD)	TC8/d	M
1.2.1.12	Glyceraldehyde-phosphate dehydrogenase (GAPDH)	TC8/d	M
2.6.1.1	Glutamate oxaloacetate transaminase (GOT-1, GOT-2)	TC8/d	L
2.7.4.3	Adenylate kinase (AK)	AC 5.8	M
2.7.3.2	Creatine kinase (CK)	AC 5.8	M
3.4.11.	Peptidase		
	Leu-gly-gly substrate (LGG-2)	phos-cit	L
	Leu-ala substrate (LA-1, LA-2)	phos-cit	L
5.3.1.8	Mannose-6-phosphate isomerase (MPI)	TC8/d	L
5.4.2.2	Phosphoglucomutase (PGM)	TC8/d	L

genetic distance between samples from the Chattahoochee and Savannah drainages will be lower than the genetic distance of either to a sample from the Nantahala River of the Tennessee drainage. (2) The genetic distance between samples from two different rivers of the Savannah drainage will be lower than the genetic distance of either to samples from the Chattahoochee or Tennessee drainages. Both predictions were tested by examining patterns of allozyme variation for populations of *Leurognathus mar-moratus*.

We collected post-metamorphic salamanders from one or more different streams within each drainage. As shown in Fig. 1B, the collection sites were 1) streams of the Chattooga River in the Savannah drainage: Holcomb Creek (N = 2) and Overflow Creek (N = 7) in Macon Co., North Carolina and an unnamed tributary of the Chattooga (N = 2) in Rabun Co., Georgia; 2) streams of the Tallulah River in the Savannah drainage: Tallulah River (N = 9) and Coleman River (N = 1) in Rabun Co., Georgia; 3) Tennessee drainage: Roaring Fork (N = 1) and Jarrett Creek (N = 2) in Macon Co., North Carolina and Buck Creek (N = 1) and Barnards Creek (N = 8) in Clay Co., North Carolina and 4) Chattahoochee drainage: Wilks Creek (N = 11) in White Co., Georgia.

We used starch gel electrophoresis to score 12 enzyme systems determining 16 presumptive loci in total. Liver and muscle tissue were separately homogenized in two volumes of distilled water and stored at -80 C. Homogenate was applied to filter paper wicks and loaded onto 10.5% starch gels (Connaught) subjected to horizontal electrophoresis at 75-90 volts for 12-16 h. Preliminary screening of tissue-buffer combinations yielded the optimal systems in Table 1. Stains were modified after Selander et al. (1971).

Seven loci were monomorphic (LA-1, PGM, GOT-2, MDH-1, LDH-1, G3PDH, and CK). Allele frequencies for the nine variable loci are summarized in Table 2. For eight of the variable loci, the Tennessee drainage samples were fixed for one allele and all other samples were fixed for a different allele. The ninth variable locus, GOT-1, showed both inter-drainage variation and within-population polymorphism.

Nei's (1978) unbiased genetic distance estimates (D) were used to examine the pattern of inter-drainage variation among the Tennessee, Savannah, and Chattahoochee drainages, and intra-drainage variation among the Tallulah and Chattooga Rivers of the Savannah drainage. We used Release 1.7 of BIOSYS-1 (Swofford and Selander, 1981) to conduct a cluster analysis using the unweighted pair group method on Nei's D values for the nine variable loci.

Genetic distance D showed patterns consistent with both predictions, suggesting that genetic divergence is related to the length of time that populations have been isolated from one another (Fig. 2). Populations from the Tallulah River and Chattooga River of the Savannah drainage were separated by the lowest genetic distance. Although a series of man-made lakes now isolates these populations, they were probably interconnected after the Pleistocene stream capture event transferred the headwaters of the ancestral Chattahoochee drainage to the Savannah drainage. This interpretation is supported by the slightly higher genetic distance between both populations of the Savannah drainage and the population from the Chattahoochee drainage. However, populations from the Tennessee drainage were separated from all other populations by a much higher genetic distance, supporting the prediction of a pre-Pleistocene vicariant event isolating populations on either side of the Eastern Continental Divide.

