

## Genetic Differentiation among Long-Toed Salamander (*Ambystoma macrodactylum*) Populations

DAVID A. TALLMON, W. CHRIS FUNK, WILLIAM W. DUNLAP, AND FRED W. ALLENDORF

We examined the genetic population structure of Long-Toed Salamanders (*Ambystoma macrodactylum*) from the Bitterroot Mountains of Idaho and Montana to better understand their evolutionary history and genetic population structure. Populations show high levels of within-population genetic variation at six polymorphic allozyme loci ( $H_s = 0.09$  for all 18 loci examined; range 0.04–0.14). There is very little divergence among populations within basins, suggesting panmixia within basins. In contrast, genetic differentiation among all populations is high ( $G_{st} = 0.30$ ). We used computer simulations to examine population structures that could have led to the observed distribution of genetic variation, assuming selective neutrality of the allozymes. To test the assumption of selective neutrality of the markers used in this study, we compared the observed divergence among the allozymes to that expected from simulations of independently segregating and selectively neutral markers. The observed genetic divergence among populations is compatible with that expected for neutral genetic markers sampled from panmictic populations within basins that exchange less than one migrant among basins each generation.

THE population structure of a species (migration rates among populations and population sizes) is a major factor controlling the diversity of genotypes exposed to natural selection. For species with a subdivided population structure, genetic drift in small populations results in a loss of within-population genetic variation, whereas migration among populations can increase within-population variation. High migration rates can inhibit local adaptation, whereas moderate or low rates of migration can infuse local populations with adequate variation for local adaptation. In large, randomly mating populations, alleles may remain near equilibrium frequencies, whereas the effects of genetic drift may prevail in changing allele frequencies in small populations.

We used protein electrophoresis to examine the genetic population structure of 34 populations of Long-Toed Salamanders (*Ambystoma macrodactylum*) in the Bitterroot Mountains of Idaho and Montana. These populations occupy high-elevation ponds and lakes that have existed less than 12,000 yr since the last major glaciation event (Mehring et al., 1977). At high elevations, adults of this species breed in standing water, are fossorial most of the year and do not always lay discrete egg masses. This life history makes it difficult to estimate the numbers of breeders and migrants using direct methods, such as mark-recapture. However, it is easy to find and sample larvae at remote breeding sites because they spend at least several months developing in shallow, standing water. Therefore, we used the indirect method of sampling larvae

for genetic analysis to better understand the structure and evolutionary history of these populations (Slatkin, 1987; Milligan et al., 1994).

We used several genetic markers to interpret the genetic structure of these populations because natural selection or historical changes in population structure can affect individual markers differently. A consistent pattern among markers lends confidence to an interpretation of evolutionary history. We further developed and tested plausible historical population structures by comparing genetic marker data from the natural populations to data from simulated populations with known population structure. The use of simulations allowed us to control the population structure of hypothetical populations and then to observe the effects of modifying this structure on the distribution of genetic variation. This combination of empirical data and simulations allowed us to identify randomly mating units or demes and to estimate the number of migrants among these demes.

### MATERIALS AND METHODS

*Study area and sample collection.*—We collected individuals from 34 populations in a 35 × 15 km region of the Bitterroot Mountains of western Montana and eastern Idaho (the main study area). Six additional populations found to the west and north of the main study area were sampled. All populations sampled are designated by three-letter abbreviations in the text and figures (see Appendix for descriptions). Many of the 34 ponds and smaller lakes in the main study area

are relatively free of direct human influence because they lie within the Bitterroot Wilderness Area. However, most of the larger and deeper lakes have been stocked with trout and contain few or no salamanders. Therefore, these 34 samples represent a large proportion of the permanent lakes and ponds in this area that can support salamander populations.

We used dip nets to collect salamander larvae, rather than adults, to maximize both the size and number of samples taken from the wilderness ponds. However, adults were sampled from JUM and DON, which are found at lower altitudes outside the wilderness study area.

*Genetic analysis.*—All animals were kept alive until frozen at  $-40$  C. Most samples were electrophoresed within two months of collection, but some were held for two years without any major protein denaturation. We examined 18 inferred allozyme loci on two buffer systems: A (Ridgway et al., 1970); and B (Clayton and Tretiak, 1972). Allozyme nomenclature follows Shaklee et al. (1990). Six allozymes are polymorphic in the main study area (*AAT*, *PGM-1*, *MPI*, *G6PDH*, *ACP-1*, *PGDH*), and two additional allozymes are polymorphic outside the main study area (*LDH-1*, *mIDPH*).

