Copia, 1989(4), pp. 1016-l02S

The Relationship Between Allozyme Variation and Life History: Non-transforming Salamanders are Less Variable

H. BRADLEY SHAFFER AND FELIX BREDEN

Several recent papers have reported low levels of genetic variation in non-transforming, paeclomorphic urodeles. Here, we report the results of an electrophoretic survey of 21 allozyme loci in two populations of the Ozark Hellbender, *Cryptobranchus alleganiensis,* confirming the extraordinarily low levels of genetic variation previously reported in a different laboratory. We then summarize the literature on 102 species of salamanders to compare genetic variation in transforming and non-transforming taxa. On average, non-transforming salamanders are significantly less variable than transforming ones, supporting the idea that low levels of allozyme variation in *Cryptobranchus* are associated with larval reproduction. We interpret this association between life history mode and genetic variation as reflecting a fundamental difference in levels of population extinction and recolonization between aquatic and terrestrial species.

IN 1977, Merkle et al. reported the results of an extensive survey of electrophoretic variation in the non-transforming salamander *Cryptobranchus alleganiensis.* Their survey demonstrated an extraordinarily low level of genetic variation: among the products of 24 loci examined from a total of 137 specimens representing 12 geographic samples from the four major river systems inhabited by hellbenders, only two loci showed any genetic variation, and nine of the 12 populations were completely monoallelic. Merkle et al. (1977) also pointed out that several other non-transforming salamanders (the mudpuppy, *Necturus maculosus;* the dwarf siren, *Pseudobranchus striatus;* and the two-toed amphiuma, *Amphiuma means)* all appear to have low levels of genetic variation and large amounts of nuclear DNA, and suggested a possible causal relationship. A prediction from the work of Merkle et al. (1977) is that there should be a reasonably strong correlation between life history variation (that is, metamorphosis vs larval reproduction) and genetic variation. However, the relationship between life history pattern and genetic variation has never been evaluated systematically.

In this paper, we first report the results of a reexamination of genetic variation in two additional populations of the hellbender, *C. alleganiensis,* confirming the results of Merkle et al. (1977) in a different laboratory using a somewhat different set of loci. To interpret these results, we present a literature survey of electrophoretic variation in over 100 species of salamanders from six families and 19 genera. We conclude that there is a strong relationship between larval reproduction and genetic variation: larval reproducers are less variable, on average, than metamorphosing salamander species. However, we interpret this relationship as a difference in the population structures of transforming and non-transforming salamanders, rather than representing a causal relationship between structural genetic variation and components of fitness as has been previously suggested (Pierce and Mitton, 1980; Mitton and Grant, 1984).

MATERIALS AND METHODS

Hellbenders were collected from two streams in the Missouri Ozarks: 50 specimens were obtained from the Gasconade River and 21 from the Big Piney (both are part of the Missouri River system). The Gasconade sample is about twice as large as any studied by Merkle et al. (1977), and provided a very good estimate of the genetic variation within that population.

Twenty-one electrophoretically detectable genetic loci encoding 14 enzymes and non-enzymatic proteins were scored in all individuals. Enzyme nomenclature and Enzyme Commission (E.C.) number follow the recommendations of the International Union of Biochemistry (IUBNC, 1984); abbreviations generally follow Harris and Hopkinson (1976). Loci whose homologies with other vertebrates are known are identified by letter. The "m" or "s" prefixes

