MEASURING GENE FLOW AMONG POPULATIONS HAVING HIGH LEVELS OF GENETIC FRAGMENTATION

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ABSTRACT

We present an analysis of the genetic structures of 22 species of salamanders, with regard to levels of gene flow among populations. We estimate the gene flow parameter, *Nm* (the product of the effective population number and rate of migration among populations) using two alternative methods described by WRIGHT and SLATKIN. For most species, these two methods give approximately congruent estimates of *Nm*; when estimates differ, the method of WRIGHT produces values slightly larger than those derived by the method of SLATKIN. We analyze these results in light of independently derived historical inferences of the fragmentation of populations. This analysis suggests that the *Nm* values calculated from protein polymorphisms may contain information more relevant to historical patterns of gene exchange than to the current population dynamics; moderately large values of *Nm* may be calculated for species containing populations known to be no longer exchanging genes. Application of a method for estimating the maximum possible rate of gene exchange among populations indicates that, for most species studied here, gene flow among populations probably is no greater than the mutation rate. We suggest that most plethodontid species cannot be viewed as units whose cohesion is maintained by continuing gene exchange. Furthermore, we suggest that phenotypic uniformity among populations is not easily explained by hypotheses of continual stabilizing selection and propose that future work concentrate upon clarification of the genetic and epigenetic factors conferring self-maintenance or autopoietic properties on living systems.

THE importance of gene flow as a factor maintaining phenotypic cohesion among natural populations has been a source of much controversy. EHRLICH and RAVEN (1969) argue that in most cases species cannot be viewed as evolutionary units held together by the cohesive force of gene flow; selection is proposed as both the primary cohesive and disruptive force in phenotypic evolution. This argument is challenged by JACKSON and POUNDS (1979) who observe an inverse relationship between differentiation among conspecific populations and the opportunity for gene flow (see also POUNDS and JACKSON 1981). They question also the applicability of selection models and experiments that are used to support the interpretation that natural selection, rather than gene flow, serves as the primary cohesive and disruptive force in evolution. Also, some workers who agree with EHRLICH and RAVEN (1969) that gene flow

cannot be viewed as the primary cohesive force among natural populations dispute the claim that natural selection serves as the primary cohesive force. For example, WAKE, ROTH and WAKE (1983) observe that a standard organismal phenotype may be observed in different populations that have been disjunct for long periods of time and have experienced considerable environmental change; they suggest that internal organizational properties of organisms that are self-maintained (autopoietic) may underlie phenotypic stasis and apparent cohesion of populations (for a detailed development of the concept of autopoiesis, see MATURANA and VARELA 1980).

The lack of a generally applicable direct method for measuring gene flow hinders the evaluation of gene flow as a potential cohesive force among conspecific populations (see SLATKIN 1981). However, indirect methods based upon the distribution of allelic frequencies may permit the evaluation of gene flow parameters (see, for example, WRIGHT 1943; NEI 1975; SLATKIN 1981). The usage of indirect methods entails several problems, including the lack of independent results against which their predictions can be evaluated and the difficulty of distinguishing the effects of historical fragmentation events and current population dynamics. The purpose of this paper is twofold: (1) to estimate the importance of gene flow using indirect methods in a morphologically conservative group, the plethodontid salamanders, and (2) to evaluate the performance of several indirect methods relative to each other and relative to independently inferred historical patterns of fragmentation.

We use three indirect methods that can be applied readily to protein polymorphisms revealed by electrophoretic analysis. Two of these methods can be used to evaluate the gene flow parameter Nm, the product of the effective size of individual populations (N) and the rate of migration among them (m). The first method is a graphical one devised by SLATKIN (1980, 1981), which examines the relationship between the conditional average frequency of an allele, $\hat{p}_i$ (the average frequency of an allele among the populations in which it is found) and its incidence, i/d [the number of populations in which an allele is observed (i) divided by the total number of populations examined (d)]. Computer simulations suggest that this relationship may depend largely on the value of Nm, and SLATKIN (1981) presents island model simulations for various values of Nm. Graphs derived from allelic frequency data are compared with the island model simulations to derive an approximation of Nm for natural populations.

The second method utilizes the standardized genetic variance among populations (Fst; WRIGHT 1965). WRIGHT (1943) suggests that the formula $Fst = 1/(4Nm + 1)$ provides a satisfactory approximation if m is small; however, NEI, CHAKRAVARTI and TATENO (1977) warn that this formula is subject to error if the number of subdivided populations examined is small.

The third method used gives an estimate of the maximum possible migration rate among populations consistent with the observed polymorphisms. NEI (1975, p. 194) presents the formula $I = \exp(-D) = m/(m + v)$, where I is the genetic identity between populations, D is the genetic distance between populations, and v is the mutation rate.

SLATKIN (1981) concluded from applications of his method that levels of gene flow differ greatly among species. He suggests that some species may behave as single, large panmictic units as conceived by MAYR (1963) and STANLEY (1979), whereas others conform to the model of EHRLICH and RAVEN (1969). SLATKIN (1981) hypothesizes high levels of gene flow among populations of Drosophila, a fish (*Chanos chanos*), a mussel (*Mytilus edulis*) and a composite plant (*Stephanomeria exigua*). Intermediate levels are reported for several vertebrates, including two genera of rodents (Peromyscus and Thomomys), a frog (*Hyla regilla*) a lizard (*Lacerta melisellensis*) and two plethodontid salamanders (*Batrachoseps pacificus* and *Plethodon ouachitae*). Of the species sampled by SLATKIN (1981), all inferred to have low levels of gene flow are plethodontids (*Batrachoseps campi, Plethodon cinereus, P. dorsalis*). SLATKIN (1981) notes that it may be impossible with his method to discriminate low levels of continuing gene flow and complete isolation.

MATERIALS AND METHODS

We analyze patterns of genetic exchange among populations of 21 species of plethodontid salamanders and one dicamptodontid salamander, *Rhyacotriton olympicus*. Five of the plethodontid species are broken into subsets of the total geographical range for analysis: *B. pacificus, Bolitoglossa rufescens, Ensatina eschscholtzii, P. cinereus and P. dorsalis.* The distributions of protein polymorphisms within species have been gathered from previous studies (see Table 1). Also included in Table 1 is the following sampling information: (1) number of populations surveyed, (2) total number of proteins scored, (3) total number of electromorphs resolved and (4) average and minimum sample sizes per population. As an approximate indication of the geographical expanse of the units sampled, linear geographical distance between the most distant populations sampled is estimated. All species listed in Table 1 are analyzed by the method of SLATKIN (1981).