*Genetic variation within local populations.*—Short-term fluctuations in allele frequencies can make interpretation of geographic structure or evolutionary history from genetic marker data inaccurate or impossible. We directly tested the temporal stability of gene frequencies by repeated sampling of a population throughout a single year. Because most of the high-altitude ponds in the Bitterroot Mountains that contain larval populations are difficult to access during winter months, the more accessible population DON, located northwest of the main study area, was sampled five times during a 15-month interval. Adults were sampled during the breeding seasons of 1977 and 1978, and larvae were sampled once in 1977 and twice in 1978. Also, larvae were sampled from two populations, DMC and TCR, in the main study area in both 1977 and 1978. We used chi-square tests for heterogeneity to examine the stability of allele frequencies within populations between sampling periods and between populations. Samples from all populations were tested for conformity to Hardy-Weinberg proportions using chi-square goodness-of-fit tests.

In addition to the possibility of rapid temporal fluctuations in allele frequencies, microgeographic variation within samples can lead to an erroneous interpretation of population structure. To test for within-sample geographic varia-

tion, we again sampled individuals from DON because this pond dries each year into four smaller ponds. Although this population is outside the main study area and at a lower elevation than most populations, we assumed that the drastic seasonal changes in this pond would result in within-population subdivision here if it occurs anywhere. Collectively, 13 larval samples were taken from three of the remnant ponds during a single month and analyzed to examine the stability of allele frequencies within this population. In addition, we tested these samples, after pooling data across remnant ponds, for the Wahlund effect, or an excess of homozygotes, that occurs when several demes are included in a single sample.

*Genetic divergence among populations.*—We used Nei's gene diversity analysis ( $G_{st}$ ) to quantify large-scale patterns in genetic variation. This estimator uses standardized variances of gene frequencies among populations to measure divergence and allows us to apportion the genetic diversity between levels of our salamander populations. By apportioning the gene diversity within and between populations in basins ( $G_{pb}$ ), basins on ridges ( $G_{br}$ ), and ridges in the main study area ( $G_{st}$ ), we can estimate the number of migrants that could have produced the observed pattern of variation. In addition, we can test the hypothesis that population structure is controlling the pattern of genetic variation by comparing the amount of differentiation estimated from Nei's diversity analysis at different allozymes to simulated values.

Principal component analysis (PCA) provides another means to examine a large amount of the variance in gene frequencies among populations in a simplified way. We used PCA to examine the genetic similarities of populations in relation to geographic proximity.

*Simulation model.*—Empirical data from the main study area were compared to results from a simulation model we constructed to examine possible historical structures for these populations. The simulation model was written in TurboPascal (Borland International, Inc., 1992) and is available upon request.

We used the model to examine numbers of migrants that might yield observed mean divergence values. Our interpretation of historical population structures assumes selective neutrality of the molecular markers. Therefore, we used the simulation model as a test of this assumption following the conceptual example of Lewontin and Krakauer (1973). These authors showed that, if genetic marker loci are neutral, then their

distribution is determined primarily by breeding structure, and the divergence estimates among loci ( $G_{st}$ ) should be similar. However, if selection is affecting one or more markers, then affected markers should show divergence estimates different from the others.

Selection is not included in our simulation model. Therefore, if the observed range of single-locus divergence estimates from the natural populations exceeds that from the simulations, one or more loci may well be affected, directly or indirectly, by selection. In contrast, if the variance in divergence estimates from the model is greater than the variance of the divergence estimates from the observed data, then we may infer that the observed distribution of genetic variation is due to drift and migration. Thus, we can use the simulation results as a test of the neutrality of our markers by comparing the range of  $G_{st}$  estimates for the observed and simulated populations.

Our model is postulated with little empirical information about present population structure and represents just one of many ways the observed patterns could be produced. However, we did use some information about effective population sizes and gene flow patterns to construct our model. Comparisons of allele frequency changes in samples taken in 1978 and 1996–1997 from several populations suggest effective population sizes of less than 100 individuals (Funk et al., In press).

We used a generalized gene flow pattern derived from a comparison of genetic differentiation and geographic distance with the DIST and MANTEL options of the GENEPOP computer program (Raymond and Rousset, 1995). A rank-correlation of genetic differentiation and geographic distance suggests isolation by distance ( $R_s = 0.401$ ,  $P < 0.05$ ). Accordingly, we structured our model as a two-dimensional lattice ( $4 \times 5$ ) of 20 populations of 100 individuals each, linked by a stepping-stone pattern of migration.