refer to mitochondrial or supernatant/cytosolic subcellular locations, if relevant. Loci whose homologies with those of other vertebrates are not known were assigned sequential numbers within a multilocus protein system, with the locus with the most anodally migrating products labelled **"1."** We resolved up to three alleles per locus; for discussion, they are designated according to relative anodal electrophoretic mobility of their products as "fast" (the most anodally migrating allelic product), "medium" (used only for triallelic systems), or "slow" (the most cathodally migrating form). We scored the following loci for all individuals: E.C. 2.7.5.1 phosphoglucomutase (Pgm-1,2,3); E.C. 4.2.1.11 enolase (Eno-1,2); E.C. 2.6.1.1 aspartate aminotransferase (sAat-A); E.C. 1.1.1.27 lactate dehydrogenase (Ldh-A,B); E.C. 1.1.1.118 glucose dehydrogenase (Gcdh-1,2); E.C. 1.1.1.8 glycerol-3-phosphate dehydrogenase (G3pdh-1); E.C. 1.1.1.42 isocitrate dehydrogenase (sldh-A); E.C 1.2.1.12 glyceraldehyde-phosphate dehydrogenase (Gapdh-1); E.C. 1.1.1.1 alcohol dehydrogenase (ethanol substrate, Adh-1,2); E.C. 1.1.1.37 malate dehydrogenase (mMdh-A, sMdh-A); E.C. 3.4.11.-peptidase (L-leucyl-gly-gly substrate, Pep-I); and two proteins identified with the Amido Black general protein stain (Pt-1,2).

Because these specimens are part of a long-term mark and recapture study (Peterson et al., 1983), animals were not sacrified. Instead, they were anesthetized and a small (approx. 1 cm^5) piece of muscle was removed from the tail. Each individual was measured, sexed, marked with a permanent heat brand, and released. Tissue samples were frozen in liquid nitrogen until they were returned to the lab, at which time they were stored at -80 C until electrophoresis. Electrophoretic methods followed Harris and Hopkinson (1976) and Shaffer (1983, 1984), with many of the same buffer conditions as those employed by Merkle et al. (1977) for loci common to both studies.

Average heterozygosity per individual (H) and percent polyallelic loci **(P)** were calculated within geographic samples, and then averaged over all samples for a species (Soule, 1976). Whenever possible, direct counts for **H** were used. In cases from the literature where these were not provided, we calculated the **H** expected under Hardy-Weinberg equilibrium by the formula

$$H = 1/N \sum_j (1 - \sum_i Pl)$$

where pij is the frequency of the ith allele at locus j, and **H** is averaged over all N loci.

RESULTS AND DISCUSSION

Allozyme variation in Cryptobranchus.-Eighteen of 21 loci were monoallelic in all individuals sampled. sMdh-A showed a single heterozygote involving a slower migrating allelic product in the Gasconade population, but was fixed for the common "fast" allele in the Big Piney sample. Two loci showed moderate variation: mMdh-A had a fast allele (frequency in the Gasconade = 0.061; in the Big Piney= 0.025), and Pt-I was segregating for three alleles in the Gasconade ("slow," frequency = 0.135; "fast," frequency = 0.020; "medium," frequency= 0.84) and two alleles in the Big Piney ("slow," frequency = 0.16; "medium," frequency = 0.84). Thus, overall measures of H and P, averaged over loci were: Gasconade, H = 0.021, P = 0.143; Big Piney, H = 0.018, P = 0.119. Because of the similarity of these results with those of Merkle et al. (1977), who found the same polymorphisms at the mMdh-A and Pt-I loci, we feel confident that they are not artifacts of lab conditions or individuals scoring the gels. In addition, we have analyzed many other species of transforming and non-transforming salamanders and found much higher levels of genetic variation (Shaffer, 1983, 1984, unpubl.). Thus, we feel confident that *Cryptobranchus* maintains extraordinarily low levels of genetic variation.

Electrophoretic and life history variation in salamanders.-Since the initial survey of Merkle et al. (1977), an enormous number of species and geographic samples of salamanders have been studied electrophoretically, including a large proportion of the known non-transforming taxa. Our electrophoretic results confirm those of Merkle et al. (1977): *C. alleganiensis* has extremely little genetic variation at the level revealed by protein electrophoresis. The question remains as to whether this is a general pattern for non-transforming salamanders, or if *Cryptobranchus* is very homozygous and coincidentally fails to metamorphose.