Graphical representations of the relationship between the conditional average frequency of an allele ($\bar{p}_i$) and the fraction of total populations in which it occurs (i/d) follow the method of SLATKIN (1981). The procedure for constructing the empirical curves is as follows: (1) the conditional average frequency ($\bar{p}_i$) is calculated for each protein variant scored, (2) protein variants are grouped according to the fraction of total populations studied in which they are observed (i/d), (3) the average value of $\bar{p}_i$ is calculated for protein variants having the same value of i/d and (4) these values are plotted with i/d on the abscissa and $\bar{p}_i$ on the ordinate. The empirical curves are compared to theoretical results obtained by simulations using an island model with mutation rate equal to 0.0001, and ten populations per species (SLATKIN 1981). SLATKIN (1981) presents theoretical curves corresponding to Nm values of 0.025, 0.125, 0.25 and 1.25; however, he does not suggest a quantitative way in which the theoretical and empirical data curves can be compared. We have compared the results of the salamander studies with SLATKIN'S theoretical curves for the island model in the following manner: (1) the abscissae of both the empirical and theoretical curves are marked into five intervals of equal width (corresponding to values of i/d = 0–0.19, 0.2–0.39, 0.4–0.59, 0.6–0.79 and 0.8–1.0), (2) the average value of $\bar{p}_i$ for all points falling within each interval is determined, (3) the empirical and theoretical results are compared by calculating the sum of squared differences between the average values of $\bar{p}_i$ for corresponding intervals and (4) the salamander populations are categorized according to which theoretical scheme gives the lowest sum of squared differences. In the empirical results, some intervals occasionally will not be represented by actual observations; such intervals are excluded from the calculations.

The standardized genetic variance among populations, *Fst*, has been reported by LARSON (1984) for 22 of the species analyzed using SLATKINS'S method; these values are calculated and corrected for sampling error according to WRIGHT (1978). The *Fst* values presented are averaged over all proteins for all populations examined. The *Fst* values for each protein represent the averaged standardized variances of all allelic variants. Estimates of Nm are derived from *Fst* using the formula

TABLE 1

Species used for analysis of gene flow among populations

Species	Adaptive zone[a]	No. of populations	Geographical distance (km)[b]	No. of proteins	No. of alleles	Sample Size		References[c]
						Average	Minimum	
Aneides flavipunctatus	t	16	450	23	90	24	7	6
Batrachoseps attenuatus	f	11	450	19	55	19	9	15
B. campi	f	9	32	33	60	10	7	16
B. pacificus (Sta. Lucia ssp.)	f	10	160	19	53	22	9	15
B. pacificus (Gabilan ssp.)	f	16	210	19	65	23	9	15
Bolitoglossa rufescens 1[d]	a	5	550	30	85	9	6	7
B. rufescens 2	a	8	800	30	113	9	6	7
B. subpalmata	t	5	110	18	59	13	4	3
Desmognathus fuscus	w	13	1050	18	46	15	5	11
Ensatina eschscholtzii 1	t	18	5100	26	125	10	5	14
E. eschscholtzii 2	t	14	170	26	92	11	7	14
Eurycea lucifuga	w	26	1400	12	18	13	7	10
Hydromantes shastae	t	5	33	19	31	8	5	12
Plethodon cinereus (eastern Maryland)	t	36	45	5	15	31	8	4
P. cinereus (glaciated)	t	7	1700	24	32	24	16	5
P. cinereus (unglaciated)	t	8	1000	24	53	21	9	5
P. dorsalis angusticlavius	t	5	325	26	48	25	9	8
P. dorsalis dorsalis	t	13	675	26	56	23	7	8
P. glutinosus (Ouachita Mtns.)	t	9	50	23	55	15	6	1
P. ouachitae	t	10	70	23	94	31	28	1
P. serratus	t	9	1050	24	51	22	12	5
P. websteri	t	8	725	26	50	28	19	8
Pseudoeurycea leprosa	t	6	240	18	43	17	5	9
Rhyacotriton olympicus	w	7	940	24	82	12	5	13
Thorius macdougalli	t	10	45	16	65	15	8	2
T. maxillabrochus	t	4	75	20	55	16	6	2
T. narisovalis	t	5	65	15	56	18	16	2
T. sp. F	t	6	400	16	49	16	9	2

[a] Adaptive zones are: (a) arboreal, (f) semifossorial, (t) terrestrial, (w) semiaquatic.

[b] Geographical distances are approximate airline distances between the two most distant populations sampled.

[c] References are: (1) DUNCAN and HIGHTON (1979), (2) HANKEN (1980), (3) HANKEN and WAKE (1982), (4) HIGHTON (1977), (5) HIGHTON and WEBSTER (1976), (6) LARSON (1980), (7) LARSON (1983), (8) LARSON and HIGHTON (1978), (9) LYNCH, WAKE and YANG (1983), (10) MERKLE and GUTTMAN (1977), (11) TILLEY and SCHWERDTFEGER (1981), (12) WAKE, MAXSON and WURST (1978), (13) D. B. WAKE and G. Z. WURST (unpublished data), (14) D. B. WAKE and K. P. YANEV (unpublished data), (15) YANEV (1978), (16) YANEV and WAKE (1981).

[d] *B. rufescens* 1 comprises samples 17–21 of LARSON (1983); *B. rufescens* 2 comprises these samples plus samples 23–25.

given previously. The same data sets are used for the SLATKIN analysis and for *Fst* calculations with two differences: (1) all proteins are used in the SLATKIN analysis, whereas only polymorphic proteins are used to calculate *Fst*. The criterion for polymorphism is that no allele have an average frequency across populations exceeding 0.95. (2) Because *Fst* is corrected for sampling bias, the criterion for minimum sample size ($n = 4$) chosen by LARSON (1984) is less strict than that used for many species in the SLATKIN analysis; therefore, *Fst* calculations are based upon a slightly larger number of populations for several species.