We varied the migration rate ( $m$ ) among these populations to determine what range of absolute numbers of migrants ( $Nm$ ) could produce the observed divergence among the basins, how quickly this divergence could occur, and to test the neutrality of the allozyme markers. To begin each simulation, 100 genetically identical, homozygous individuals were assigned to each population. Following migration, 50 random pairings from a common pool of female and male migrants ( $m$ ) and residents ( $1 - m$ ) produced the next generation of 100 individuals. Genetic variation was added via mutation during zygote formation. Genetic diversity measures, corresponding to those calculated for the natural popula-

tions, were recorded at generations 50, 100, 200, and 1000. Each set of initial conditions was run 50 times.

## RESULTS

*Genetic variation within populations.*—The amount of genetic variation in the main study area is extremely high within populations. Mean expected heterozygosity across all populations is  $\bar{H}_s = 0.09$  (for all 18 loci examined), and ranges from 0.04–0.14 in these populations. Nearly all populations contain at least two alleles at all six polymorphic allozyme loci except *PGDH*, which is polymorphic in about one-fourth of the populations.

All tests of the stability and distribution of genetic variation suggest that our sampling methods are reliable and that these populations are stable over the short term. Specifically, no allele frequency changes between sampling periods were detected at any of the polymorphic loci in any of the samples collected over two summers from TCR, DMC, and DON, over two summers. In addition, fewer than 5% of chi-square goodness-of-fit tests for deviations from Hardy-Weinberg proportions in all populations were significant. This proportion of significant results is expected from type I error alone.

Exhaustive sampling of DON suggests that within-population subdivision is unlikely. Significant heterogeneity of allele frequencies was present at only one locus (*mIDPH*) in one of 13 larval samples taken from remnant ponds of DON. Again, more than one statistically significant result of 78 (13 samples  $\times$  6 loci) is expected due to type I error. Similarly, there was no evidence of an excess of homozygotes when pooling samples collected from these remnant ponds. We conclude that none of these tests indicates that our sampling methods are unreliable or that more extensive population subdivision occurs within sampling units.

*Genetic divergence among populations.*—Total genetic diversity and differentiation among populations are high. Many of the polymorphic allozymes show notable allelic diversity within the main study area, with three, four, and five alleles found at *AAT*, *MPI*, and *PGM-1*, respectively (Table 1).

Divergence in allele frequencies within the main study area is high. For example, DMC and GLE, which are separated by several kilometers, are nearly fixed for different alleles at *MPI* and differ in allele frequencies at *AAT* and *G6PDH* by approximately 40% (Table 1). STG contains a private *PGM-1* allele at relatively high frequency (0.30). Such extreme variation between popula-

TABLE 1. ALLELE FREQUENCIES OF SIX ALLOZYME LOCI POLYMORPHIC IN SAMPLES FROM 34 POPULATIONS (*Pep*) IN THE BITTERROOT MOUNTAINS OF IDAHO AND MONTANA (COM-FEZ) AND SIX POPULATIONS OUTSIDE OF THE MAIN STUDY AREA (JUM-TUR). (The frequency of one allele per locus is omitted.)