Therefore, the observed pattern of genetic variation among populations was congruent with the presumptive geologic history of the three Southern Appalachian drainages, and supported both of the predictions. The data supporting the first prediction are comparatively robust because half of the loci surveyed were fixed in the Tennessee drainage for an allele not found elsewhere. The resulting large genetic distance between the Tennessee drainage and the others is therefore unlikely to be substantially affected by increasing sample sizes or the number of loci scored. The data supporting the second prediction are less robust, based entirely on variation at the single locus GOT-1. Discovery of an additional polymorphic locus could change the value of the genetic

TABLE 2. Allele frequencies at variable enzyme loci in populations of *Leurognathus marmoratus*. Collection sites are shown in Fig. 1B. Alleles are coded according to the mobility of their product relative to the fastest observed (=100) at each locus. Genotype frequencies are shown for GOT-1, the only locus showing polymorphism within a site.

Region		Tennessee Drainage	Chattahoochee Drainage	Savannah Drainage	
Collection site		3	4	Tallulah R.	Chattooga R.
Sample size		N = 12	N = 11	2	1
Locus	Allele			N = 10	N = 11
6-PGD	100	0.00	1.00	1.00	1.00
	76	1.00	0.00	0.00	0.00
GAPDH	100	1.00	0.00	0.00	0.00
	87	0.00	1.00	1.00	1.00
AK	100	1.00	0.00	0.00	0.00
	83	0.00	1.00	1.00	1.00
MPI	100	1.00	0.00	0.00	0.00
	95	0.00	1.00	1.00	1.00
LDH-2	100	1.00	0.00	0.00	0.00
	58	0.00	1.00	1.00	1.00
MDH-2	100	0.00	1.00	1.00	1.00
	77	1.00	0.00	0.00	0.00
LGG-2	100	1.00	0.00	0.00	0.00
	16	0.00	1.00	1.00	1.00
LA-2	100	1.00	0.00	0.00	0.00
	75	0.00	1.00	1.00	1.00
Genotype					
GOT-1	100/100	1.00	0.00	0.10	0.55
	100/70	0.00	0.00	0.80	0.36
	70/70	0.00	1.00	0.10	0.09

distance calculated within and between the Savannah and Chattahoochee drainages; however all these values would still be very small compared to the larger D's separating the Tennessee drainage from the others.

Extremely low levels of genetic variation were found within drainages. Attempts to account for observed levels of genetic variation in salamander populations have invoked effects of genome size (Merkle et al., 1977; Pierce and Mitton, 1980; Larson, 1981), life-history mode (Shaffer and Breden, 1989), and species-

specific characteristics (Matsui and Hayashi, 1992). Paedomorphic species generally have lower genetic variability than those that metamorphose (Shaffer and Breden, 1989). *Leurognathus marmoratus* does undergo metamorphosis but both larval and adult life phases are aquatic; thus whatever ecological factors that reduce genetic variation in paedomorphic species may be operating on *Leurognathus marmoratus* as well. Additional studies on genome size, population structure, and behavior are necessary to evaluate the relative importance of all these factors in accounting for low levels of genetic variation in this species.

The high genetic distance between the Tennessee drainage and the others indicates that a taxonomic revision of *Leurognathus marmoratus* is probably warranted. Martof (1962) reported a high degree of geographic variation in morphological features and pigmentation in the drainages that we sampled. Although Martof (1956) initially argued for three distinct subspecies of *Leurognathus marmoratus*, he later (1962) decided that a taxonomic revision based on existing morphological differences was not the best solution. More recently, Titus (1992) used mitochondrial DNA to examine the relationship of *Leurognathus* to the desmognathines. We are currently examining the relationship between morphological and allozyme variation among populations of *Leurognathus* in these and additional drainages in an attempt to gain a better understanding of the taxonomic status and vicariance biogeography of *Leurognathus*.

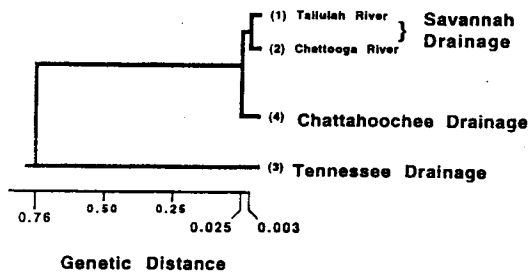


FIG. 2. Phenogram depicting an unweighted pair group method cluster analysis based upon the matrix of Nei's (1978) unbiased genetic distances between populations of *Leurognathus marmoratus*. Numbers in parentheses are collection sites indicated in Fig. 1B.