The method of NEI (1975, p. 194) is used to estimate the maximum possible value of m. Genetic identities (I; NEI 1975) are averaged among all pairwise comparisons per species. The maximum estimate of m, therefore, is the average of all possible pairwise exchanges among the populations sampled. Although NEI's formula is an approximation which assumes that migration rate greatly exceeds mutation rate, it still provides an estimate of the maximum possible migration rate when the two values are approximately equal (M. NEI, personal communication). The value used for the mutation rate is 2×10^{-6} mutations per locus per generation, which is used by NEI (1975) in a similar calculation for human populations. This is very similar to the value reported for protein variants in Drosophila by MUKAI and COCKERHAM (1977; $v = 1.8 \times 10^{-6}$ mutations/locus/generation, 95% confidence interval: 3.7×10^{-7} to 5.3×10^{-6}). The rate of mutation for protein variants in plethodontids has not been measured and conceivably could differ, but this value is used as an approximation. A minimum estimate of the average effective size of individual populations is derived by dividing Nm (as calculated from *Fst*) by m, as calculated here.

Historical relationships among several of these populations can be inferred from diverse observations. Several species are known, on the basis of current distribution, to comprise geographical isolates among which continuing genetic exchange is not possible. Because the isolates are separated by unfavorable habitat, recolonization of an isolate following an extinction event would not occur unless fundamental alteration of the intervening areas were achieved. Such species include *Aneides flavipunctatus*, which comprises at least three isolates (northwestern California, Santa Cruz mountains, Shasta populations; see LYNCH 1981; LARSON 1980), *B. campi*, composed of a series of isolated populations in an otherwise uninhabitable, desert region (see MARLOW, BRODE and WAKE 1979; YANEV and WAKE 1981) and *Plethodon serratus*, which comprises at least five widely separated isolates in eastern North America (see HIGHTON and WEBSTER 1976).

Populations of *P. cinereus* offer a unique opportunity for evaluating the effects of historical events on fragmentation patterns (see HIGHTON and WEBSTER 1976; HIGHTON 1977). This species has a continuous geographical distribution, the northern portions of which were glaciated during the Pleistocene epoch. These northern populations of *P. cinereus* must represent a relatively recent invasion, and the results of HIGHTON and WEBSTER (1976) suggest that they comprise a single invasion from unglaciated territory. The retreat of the last Pleistocene glaciation occurred approximately 5000 to 10,000 years ago (see BLACK 1974), and the generation time for *P. cinereus* is approximately 3 years (SAYLER 1966); therefore, it may be inferred that *P. cinereus* has invaded previously glaciated territory over a period of several thousand generations. Populations south of the glaciation, although apparently continuous in distribution, were subjected to fragmentation associated with climatic factors resulting from Pleistocene glaciations and earlier events; these populations have been studied on a broad scale by HIGHTON and WEBSTER (1976) and on a very fine scale by HIGHTON (1977). In the latter case, an apparent secondary contact and reestablishment of gene flow among previously separated populations is suggested (HIGHTON 1977). Additional North American species whose populations probably were fragmented by climatic changes of the Pleistocene and earlier epochs include *Batrachoseps attenuatus, B. pacificus, Desmognathus fuscus, E. eschscholtzii, Eurycea lucifuga, P. dorsalis, P. glutinosus, P. ouachitae* and *P. websteri*. Ecological studies have shown that plethodontids may be very susceptible to climatic fluctuations that expose them to dryness or heat (see, for example, HENDRICKSON 1954; ROSENTHAL 1957; MAIORANA 1974, 1977); therefore, local fluctuations of this nature may restrict gene exchange even on a very local scale. Also, the presence of closely related congeners may exclude salamanders from otherwise favorable habitats and promote geographical isolation of conspecific populations (see JAEGER 1971). Observations of home range fidelity and lack of long-distance dispersal (see, for example, HENDRICKSON 1954; STEBBINS 1954; GORDON 1961; VIAL 1968; MADISON 1969; MERCHANT 1972) support further the probability of very strong limits to long-range gene exchange among plethodontids.

The species chosen for analysis cover a variety of adaptive zones: semiaquatic, terrestrial, semifossorial and arboreal (see WAKE 1966; Table 1). This permits evaluation of the effect of different life-styles on the genetic fragmentation of populations.

RESULTS

Graphical representations of the relationship between conditional average frequency ($\bar{p}_i$) of an allele and its incidence among populations (i/d) are presented in Figures 1–3. Comparisons of these patterns to those expected under different levels of Nm (1.25, 0.25, 0.125, 0.025) reveal that none of the units analyzed closely approximates the highest level (Nm = 1.25). Six of the units approximate most closely the theoretical result for Nm = 0.25 (Figure 1), 16 approximate most closely the theoretical result for Nm = 0.125 (Figure 2) and six approximate most closely the theoretical result for Nm = 0.025 (Figure 3). There is no clear partitioning of levels of genetic exchange according to adaptive zone; terrestrial and semifossorial forms appear in all three categories, arboreal forms fall within the intermediate category and semiaquatic forms are found in the intermediate and low categories.

Values of *Fst* (WRIGHT 1965) are presented for 22 of the species used in the gene flow analysis (see Table 2). The average value of *Fst* among these species is 0.53 with a standard deviation of 0.19; on the average, more than half of the measured genetic variance within these species is partitioned among populations. This indicates strong isolation, particularly in light of theoretical results indicating that even a low level of migration generally is sufficient to maintain genetic homogeneity for selectively neutral alleles (see KIMURA and MARUYAMA 1971; SPIETH 1974). SLATKIN (1982) applied a test for selective

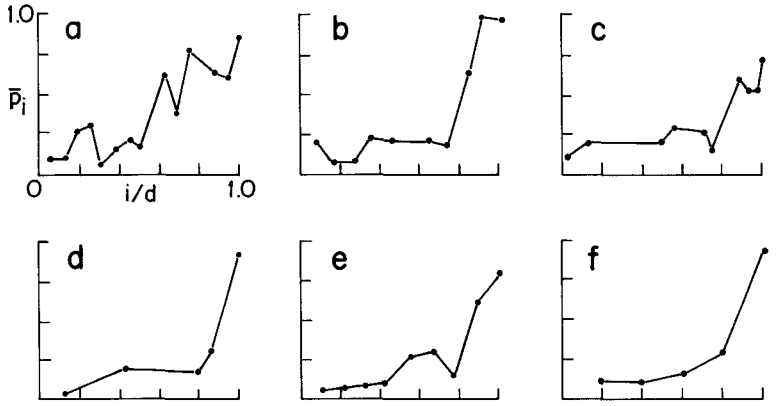

FIGURE 1.—The relationship between the conditional average frequency of a protein variant, $\bar{p}_i$ (the average frequency of a protein variant in the populations in which it is found), and its incidence [the number of populations in which the variant is observed (i) divided by the total number of populations examined (d)]. The points plotted represent the average value of $\bar{p}_i$ for all variants having the same value of i/d. These curves are approximately congruent with an island model simulation for Nm = 0.25 (SLATKIN 1981). (a) *A. flavipunctatus*, (b) *B. attenuatus*, (c) *P. cinereus* (eastern Maryland), (d) *P. cinereus* (glaciated), (e) *P. glutinosus* (Ouachita Mtns.), (f) *T. narisovalis*.