Pop	AAT			PGMH				MPI			GGPGH			ACPI		PGDH
	a	b	a	a	b	c	d	a	b	c	a	b	c	a	b	a
COM	—	1.000	1.000	—	—	—	—	0.025	0.975	—	1.000	—	—	0.756	0.244	0.486
GAS	—	1.000	0.781	—	—	—	—	0.367	0.632	—	0.970	—	—	0.924	0.075	1.000
GLE	0.293	0.706	1.000	—	—	—	—	0.034	0.965	—	0.069	—	—	1.000	—	0.954
TCR	0.225	0.774	0.967	—	—	—	—	0.048	0.951	—	0.080	—	—	1.000	—	0.903
UHD	0.094	0.905	0.962	—	—	—	—	0.641	0.358	—	—	—	—	0.829	0.170	1.000
STG	0.212	0.787	0.637	—	—	—	0.326	0.412	0.575	—	0.512	—	—	0.644	0.355	1.000
UPR	0.298	0.701	0.764	—	—	—	—	0.392	0.607	—	0.625	—	—	0.843	0.156	1.000
SH2	0.275	0.725	1.000	—	—	—	—	0.816	0.183	—	0.440	—	—	0.913	0.086	0.923
SH3	0.191	0.808	1.000	—	—	—	—	0.762	0.237	—	0.400	—	—	1.000	—	0.866
LDS	0.303	0.696	1.000	—	—	—	—	0.796	0.203	—	0.345	—	—	1.000	—	0.848
UDS	0.044	0.955	1.000	—	—	—	—	0.676	0.323	—	0.450	—	—	1.000	—	0.893
SMC	0.483	0.516	1.000	—	—	—	—	0.935	0.064	—	0.409	—	—	1.000	—	1.000
DMC	0.530	0.469	1.000	—	—	—	—	0.907	0.092	—	0.462	—	—	0.986	0.013	0.972
LSM	0.159	0.840	1.000	—	—	—	—	0.625	0.375	—	0.215	—	—	0.465	0.035	1.000
UDG	0.264	0.735	0.867	—	—	—	—	0.941	0.058	—	0.088	—	—	0.323	0.676	1.000
LBV	—	1.000	0.975	—	—	—	—	1.000	—	—	0.500	—	—	0.426	0.574	1.000
UBV	0.071	0.928	0.892	—	—	—	—	0.940	0.059	—	0.345	—	—	0.462	0.538	1.000
ALD	0.156	0.843	0.750	—	—	—	—	1.000	—	—	0.343	—	—	0.187	0.813	1.000
LAP	—	1.000	1.000	—	—	—	—	0.791	0.208	—	—	—	—	0.277	0.723	1.000
LSJ	0.108	0.850	0.983	—	—	—	—	0.816	0.183	—	0.508	—	—	0.850	0.150	0.333
ASC	0.350	0.614	0.921	—	—	—	—	0.816	0.183	—	0.121	—	—	0.458	0.542	1.000
SWE	0.516	—	0.573	—	—	—	—	0.803	0.196	—	0.672	—	—	0.409	0.591	0.795
DUF	0.066	0.933	0.988	—	—	—	—	0.133	0.866	—	0.411	—	—	0.512	0.487	1.000
NDF	0.285	0.700	1.000	—	—	—	—	0.200	0.800	—	0.485	—	—	0.551	0.449	0.971
MOH	0.179	0.820	1.000	—	—	—	—	0.166	0.833	—	0.370	—	—	0.759	0.240	1.000
WOO	0.590	0.363	0.727	—	—	0.025	—	0.568	0.409	—	0.545	—	—	0.357	0.642	0.977
BF4	0.745	0.254	0.441	—	—	—	—	0.264	0.735	—	0.676	—	—	0.490	0.509	1.000
BF3	0.791	0.175	0.675	—	—	—	—	0.341	0.658	—	0.625	—	—	0.316	0.683	1.000
BF2	0.800	0.193	0.783	—	—	—	—	0.400	0.600	—	0.500	—	—	0.533	0.466	1.000
BF1	0.675	0.289	0.728	—	—	—	—	0.438	0.561	—	0.543	—	—	0.350	0.649	1.000
TER	0.787	0.202	0.923	—	—	—	—	0.608	0.391	—	0.670	—	—	0.047	0.952	1.000
SRY	0.803	0.122	0.844	—	—	—	—	0.696	0.278	—	0.631	—	—	0.400	0.600	1.000

TABLE 1. CONTINUED.

Pop	AAT		PGM1				MPI			G6PGH		ACP1		PGDH
	a	b	a	b	c	d	a	b	c	a	b	a	b	a
MFP	0.843	0.109	0.671	0.281	0.048	—	0.796	0.171	—	0.703	—	0.638	0.361	1.000
FFZ	0.594	0.310	0.567	0.418	0.015	—	0.743	0.256	—	0.757	—	0.100	0.900	0.986
JUM	0.443	0.488	0.840	0.159	—	—	0.909	0.090	—	0.693	0.306	0.738	0.261	1.000
DON	0.500	0.403	0.645	0.266	0.089	—	0.976	0.023	—	0.650	0.349	0.604	0.395	1.000
JMI	0.682	—	0.714	—	0.286	—	1.000	—	—	0.250	0.750	0.974	0.025	1.000
LEW	0.763	0.157	0.750	0.250	—	—	0.973	—	0.027	0.263	0.315	0.527	0.444	1.000
ETW	0.724	0.061	0.734	0.163	0.003	—	0.989	0.010	—	0.687	0.312	0.732	0.267	1.000
TUR	0.451	0.483	0.919	—	0.081	—	0.871	0.128	—	0.790	0.209	1.000	—	1.000

tions is possible only if populations are nearly isolated from each other or if selection is acting on these loci.

There are also broad-scale patterns of genetic variation within the entire region. Populations in the northern part of the main study area harbor rare alleles at *MPI*, *PGM-1*, and *AAT*, found in samples taken from populations to the north and west of the main study area. The *PGDH*\*(b) allele occurs at low to moderate frequencies along the eastern edge of the main study area and is common to the south in COM. STG, in the southwest corner of the main study area, has private alleles at *PGM-1* and *MPI*, and ASC contains a private *PGM-1* allele at very low frequency. Each of these rare alleles occurs in a single population or geographically clustered populations and is not patchily distributed throughout the study area. Otherwise, one or two alleles are common at these loci and are present throughout the main study area.