To examine this issue, we conducted a literature survey of salamander electrophoretic studies, and recorded (or computed) Hand P for 102 species from 19 genera and six families ofurodeles. We have tried to be comprehensive and use all literature values as long as: 1) both polyallelic and monoallelic loci were recorded; 2) the animals were derived from field samples; and 3) in studies where more than one population was available, populations were recorded

TABLE 1. AVERAGE HETEROZYGOSITY (H), PROPORTION OF POLYALLELIC LOCI (P), NUMBER OF LOCI EXAMINED AND LITERATURE CITATION FOR ALL SPECIES USED IN THIS SURVEY. T /L refers to transforming (T) or larval reproducing (L) species or populations.

Species	T/L	H	P	Number of loci	Reference
Ambystoma macrodactylum	T	0.066	0.245	21	Howard and Wallace, 1981
A. andersoni	L	0.031	0.094	32	Shaffer, 1984
A. dumerilii	L	0.031	0.063	32	Shaffer, 1984
A. fiavipiperatum	T	0.044	0.094	32	Shaffer, 1984
A. granulosum	T	0.102	0.250	32	Shaffer, 1984
A. mexicanum	L	0.073	0.219	32	Shaffer, 1984
A. rosaceum	T	0.139	0.396	29	Shaffer, 1983
A. taylori	L	0.091	0.250	32	Shaffer, 1984
A. tigrinum	T	0.055.	0.086	32	Shaffer, 1984
A. tigrinum	L	0.180	0.312	32	Shaffer, 1984
Aneides fiavipunctatus	T	0.114	0.462	21	Nevo et al., 1984
A. hardii	T	0.039	0.131	21	Nevo et al., 1984
Batrachoseps campi	T		0.210	33	Nevo et al., 1984
B. wrighti	T		0.182	33	Nevo et al., 1984
Bolitoglossa adspersa	T	0.075	0.28	17	Hanken and Wake, 1982
B. borburata	T	0.111	0.11	17	Hanken and Wake, 1982
B. cuchumatana	T	0.090	0.200	30	Larson, 1983
B. dofleini	T	0.12	0.267	30	Larson, 1983
B. dunni	T	0.100	0.217	30	Larson, 1983
B. engelhardti	T	0.140	0.367	30	Larson, 1983
B. flavimembris	T	0.045	0.167	30	Larson, 1983
B. franklini	T	0.134	0.46	17	Wake and Lynch, 1982
B. helmrichi	T	0.117	0.267	30	Larson, 1983
B. hermosa	T	0.048	0.37	19	Papenfuss et al., 1983
B. lincolni	T	0.040	0.100	30	Larson, 1983
B. lincolni	T	0.099	0.29	17	Wake and Lynch, 1982
B. macrinii	T	0.136	0.35	19	Papenfuss et al., 1983
B. marmorea	T	0.094	0.440	18	Hanken and Wake, 1982
B. meliana	T	0.138	0.35	17	Wake and Lynch, 1982
B. morio	T	0.100	0.258	30	Larson, 1983
B. nigrescens	T	0.222	0.22	18	Hanken and Wake, 1982
B. occidentalis	T	0.120	0.317	30	Larson, 1983
B. pandi	T	0.111	0.11	17	Hanken and Wake, 1982
B. .resplendens	T	0.088	0.18	17	Wake and Lynch, 1982
B. riletti	T	0.039	0.42	19	Papenfuss et al., 1983
B. rostrata	T	0.060	0.133	30	Larson, 1983
B. rufescens	T	0.146	0.367	30	Larson, 1983
B. "soacha"	T	0.111	0.28	17	Hanken and Wake, 1982
B. subpalmata	T	0.221	0.580	18	Hanken and Wake, 1982
B. vallecula	T	0.136	0.39	18	Hanken and Wake, 1982
Cryptobranchus alleganiensis	L	0.007	0.026	24	Merkle et al., 1977
Cryptobranchus alleganiensis	L	0.019	0.095	21	This study
Desmognathus fuscus	T		0.182	19	Nevo et al., 1984
D. imitator	T	0.222	0.440	23	Nevo et al., 1984
D. ochrophaeus	T	0.193	0.590	15	Nevo et al., 1984
Dicamptodon copei	L	0.003	o.oi	34	Daugherty et al., 1983
D. ensatus[1]	T	0.012	0.032	34	Daugherty et al., 1983
Ensatina eschscholtzii	T	0.105	0.36	26	Wake and Yanev, 1986
Eurycea lucifuga	T	0.025	0.137	13	Nevo et al., 1984
Hydromantes platycephalus	T		0.153	18	Nevo et al., 1984
H. shastae	T		0.175	18	Nevo et al., 1984
Necturus lewisi	L	0,018	0.084	17	Nevo et al., 1984
N. maculosus	L	0.006	0.012	17	Nevo et al., 1984