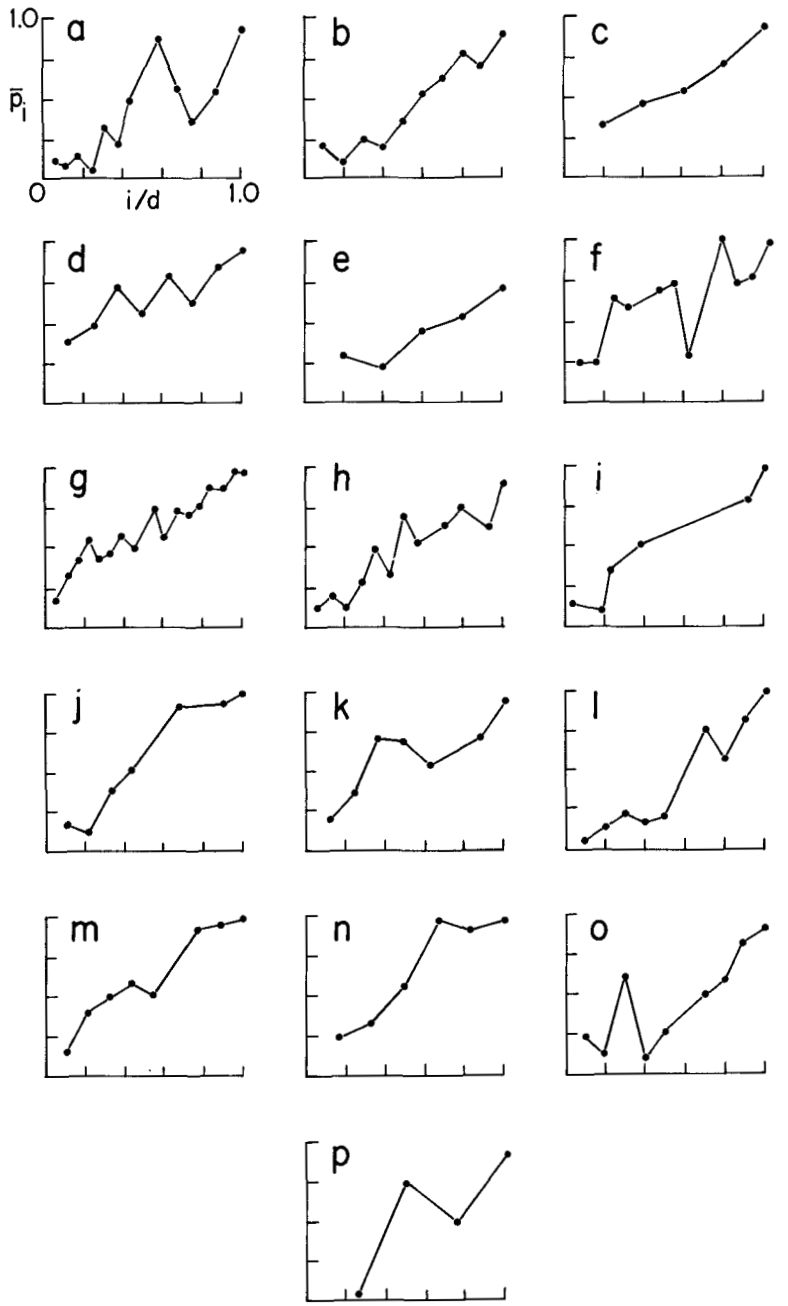

FIGURE 2.—The relationship between the conditional average frequency of a protein variant, $\bar{p}_i$ (the average frequency of a protein variant in the populations in which it is found), and its incidence [the number of populations in which the variant is observed (i) divided by the total number of populations examined (d)]. The points plotted represent the average value of $\bar{p}_i$ for all variants having the same value of i/d. These curves are approximately congruent with an island model simulation for $Nm = 0.125$ (SLATKIN 1981): (a) *B. pacificus* (Gabilan ssp.), (b) *B. pacificus* (Sta. Lucia ssp.), (c) *B. rufescens* (1), (d) *B. rufescens* (2), (e) *B. subpalmata*, (f) *D. fuscus*, (g) *E. eschscholtzii* (1), (h) *E. eschscholtzii* (2), (i) *E. lucifuga*, (j) *H. shastae*, (k) *P. cinereus* (unglaciated), (l) *P. ouachitae*, (m) *P. serratus*, (n) *P. leprosa*, (o) *T. macdougalli*, (p) *T. maxillabrochus*.

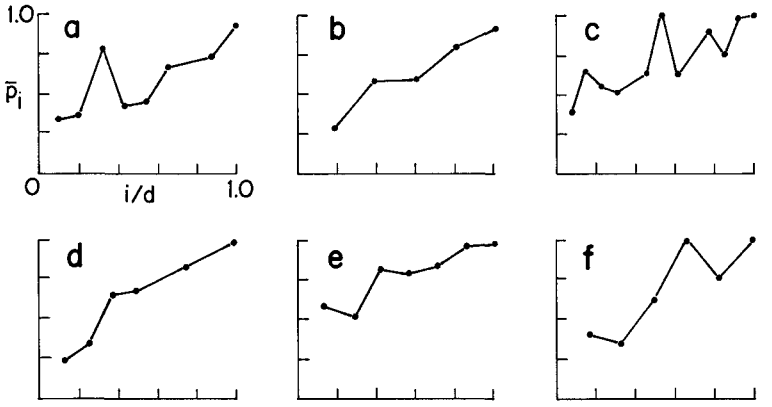

FIGURE 3.—The relationship between the conditional average frequency of a protein variant, $\bar{p}_i$ (the average frequency of a protein variant in the populations in which it is found), and its incidence [the number of populations in which the variant is observed (i) divided by the total number of populations examined (d)]. The points plotted represent the average value of $\bar{p}_i$ for all variants having the same value of i/d. These curves are approximately congruent with an island model simulation for $Nm = 0.025$ (SLATKIN 1981): (a) *B. campi*, (b) *P. dorsalis angusticlavius*, (c) *P. d. dorsalis*, (d) *P. websteri*, (e) *R. olympicus*, (f) Thorius sp. F.

neutrality to one of the data sets analyzed here (*P. dorsalis*); the results indicate that most patterns of protein variation are consistent with the predictions of selective neutrality. The results of NEI, FUERST and CHAKRABORTY (1976), FUERST, CHAKRABORTY and NEI (1977) and CHAKRABORTY, FUERST and NEI (1978, 1980) lend further support to the assumption of selective neutrality for electrophoretically resolved protein variants. Table 2 also summarizes cases of mutually exclusive allelic composition among conspecific populations for protein variants.