The geography of the main study area suggests a hierarchical population structure. The ponds are typically found in basins (glacial cirques) lying on long ridges at elevations of 1900–2500 m. In most cases, several ponds are found in each basin, and several basins occupy each ridge (Fig. 1). Most of the ponds in the main study area are found on one of four major ridges. The ponds within each basin are separated by short stretches of moist, grassy terrain; steep, rocky spur ridges lie between basins; and canyons as deep as 1000 m and several kilometers wide separate the ridges.

Only a small amount of the total variation among populations is due to differences between populations within the same basins ( $G_{pb} = 0.026$ ); much more variation is due to differences between basins on a ridge ( $G_{br} = 0.124$ ); and about half occurs between the four major ridges ( $G_{ri} = 0.149$ ; Table 2). The low divergence within basins suggests panmixia at this geographic scale and supports the lack of significance found in tests for heterogeneity in allele frequencies between populations within basins. In contrast, a great deal of variation, 30%, is due to differences among all local populations. Most tests for heterogeneity of allele frequencies between populations from different basins were significant.

The populations of the main study area are separated into three clusters by the first and second principal components. These principal components explain 39% and 26% of the variance in allele frequencies among populations, respectively (Fig. 2). The allozyme loci contribute very unevenly to the PCA, with *AAT* and *ACP-1* contributing the most to the first principal component and *MPI* contributing the most to the second

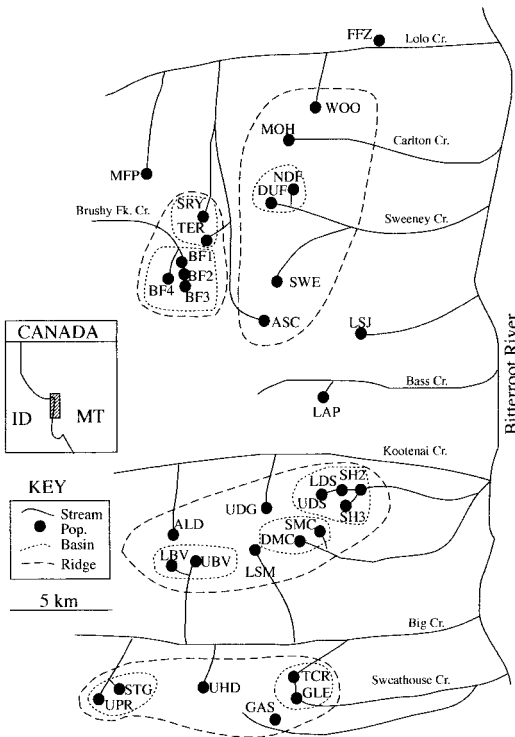


Fig. 1. Long-toed salamander populations sampled in the main study area of the Bitterroot Mountains of eastern Idaho and western Montana. Dotted lines outline basins containing multiple populations ( $\beta$ ). Dashed lines outline the four major ridges.

principal component. Fifteen populations in the center of the main study area form a cluster (UHD, SH2-ASC; cluster C, Fig. 2), as do 10 populations in the northeast (SWE, WOO-FFZ; cluster A, Fig. 2). These clusters reflect geography but not topography, because they include populations from different ridges in the same clusters. For example, ASC and UHD do not cluster with other populations located on their respective ridges. In contrast to the clusters A and C, which make geographic sense, cluster B contains populations from both the northern (DUF, NDF, MOH) and southern (COM-TCR, STG, UPR) portions of the main study area.

*Simulation model.*—We used the simulation model to examine numbers of migrants that might yield observed mean divergence values and to test the selective neutrality of these markers. The model results qualitatively follow those of previous modeling efforts in that the absolute number of migrants,  $Nm$ , determines the course of divergence (Kimura and Maruyama, 1971; Allendorf and Phelps, 1981). The absolute number of migrants is determined by the product of population size,

TABLE 2. SINGLE-LOCUS TOTAL HETEROZYGOSITY ( $H_i$ ), DIVERGENCE ESTIMATES ( $G_{ij}$ ) AND OVERALL HIERARCHICAL GENE DIVERSITY ANALYSIS ( $G_{pb}$ ,  $G_{br}$ ,  $G_{rt}$ ,  $G_{pt}$ ) OF LONG-TOED SALAMANDERS IN THE BITTERROOT MOUNTAINS OF IDAHO AND MONTANA.