Acknowledgments.—We thank M. Voss for helping to collect salamanders. This research was supported by an NSF-EPSCoR grant to the State of South Carolina and by the Clemson University Innovation Fund.

LITERATURE CITED

- CAMPBELL, M. R. 1896. Drainage modifications and their interpretation. *J. Geology* 4:657-678.
- CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fisheries Res. Board. Canada* 29: 1169-1172.
- HOBBS, H. H., JR. 1971. On the distribution and phylogeny of the crayfish genus *Cambarus*. In P. C. Holt (ed.), *Distributional History of the Biota of the Southern Appalachians. Part I: Invertebrates*, pp. 93-178. Research Division Monograph 1, Virginia Polytechnic Inst. and State Univ., Blacksburg.
- HOLSINGER, J. R. 1971. Biogeography of the freshwater amphipod crustaceans (Gammaridae) of the central and southern Appalachians. In P. C. Holt (ed.), *Distributional History of the Biota of the Southern Appalachians. Part I: Invertebrates*, pp. 19-49. Research Division Monograph 1, Virginia Polytechnic Inst. and State Univ., Blacksburg.
- JOHNSON, D. W. 1907a. Drainage modifications in the Tallulah district, Georgia. *Science* 25:428-432.
- . 1907b. River capture in the Tallulah district, Georgia. *Boston Soc. Nat. Hist. Proc.* 23:211-248.
- LARSON, A. 1981. A reevaluation of the relationship between genome size and genetic variation. *Amer. Natur.* 118:119-125.
- LYDEARD, C., M. C. WOOTEN, AND M. H. SMITH. 1991. Occurrence of *Gambusia affinis* in the Savannah and Chattahoochee drainages: previously undescribed geographic contacts between *G. affinis* and *G. holbrooki*. *Copeia* 1991:1111-1116.
- MARTOF, B. S. 1956. Three new subspecies of *Leurognathus marmorata* from the southern Appalachian Mountains. *Occ. Pap. Mus. Zool. Univ. Mich.* 575:1-14.
- . 1962. Some aspects of the life history and ecology of the salamander *Leurognathus*. *Amer. Mid. Natur.* 67:1-35.
- MATSUI, M., AND T. HAYASHI. 1992. Genetic uniformity in the Japanese giant salamander, *Andrias japonicus*. *Copeia* 1992:232-235.
- MAYDEN, R. L. 1988. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. *Syst. Zool.* 37:329-355.
- MERKLE, D. A., S. I. GUTTMAN, AND M. A. NICKERSON. 1977. Genetic uniformity throughout the range of the hellbender, *Cryptobranchus alleganiensis*. *Copeia* 1977:549-553.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.
- PIERCE, B. A., AND J. B. MITTON. 1980. The relationship between genome size and genetic variation. *Amer. Natur.* 116:850-861.
- ROSS, R. D. 1971. The drainage history of the Tennessee River. In P. C. Holt (ed.), *Distributional History of the Biota of the Southern Appalachians. Part III: Vertebrates*, pp. 11-42. Research Division Monograph 4, Virginia Polytechnic Inst. and State Univ., Blacksburg.
- SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY. 1971. Biochemical polymorphisms and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). *Studies in Genetics VI*. University of Texas Publ. 7103:49-90.
- SHAFFER, H. B., AND F. BREDEN. 1989. The relationship between allozyme variation and life history: non-transforming salamanders are less variable. *Copeia* 1989:1016-1023.
- SWIFT, C. C., C. R. GILBERT, S. A. BORTONE, G. H. BURGESS, AND R. W. YERGER. 1986. Zoogeography of the freshwater fishes of the southeastern United States: Savannah River to Lake Pontchartrain. In C. H. Hocutt and E. O. Wiley (eds.), *The Zoogeography of North American Freshwater Fishes*, pp. 213-264. John Wiley & Sons, New York.
- SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Heredity* 72:281-283.
- TITUS, T. A. 1992. A phylogenetic analysis of the Desmognathinae (Caudata: Plethodontidae): evolutionary patterns inferred from mitochondrial DNA sequences. Ph.D. Diss., Univ. Kansas, Lawrence.

Accepted: 30 April 1995.