WRIGHT (1978, p. 288) presented *Fst* values for a variety of animals. Of the 31 values presented by WRIGHT (1978), only three exceed the average value for the plethodontid populations presented here; these are the lizards *Anolis brevirostris* (0.78) and *Anolis carolinensis* (0.61) and cave populations of the fish, *Astyanax mexicanus* (0.65). Values for eight Drosophila species vary from 0.01 (*D. pechea*) to 0.27 (*D. pseudoobscura*); the value for *D. pseudoobscura* within the United States alone is 0.03. The *Fst* value for human populations on a worldwide basis is approximately 0.15 (CAVALLI-SFORZA 1966).

There is not a simple relationship between *Fst* and average genetic identity ($\bar{I}$; NEI 1975) for the plethodontid species analyzed here. This results from the fact that *Fst* is calculated only from polymorphic proteins, whereas $\bar{I}$ is calculated from polymorphic and monomorphic proteins. Two species may differ greatly in the proportion of proteins that are polymorphic and show interpopulational differentiation and, yet, may be very similar in the portion of total genetic variance that is partitioned among populations. For example, *E. eschscholtzii* and *P. ouachitae* have similar values of *Fst* (0.71 and 0.67, respectively); however, *E. eschscholtzii* has an $\bar{I}$ value of 0.69, and 25 of the 26 proteins examined are polymorphic, whereas *P. ouachitae* has an $\bar{I}$ value of 0.91 and

TABLE 2

Estimates of five population genetic parameters for plethodontids

Species	No. of populations	No. of polymorphic proteins	F_{ST}	±SE	*Nm* (W)	$\bar{I}$	Maximum *m*	*N*	References[d]
I. *Nm* (S) = 0.25									
Aneides flavipunctatus	22	11 (7)[b]	0.44	0.07	0.32	0.91[c]	2×10^{-5}	16,000	8
Batrachoseps attenuatus	13	7 (5)	0.56	0.11	0.20	0.90	2×10^{-5}	11,000	5
Plethodon cinereus (glaciated)	7	4 (0)	0.13	0.03	1.67[a]	0.99	2×10^{-4}	8,000	1
P. cinereus (eastern Maryland)	36	5 (2)	0.33	0.05	0.51				2
P. glutinosus (Ouachita Mtns.)	9	9 (2)	0.19	0.06	1.07[a]	0.95	4×10^{-5}	28,000	6
Thorius narisovalis	5	6 (1)	0.15	0.08	1.42[a]	0.94	3×10^{-5}	43,000	7
II. *Nm* (S) = 0.125									
Batrachoseps pacificus (Gabilan ssp.)	19	12 (5)	0.47	0.07	0.28[a]	0.91	2×10^{-5}	14,000	5
B. pacificus (Sta. Lucia ssp.)	11	10 (4)	0.45	0.08	0.31[a]	0.88	1×10^{-5}	21,000	5
Bolitoglossa rufescens[e]	9	24 (22)	0.72	0.04	0.10	0.57	3×10^{-6}	38,000	12
B. subpalmata	5	13 (4)	0.37	0.07	0.43[a]	0.77	7×10^{-6}	64,000	11
Desmognathus fuscus	13	10 (8)	0.65	0.08	0.13	0.81	9×10^{-6}	15,000	9
Ensatina eschscholtzii 1	19	25 (17)	0.71		0.10	0.69	4×10^{-6}	23,000	14
Eurycea lucifuga	26	2 (1)	0.59	0.06	0.17				3
Plethodon cinereus (unglaciated)	8	9 (4)	0.65	0.06	0.13	0.83	1×10^{-5}	13,000	1
P. ouachitae	10	6 (6)	0.67	0.07	0.12	0.91	2×10^{-5}	6,000	6
Pseudoeurycea leprosa	6	10 (6)	0.66	0.07	0.13	0.83	1×10^{-5}	13,000	13
Thorius macdougalli	11	9 (4)	0.42	0.10	0.35[a]	0.85	1×10^{-5}	31,000	7
III. *Nm* (S) = 0.025									
Batrachoseps campi	12	11 (4)	0.59	0.11	0.17[a]	0.88	1×10^{-5}	14,000	10
Plethodon dorsalis dorsalis	13	8 (8)	0.80	0.06	0.06	0.87	8×10^{-6}	17,000	4
P. d. angusticlavius	5	7 (4)	0.67	0.09	0.12[a]	0.89	2×10^{-6}	8,000	4
P. websteri	8	4 (3)	0.74	0.07	0.09	0.92	2×10^{-5}	4,000	4
Thorius sp. F	6	12 (5)	0.59	0.08	0.17[a]	0.72	5×10^{-6}	34,000	7
Mean	12.5	9.5 (5.6)	0.53		0.37	0.85	2×10^{-5}	21,000	

F_{ST} = the fraction of total genetic variance partitioned among populations; *Nm* = the product of effective population size and migration rate estimated by the methods of Wright (W) and Slatkin (S); $\bar{I}$ = average genetic identity among populations, maximum *m* = maximum estimate of migration rate; *N* = estimate of effective population size. Species are organized into three groups according to levels of *Nm* as estimated by the method of SLATKIN (1981).

[a] Cases showing a discrepancy between *Nm* values estimated by the methods of WRIGHT (1943) and SLATKIN (1981).

[b] Numbers in parentheses denote the number of polymorphic proteins showing at least one case of mutually exclusive allelic composition (fixed difference) among the populations compared.

[c] For *A. flavipunctatus*, sample size is 13.

[d] References are: (1) HIGHTON and WEBSTER (1976), (2) HIGHTON (1977), (3) MERKLE and GUTTMAN (1977), (4) LARSON and HIGHTON (1978), (5) YANEV (1978), (6) DUNCAN and HIGHTON (1979), (7) HANKEN (1980), (8) LARSON (1980), (9) TILLEY and SCHWERDTFEGER (1981), (10) YANEV and WAKE (1981), (11) HANKEN and WAKE (1982), (12) LARSON (1983), (13) LYNCH, WAKE and YANG (1983), (14) D. B. WAKE and K. P. YANEV (unpublished data).