Locus	$H_i$	$G_{ij}$
AAT	0.493	0.350
PGM-1	0.242	0.210
MPI	0.481	0.340
G6PGH	0.493	0.212
ACP-1	0.477	0.357
PGDH	0.091	0.322

$G_{pb} = 0.026$  Diversity between populations in a basin  
 $G_{br} = 0.124$  Diversity between basins on a ridge  
 $G_{rt} = 0.149$  Diversity between ridges  
 $G_{pt} = 0.299$  Total diversity between populations

$N$ , and the proportion of the population that is migrants,  $m$ . Our results suggest the observed divergence among genetically homogeneous basins would be expected at equilibrium only if fewer than one migrant is exchanged among populations each generation ( $Nm < 1$ ; Fig. 3). If the number of migrants exchanged is very small ( $Nm = 0.05$ ), then populations with effective sizes of approximately 100 individuals would be expected to diverge to very high levels within 100 generations. If more migrants are exchanged each generation ( $Nm = 0.90$ ), then the observed di-

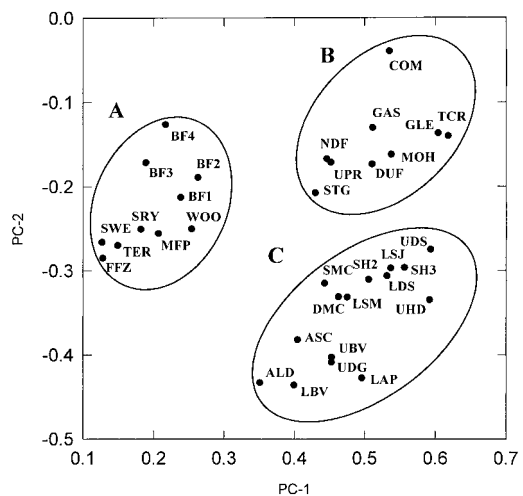


Fig. 2. Plot of the first two principal component scores of the most common allele at six polymorphic loci among 34 long-toed salamander populations in the Bitterroot Mountains. (The axes are scaled by the square root of the associated eigenvalue.) These 34 populations aggregate loosely into three clusters lettered A, B, and C.

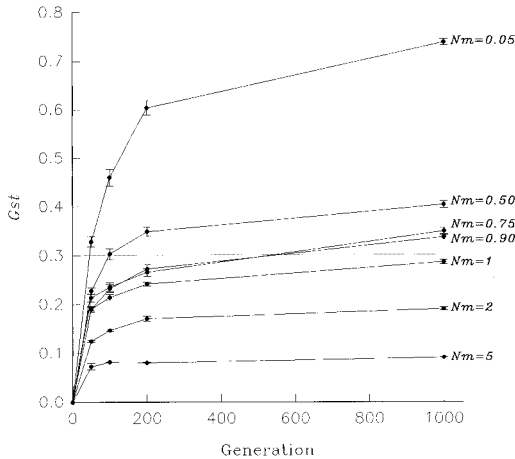


Fig. 3. Mean and variance in divergence among populations ( $G_{st}$ ) as a function of the number of migrants per generation ( $Nm$ ) between populations. Effective population size is 100 for each population. (The dotted line indicates the observed mean level of divergence among long-toed salamander populations.)

vergence could be produced within several hundred generations.

It is important to note that there is a great deal of variation around each simulated mean  $G_{st}$ -value (Table 3). Even the smallest 95% confidence interval around the divergence estimator at any point in the simulations spans a greater range of values than that observed for the six polymorphic allozymes (0.21–0.35). This result suggests selective neutrality of the allozyme markers used in this study. Therefore, if the number of migrants exchanged among populations each generation is less than one and effective population sizes are approximately 100 individuals, the observed variation in divergence estimates of the six polymorphic allozyme loci could be achieved rapidly and maintained for many hundreds of generations.

## DISCUSSION

We found substantial genetic variation in these populations of long-toed salamanders and gained insight into several evolutionarily important genetic population characteristics. The genetic similarity among ponds within basins, shown by the lack of significance of tests for heterogeneity in allele frequencies and the gene diversity analysis, suggests there is sufficient migration among populations within basins to make them panmictic. In other words, salamanders within basins compose randomly mating units, or demes.

The distribution of many alleles throughout the main study area suggests gene flow may be an important factor in the evolution of these populations. However, the patterns in allele frequencies and high divergence estimates among basins and ridges indicate there has not been sufficient gene flow to homogenize these populations beyond the basin level. The correlation of geographic distance and genetic differentiation suggests isolation by distance of these populations.

The high estimated mean level of divergence is due to the exchange of only a small number of migrants among populations. More specifically, these data are consistent with the exchange of slightly less than one migrant per generation among homogeneous basins linked by a stepping-stone pattern of migration. The observed levels of divergence among populations could be produced within tens to hundreds of generations if less than one migrant is exchanged among basins each generation ( $Nm < 1$ ). This level of migration can be attributed to a low migration rate ( $m$ ) among populations or small effective population sizes ( $N$ ). Analyses of recently collected data suggest the latter is the case (Funk et al., In press). If effective population sizes are smaller than the 100 individuals used in the simulation model, then the observed level of divergence could be achieved much faster for identical rates

TABLE 3. THE MEAN (AND SD) GENETIC DIVERGENCE ( $G_{st}$ ) FROM STOCHASTIC SIMULATIONS OF 20 POPULATIONS OF 100 INDIVIDUALS EACH, ARRANGED IN A TWO-DIMENSIONAL STEPPING-STONE PATTERN.  $Nm$  is the expected number of migrants exchanged among populations each generation.

$Nm$	Generation			
	50	100	200	1000
0.90	0.19 (0.070)	0.23 (0.088)	0.27 (0.096)	0.34 (0.085)
0.75	0.21 (0.089)	0.24 (0.095)	0.27 (0.088)	0.35 (0.085)
0.50	0.23 (0.082)	0.30 (0.105)	0.35 (0.098)	0.40 (0.084)
0.05	0.33 (0.101)	0.46 (0.129)	0.60 (0.124)	0.74 (0.080)

of migration among basins, as shown by Kimura and Maruyama (1971). Therefore, the use of 100 individuals in each simulated population (the upper bound estimated by Funk et al., In press) makes conservative our estimates of the time required to reach the simulated levels of equilibrium divergence.

Although it is difficult to eliminate the possibility that selection has produced any pattern of genetic variation, the observed range of divergence estimates is consistent with that expected from six neutral loci, assuming our estimates of population size and rates and patterns of gene flow are reasonable. We acknowledge that the statistical power to reject the null hypothesis of neutral markers is unknown and may be low for our model.

If these allozyme markers are not in drift-migration equilibrium, the observed distribution of genetic variation may be due to historical events or on-going processes. At one extreme, it is possible that the estimated level of divergence is the result of historical associations of these populations and a lack of any gene flow following population founding (Larson et al., 1984). Indeed, although two PCA clusters suggest geographic association of populations, a third cluster (Cluster B; Fig. 2) includes populations unlikely to be linked by gene flow because intervening populations are excluded. However, the randomization test, which suggests isolation by distance of these populations, along with the high levels of within-population variation, provides evidence that these populations are probably not evolving in complete isolation. Alternatively, these populations could be in the process of converging in allele frequencies due to high levels of ongoing gene flow that are erasing founder effects that produced the observed divergence among populations. This hypothesis may gain support from the remarkably high levels of polymorphism observed in these small populations.

The allozyme data we collected provided insight into several important aspects of the structure of these populations. Populations within basins are largely genetically indistinguishable from one another, or panmictic. Although definitive conclusions about large-scale patterns are more difficult to attain, these populations appear to be linked by low levels of gene flow following a stepping-stone pattern of migration. It also seems likely that these populations were founded in the last few hundred generations, given the high levels of genetic variation within these small populations.

#### ACKNOWLEDGMENTS

G. Luikart and P. Spruell provided valuable comments on early drafts of this manuscript, and J. Citta was invaluable in producing Figure 3. J. Howard kindly provided samples from LEW. DAT thanks the Five Valleys Chapter of the Audubon Society and a National Science Foundation Training Grant (DGE9663611) for support during preparation of this manuscript. WCF was supported by a National Science Foundation Graduate Research Fellowship (DGE9616153) and a seed grant from the Declining Amphibian Populations Task Force.