[e] Based upon samples 17–25 of LARSON (1983).

only six of the 23 proteins examined are polymorphic. There are several possible explanations for this observation. The populations of the two species may be comparable in degree of genetic isolation, but the ancestral populations may have differed greatly in levels of polymorphism. Alternatively, the populations of the two species may have comparable levels of genetic isolation, but one may have maintained such isolation over longer periods of time, permitting a greater number of proteins to diverge.

Values of *Nm* estimated according to the methods of SLATKIN (1981) and WRIGHT (1943) are compared in Table 2. The species are organized in Table 2 in three categories according to approximate values of *Nm* indicated by the SLATKIN (1981) analysis. Of the 22 sets of populations analyzed, 12 show approximate congruence between the two methods. In ten additional species the method of WRIGHT (1943) gives estimates of *Nm* that are consistently larger than those calculated by the SLATKIN (1981) method. In all three categories, there are species whose *Nm* values, as estimated from *Fst*, would place them in the next higher category. For example, estimates of *Nm* from *Fst* indicate that the *P. cinereus* populations from formerly glaciated territory, the *P. glutinosus* populations from the Ouachita mountains and *Thorius narisovalis* populations should approximate more closely the SLATKIN (1981) curve representing $Nm = 1.25$, rather than the one representing $Nm = 0.25$ as observed. Similarly, four species in the category $Nm = 0.125$ based on the SLATKIN (1981) analysis would fall into the category $Nm = 0.25$ based on the *Fst* analysis. Three species in the category $Nm = 0.025$ based upon the SLATKIN (1981) analysis would be in the category $Nm = 0.125$ based on the *Fst* analysis.

Maximum estimates of m, derived by the method of NEI (1975), indicate that gene flow among plethodontid populations is very restricted and approximately the same order of magnitude as mutation rate (see Table 2). The highest value of m reported is that for the *P. cinereus* populations on formerly glaciated territory, and even this value is not large ($m = 0.0002$). This value is nearly equivalent to values estimated in a similar manner for migration rates between major human racial groups (see NEI 1975, p. 194). By dividing estimates of *Nm* (in this case, those estimated from *Fst*) by maximum estimates of m, estimates of N can be derived. The results in Table 2 indicate that values of N for plethodontids are in the thousands (from approximately 8000 to 64,000). These results are consistent with census data indicating that plethodontids are present in locally high densities where they occur (see, for example, HENDRICKSON 1954; STEBBINS 1954; VIAL 1968; MERCHANT 1972; BURTON and LIKENS 1975; and review by LARSON 1984). Although there may be large discrepancies between the total number of individuals and the effective number, estimates based upon protein polymorphisms at least are not unrealistic relative to the census data.

DISCUSSION

SLATKIN (1981) notes that the use of protein polymorphisms to infer patterns of gene exchange assumes that patterns of migration among populations are stable over time. For low migration species, a radiation model, in which a

single deme at some time in the past radiated or was fractionated into several demes that do not now exchange migrants, may be more appropriate. The differences between the radiation model and those used for SLATKIN'S analysis may be critical for evaluating gene flow as a determinant of cohesion among conspecific populations. Analysis of gene flow among *P. cinereus* populations suggests that protein variants may contain information more relevant to historical patterns of gene exchange than to current patterns. Populations inhabiting territory that was covered with ice during the last Pleistocene glaciation show little genetic differentiation, although the geographical area covered by these samples is very large (about 1700 km in diameter). Geographically widespread populations of *P. cinereus* from formerly glaciated areas (HIGHTON and WEBSTER 1976) and populations from a small portion of the unglaciated region (no larger than 45 km in diameter; HIGHTON 1977) both approximate the $Nm = 0.25$ level according to the SLATKIN (1981) method; calculated using WRIGHT'S (1943) formula, Nm is considerably larger for the geographically widespread populations on formerly glaciated territory ($Nm = 1.67$) than for those on the small section of unglaciated territory ($Nm = 0.51$). The smaller geographical unit partitions a greater portion of its total variation among populations than does the larger one (see Table 2). If gene flow among populations were a continuing phenomenon, it is unlikely that the smaller unit would be the more extensively differentiated one. The historical hypothesis, that populations from the glaciated region recently radiated from a single ancestral population and that the populations from nonglaciated territory were separated for longer periods of time, is more consistent with these observations than is a hypothesis based entirely on existing levels of gene flow.

HIGHTON and WEBSTER (1976) also sampled *P. cinereus* populations throughout the unglaciated portion of the range; these populations demonstrate lower apparent levels of gene flow than the conspecific populations on formerly glaciated territory according to both the WRIGHT (1943) and SLATKIN (1981) methods ($Nm = 0.13$), although their geographical expanse is smaller. It is likely that these populations were effectively isolated from each other by events of the Pleistocene and earlier epochs, and that gene flow among them has not been restored completely. This hypothesis is strengthened by analysis of protein variants in the closely related species, *P. serratus*. This species also inhabits unglaciated territory in eastern North America and is known to contain widely separated geographical isolates (on the basis of distributional data). Many pairs of *P. serratus* populations are now completely isolated from genetic exchange; however, the value of Nm estimated according to SLATKIN (1981) is the same as that determined for the *P. cinereus* populations from unglaciated territory. Although *P. cinereus* does not demonstrate large distributional gaps comparable to those of *P. serratus*, it may contain populations that are effectively isolated from gene exchange by distance or less obvious barriers to dispersal. The *P. cinereus* populations from the small portion of the unglaciated territory discussed before (see HIGHTON 1977) also are relevant to this argument; these populations are apparently continuous and show evidence of a secondary contact between previously separated populations, but gene flow has not been

effective enough to eradicate fixed differences occurring between populations no more than 45 km apart. Other widely ranging plethodontid species that inhabit unglaciated territory also probably contain populations effectively isolated from genetic exchange. These include: *B. attenuatus*, *B. pacificus*, *Desmognathus fuscus*, *E. eschscholtzii*, *E. lucifuga*, *P. dorsalis* and *P. websteri*.