#### LITERATURE CITED

- ALLENDORF, F. W., AND S. R. PHELPS. 1981. Use of allelic frequencies to describe population structure. *Can. J. Fish. Aqua. Sci.* 38:1507–1514.
- CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Bd. Canada* 29:1169–1172.
- FUNK, W. C., D. A. TALLMON, AND F. W. ALLENDORF. In press. Small effective size in the long-toed salamander. *Mol. Ecol.*
- KIMURA, M., AND T. MARUYAMA. 1971. Pattern of neutral polymorphism in a geographically structured population. *Genet. Res.* 18:125–131.
- LARSON, A., D. B. WAKE, AND K. P. YANEV. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. *Genetics* 106:293–308.
- LEWONTIN, R. C., AND J. KRAKAUER. 1973. Distribution of gene frequency as a test of the selective neutrality of polymorphisms. *Ibid.* 74:175–196.
- MEHRINGER, P., S. F. ARNO, AND K. L. PETERSEN. 1977. Postglacial history of Lost Trail Pass bog, Bitterroot Mountains, Montana. *Arc. Alpine Res.* 9:345–368.
- MILLIGAN, B. G., J. LEEBENS-MACK, AND A. E. STRAND. 1994. Conservation genetics: beyond the maintenance of marker diversity. *Mol. Ecol.* 3:423–435.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP. Version 1.2. A population genetics software for exact tests and ecumenicism. *J. Hered.* 86:248–249.
- REDGWAY, G. J., S. SHERBURNE, AND R. LEWIS. 1970. Polymorphisms in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* 99:147–151.
- SHAKLEE, J. B., F. W. ALLENDORF, D. C. MORIZOT, AND G. S. WHITT. 1990. Gene nomenclature for protein-coding loci in fish. *Ibid.* 119:2–15.
- SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787–792.
- (DAT, WCF, FWA) DIVISION OF BIOLOGICAL SCIENCES, UNIVERSITY OF MONTANA, MISSOULA, MONTANA 59812; AND (WWD) 2043 MT. VERNON-BIG LAKE ROAD, MOUNT VERNON, WASHINGTON 98724. E-mail: (DAT) dtatum@selway.umt.edu. Send reprint requests to DAT. Submitted: 12 Nov. 1998. Accepted: 8 June 1999. Section editor: J. D. McEachran.

APPENDIX. SAMPLE SITE LOCATIONS. Populations and sample sizes (n) are listed; including legal description (Township, Range, Section, and Quadrat), elevation, and location. COM–FFZ are in the main study area; JUM–DON are found to the west, north, and east of the main study area.

Population	n	Legal description	Elev. (m)	Location
COM	40	T4N R21W S30 SW	1298	Lk. Como slough
GAS	34	T8N R21W S30 SW	1871	near Gash Cr.
GLE	29	T8N R22W S13 NE	2299	Glenn Lk.
TCR	62	T8N R22W S13 NE	2388	above Glenn Lk.
UHD	53	T8N 4222 S11 SW	2237	above Hidden Lk.
STG	40	T8N R22W S16 NE	2207	unnamed pond
UPR	52	T8N R22W S17 NE	2229	above Pearl Lk.
SH2	60	T9N R21W S22 SE	2213	Sharrot Cr. basin
SH3	60	T9N R21W S27 NE	2316	Sharrot Cr. basin
LDS	33	T9N R21W S27 NW	2316	Sharrot Cr. basin
UDS	34	T9N R21W S27 NW	2438	Sharrot Cr. basin
SMC	31	T9N R21W S28 SE	2432	McCalla Cr. basin
DMC	54	T9N R21W S33 NW	2438	McCalla Cr. basin
LSM	44	T9N R21W S31 NE	2253	Little St. Joe Pk.
UDG	34	T9N R21W S29 NW	2070	unnamed pond
LBV	41	T9N R22W S24 SW	2213	Beaver Cr. basin
UBV	42	T9N R22W S24 SE	2286	Beaver Cr. basin
ALD	16	T9N R22W S24 NW	2006	Alder Lk.
LAP	12	T9N R21W S4 NE	2316	Lappi Lk.
LSJ	60	T10N R21W S25 SE	1914	Little St. Joe
ASC	70	T20N R21W S30 NW	2164	Ascapus Lk.
SWE	61	T10N R21W S20 NW	2337	Sweeney Lk.
DUF	45	T10N R21W S9 NW	2231	pond by Duffy Lk.
NDF	35	T10N R21W S4 SW	2469	N of Duffy Lk.
MOH	27	T11N R21W S33 NE	2408	Reed Lk.
WOO	22	T11N R21W S22 SW	2271	NE of Carlton Pk.
BF4	51	T38N R17E S20/21	2079	Brushy Fk. basin
BF3	60	T38N R17E S21	2204	Brushy Fk. basin
BF2	60	T38N R17E S16/21	2192	Brushy Fk. basin
BF1	57	T38N R17E S16	2154	Brushy Fk. basin
TER	47	T10N R22W S1 SW	2278	unnamed pond
SRY	61	T10N R22W S2 NE	2106	unnamed pond
MFP	32	T11N R22W S15/22	1753	Mary's Frog Pond
FFZ	37	T11N R21W S1 SW	1036	Fort Fizzle slough
JUM	44	R13N R19W S12 SE	1201	Mt. Jumbo pond
DON	62	T12N R17W S10 NE	1829	unnamed pond
JM1	42	T21N R18W S9 NE	1904	Jim Cr. Basin
LEW	19	Lewiston, ID	226	Linsay Cr.
ETW	49	T14N R17W S26 NE	1280	near E. Twin Cr.
TUR	31	T12N R17W S1	1021	Turah rest slough