Direct observation of patterns of allelic distribution may give important clues concerning the degree of isolation of these populations. If there is evidence that two populations are completely different in allelic composition for several proteins, gene flow between the two populations probably is nonexistent and probably has not occurred for a considerable period of time (at least the amount of time required for alternative allelic fixations). Table 2 presents counts of proteins for which patterns of mutually exclusive allelic composition are observed among populations. On average, the studies reviewed in Table 2 reveal about five proteins per species, or subset thereof, that demonstrate at least one instance of mutually exclusive allelic composition among populations. With the possible exception of populations of *P. cinereus* from formerly glaciated territory, it is unlikely that any of these species comprises a series of populations currently connected by gene exchange. Of the 22 sets of populations examined (Table 2), only one (populations of *P. cinereus* from formerly glaciated territory) shows no fixed differences among populations. As noted earlier, historical considerations suggest that this set of populations has recent common ancestry, whether or not gene exchange among populations continues. The opposite extreme is represented by *E. eschscholtzii*, for which 17 proteins demonstrate patterns of mutually exclusive allelic composition, and *B. rufescens*, for which 22 proteins demonstrate patterns of mutually exclusive allelic composition. In *B. rufescens*, it is possible that several reproductively isolated units are involved (LARSON 1983); LARSON (1983) suggests that phenotypic characters usually used to recognize species within the *B. rufescens* group are not valid indicators of genetic lineages.

In addition to *P. serratus*, two additional species are known from distributional data to contain populations completely isolated from genetic exchange. These species are *A. flavipunctatus* and *B. campi*. The method of SLATKIN (1981) estimates a much lower value of *Nm* for *B. campi* (0.025) than does the formula of WRIGHT (1943; $Nm = 0.17$); however, both methods are approximately concordant in estimating a sizeable value of *Nm* for *A. flavipunctatus* ($Nm = 0.25$–0.32). The use of indirect methods to estimate a single value of *Nm* for a species assumes that migration rates among all populations sampled are not subject to a large variance. This assumption probably is not realized with real examples, and will be violated particularly when sampling includes clusters of populations widely separated from other clusters. Therefore, even species whose measured value of *Nm* is moderately large may contain some pairs of populations that are completely isolated from genetic exchange.

The estimates of the maximum possible rate of migration among populations (NEI 1975) are informative. For many instances, the maximum possible migration rate is less than the estimated rate of mutation (see Table 2), and in very

few cases is it more than an order of magnitude larger than the inferred mutation rate. These results indicate that many, if not most, of the species analyzed here contain populations that are completely isolated from genetic exchange.

The methods of SLATKIN (1981) and WRIGHT (1943) for estimating *Nm* are in fairly close agreement for a majority of the species compared. These methods, therefore, may estimate the same parameter, even if this parameter contains largely information relevant to historical rather than continuing patterns of gene flow among populations. When these methods disagree, the method of WRIGHT (1943) gives estimates of *Nm* that are consistently larger than those of SLATKIN's method (SLATKIN 1981). This observation currently is without an adequate explanation. Application of NEI's (1975) method suggests that these *Nm* values may represent a balance between very large population sizes and very low effective migration among populations, to the extent that these indirect estimates of *Nm* reflect current population dynamics.

We interpret our results for plethodontids as being consistent with one conclusion of EHRLICH and RAVEN (1969): species of plethodontids generally do not comprise units connected by gene flow. We believe that the problem of species level cohesion addressed by EHRLICH and RAVEN (1969) is an important one; the maintenance of a standard organismal morphology among populations isolated from genetic exchange requires an explanation. As an alternative to the gene flow hypothesis, EHRLICH and RAVEN (1969) propose that natural selection is the primary cohesive and disruptive force in evolution; however, this hypothesis also may be inadequate to account for the stability of organismal phenotype (see JACKSON and POUNDS 1979; WAKE, ROTH and WAKE 1983). WAKE, ROTH and WAKE (1983) argue from a historical perspective that plethodontid morphologies that are uniform in at least a qualitative sense over a geographical expanse also are probably uniform over many millions of years. The observation that plethodontid organismal morphologies may be more stable in time than the ecosystems in which they occur questions the interpretation that continual stabilizing selection offers a reasonable explanation of phenotypic uniformity (WAKE, ROTH and WAKE 1983). We suggest instead that research focus upon the genetic and epigenetic factors conferring stability to biological systems, allowing them to compensate fragmentation of the gene pool and altered environments without fundamental changes in morphology.

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LITERATURE CITED

BLACK, R. F., 1974 Geology of the Ice Age National Scientific Reserve of Wisconsin. National Park Service Scientific Monograph Series **2:** 1–234.

BURTON, T. M. and G. E. LIKENS, 1975 Salamander populations and biomass at the Hubbard Brook Experimental Forest, New Hampshire. Copeia **1975:** 541–546.

CAVALLI-SFORZA, L. L., 1966 Population structure and human evolution. Proc. R. Soc. Lond. (Biol) **164:** 362–379.

CHAKRABORTY, R., P. A. FUERST and M. NEI, 1978 Statistical studies on protein polymorphism in natural populations. II. Gene differentiation between populations. Genetics **88:** 367–390.

CHAKRABORTY, R., P. A. FUERST and M. NEI, 1980 Statistical studies on protein polymorphism in natural populations. III. Distribution of allele frequencies and the number of alleles per locus. Genetics **94:** 1039–1063.

DUNCAN, R. and R. HIGHTON, 1979 Genetic relationships of the eastern large *Plethodon* of the Ouachita mountains. Copeia **1979:** 95–110.

EHRLICH, P. R. and P. H. RAVEN, 1969 Differentiation of populations. Science **165:** 1228–1232.

FUERST, P. A., R. CHAKRABORTY and M. NEI, 1977 Statistical studies on protein polymorphism in natural populations. I. Distribution of single locus heterozygosity. Genetics **86:** 455–485.

GORDON, R. E., 1961 The movement of displaced green salamanders. Ecology **42:** 200–202.

HANKEN, J., 1980 Genetic and morphological investigations of miniaturization in salamanders. Ph.D. Dissertation, University of California, Berkeley.

HANKEN, J. and D. B. WAKE, 1981 Genetic differentiation among plethodontid salamanders (genus *Bolitoglossa*) in Central and South America: implications for the South American invasion. Herpetologica **38:** 272–287.

HENDRICKSON, J. R., 1954 Ecology and systematics of the salamanders of the genus *Batrachoseps.* Univ. California Publ. Zool. **54:** 1–46.

HIGHTON, R., 1977 Comparisons of microgeographic variation in morphological and electrophoretic traits. Evol. Biol. **10:** 397–436.

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

JACKSON, J. F. and J. A. POUNDS, 1979 Comments on assessing the dedifferentiating effects of gene flow. Syst. Zool. **28:** 78–85.

JAEGER, R. G., 1971 Competitive exclusion as a factor influencing the distribution of two species of terrestrial salamanders. Ecology **52:** 632–637.

KIMURA, M. and T. MARUYAMA, 1971 Pattern of neutral polymorphism in a geographically structured population. Genet. Res. **18:** 125–131.

LARSON, A., 1980 Paedomorphosis in relation to rates of morphological and molecular evolution in the salamander *Aneides flavipunctatus* (Amphibia, Plethodontidae). Evolution **34:** 1–17.

LARSON, A., 1983 A molecular phylogenetic perspective on the origins of a lowland tropical salamander fauna. I. Phylogenetic inferences from protein comparisons. Herpetologica **39:** 85–99.

LARSON, A., 1984 Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. Evol. Biol. In press.

LARSON, A. and R. HIGHTON, 1978 Geographic protein variation and divergence in the salamanders of the *Plethodon welleri* group. Syst. Zool. **27:** 431–448.

LYNCH, J. F., 1981 Patterns of ontogenetic and geographic variation in the black salamander, *Aneides flavipunctatus* (Caudata: Plethodontidae). Smithsonian Contr. Zool. **324:** 1–53.

LYNCH, J. F., D. B. WAKE and S. Y. YANG, 1983 Genic and morphological differentiation in populations of the salamander genus *Pseudoeurycea* inhabiting the transverse volcanic range of Mexico, with a description of a new species. Copeia. In press.

MADISON, D. M., 1969 Homing behaviour of the red-cheeked salamander *Plethodon jordani.* Anim. Behav. **17:** 25–39.

MAIORANA, V. C., 1974 Studies on the behavioral ecology of the plethodontid salamander *Batrachoseps attenuatus*. Ph.D. Dissertation, University of California, Berkeley.

MAIORANA, V. C., 1977 Observations of salamanders (Amphibia, Urodela, Plethodontidae) dying in the field. J. Herpetol. **11:** 1–5.

MARLOW, R. W., J. M. BRODE and D. B. WAKE, 1979 A new salamander, genus *Batrachoseps*, from the Inyo mountains of California with a discussion of relationships in the genus. Contr. Sci. Los Angeles Co. Mus. Nat. Hist. **308:** 1–17.

MATURANA, H. R. and F. J. VARELA, 1980 *Autopoiesis and Cognition—The Realization of the Living*. D. Reidel Publishing Company, Dordrecht.

MAYR, E., 1963 Animal Species and Evolution. Harvard University Press, Cambridge.

MERCHANT, H., 1972 Estimated population size and home range of the salamanders *Plethodon jordani* and *Plethodon glutinosus*. J. Wash. Acad. Sci. **62:** 248–257.

MERKLE, D. A. and S. I. GUTTMAN, 1977 Geographic variation in the cave salamander *Eurycea lucifuga*. Herpetologica **33:** 313–321.

MUKAI, T. and C. COCKERHAM, 1977 Spontaneous mutation rates at enzyme loci in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **74:** 2514–2517.

NEI, M., 1975 *Molecular Population Genetics and Evolution*. North-Holland Publishing Company, Amsterdam.

NEI, M., A. CHAKRAVARTI and Y. TATENO, 1977 Mean and variance of Fst in a finite number of incompletely isolated populations. Theor. Pop. Biol. **11:** 291–306.

NEI, M., P. A. FUERST and R. CHAKRABORTY, 1976 Testing the neutral mutation hypothesis by distribution of single locus heterozygosity. Nature **262:** 491–493.

POUNDS, J. A. and J. F. JACKSON, 1981 Riverine barriers to gene flow and the differentiation of fence lizard populations. Evolution **35:** 516–528.

ROSENTHAL, G. M., 1957 The role of moisture and temperature in the local distribution of the plethodontid salamander, *Aneides lugubris*. Univ. California Publ. Zool. **54:** 371–420.

SAYLER, A., 1966 The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. Copeia **1966:** 183–193.

SLATKIN, M., 1980 The distribution of mutant alleles in a subdivided population. Genetics **95:** 503–523.

SLATKIN, M., 1981 Estimating levels of gene flow in natural populations. Genetics **99:** 323–335.

SLATKIN, M., 1982 Testing neutrality in subdivided populations. Genetics **100:** 533–545.

SPIETH, P. T., 1974 Gene flow and genetic differentiation. Genetics **78:** 961–965.

STANLEY, S. M., 1979 *Macroevolution, Pattern and Process*. W. H. Freeman and Company, San Francisco.

STEBBINS, R. C., 1954 Natural history of the plethodontid salamanders of the genus *Ensatina*. Univ. California Publ. Zool. **54:** 47–124.

TILLEY, S. G. and P. M. SCHWERDTFEGER, 1981 Electrophoretic variation in appalachian populations of the *Desmognathus fuscus* complex (Amphibia: Plethodontidae). Copeia **1981:** 109–119.

VIAL, J. L., 1968 The ecology of the tropical salamander, *Bolitoglossa subpalmata*, in Costa Rica. Rev. Biol. Trop. **15:** 13–115.

WAKE, D. B., 1966 Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem. So. California Acad. Sci. **4:** 1–111.

WAKE, D. B., L. R. MAXSON and G. Z. WURST, 1978 Genetic differentiation, albumin evolution, and their biogeographic implications in the plethodontid salamanders (genus *Hydromantes*) of California and southern Europe. Evolution **32:** 529–539.

WAKE, D. B., G. ROTH and M. H. WAKE, 1983 On the problem of stasis in organismal evolution. J. Theor. Biol. **101:** 211–224.

WRIGHT, S., 1943 Isolation by distance. Genetics **28:** 114–138.

WRIGHT, S., 1965 The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution **19:** 395–420.

WRIGHT, S., 1978 *Evolution and the Genetics of Populations*, Vol. 4, Variability within and among Natural Populations. University of Chicago Press, Chicago.

YANEV, K. P., 1978 Evolutionary studies of the plethodontid salamander genus *Batrachoseps*. Ph. D. Dissertation, University of California, Berkeley.

YANEV, K. P. and D. B. WAKE, 1981 Genetic variation in a relict desert salamander, *Batrachoseps campi*. Herpetologica **37:** 16–28.

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