TABLE 1. CONTINUED.

Species	T/L	H	P	Number of loci	Reference
N. punctatus	L	0.026	0.084	17	Nevo et al., 1984
Notophthalmus viridescens	T	0.157	0.42	15	Merritt et al., 1984
Notophthalmus viridescens	L	0.120	0.33	15	Merritt et al., 1984
Plethodon aureolus	T	0.117	0.41	22	Highton, 1983
P. caddoensis	T	0.069	0.372	23	Nevo et al., 1984
P. cinereus	T	0.044	0.166	24	Nevo et al., 1984
P. dorsalis	T	0.040	0.137	26	Nevo et al., 1984
P. dunni	T	0.009	0.065	24	Nevo et al., 1984
P. fourchensis	T	0.028	0.254	23	Nevo et al., 1984
P. glutinosus	T	0.144	0.431	18	Nevo et al., 1984
P. jordani	T	0.086		10	Nevo et al., 1984
P. kentucki	T	0.108	0.32	22	Highton and MacGregor, 1983
P. larselli	T	0.04	0.133	30	Howard et al., 1983
P. ouachitae	T	0.056	0.334	23	Nevo et al., 1984
P. serratus	T	0.033	0.097	24	Nevo et al., 1984
P. teyahalee	T	0.072	0.34	22	Highton, 1983
P. vehiculum	T	0.089	0.330	24	Nevo et al., 1984
P. websteri	T	0.031	0.131	26	Nevo et al., 1984
P. welleri	T	0.043	0.140	26	Nevo et al., 1984
P. yonahalossee	T	0.020	0.119	22	Nevo et al., 1984
Pseudoeurycea altamontana	T	0.127	0.270	18	Lynch et al., 1983
P. leprosa	T	0.057	0.23	18	Lynch et al., 1983
P. longicauda	T	0.064	0.39	18	Lynch et al., 1983
P. robertsi	T	0.167	0.44	18	Lynch et al., 1983
P. smithii	T	0.091	0.500	22	Nevo et al., 1984
P. unguidentis	T	0.041	0.182	22	Nevo et al., 1984
Rhyacosiredon altimirani	T	0.066	0.109	32	Shaffer, 1984
R. rivularis	T	0.231	0.469	32	Shaffer, 1984
Rhyacotriton olympicus• (N)	T	0.036	0.124	29	Good et al., 1987
Rhyacotriton olympicus• (S)	T	0.073	0.221	29	Good et al., 1987
Rhyacotriton olympicus• (C)	T	0.049	0.192	29	Good et al., 1987
Taricha granulosa	T	0.091	0.393	29	Nevo et al., 1984
T. rivularis	T	0.067	0.228	36	Nevo et al., 1984
T. torosa	T	0.058	0.253	25	Nevo et al., 1984
Thorius dubitus	T	0.06	0.19	16	Hanken, 1983
T. macdougalli	T	0.20	0.59	16	Hanken, 1983
T. minutissimus	T	0.08	0.312	16	Hanken, 1983
T. narisovalis	T	0.115	0.560	16	Hanken, 1983
T. pennatulus	T	0.095	0.22	16	Hanken, 1983
T. pulmonaris	T	0.080	0.375	16	Hanken, 1983
T. schmidti	T	0.085	0.25	16	Hanken, 1983
T. troglodytes	T	0.14	0.375	16	Hanken, 1983
T. sp A	T	0.09	0.188	16	Hanken, 1983
T. sp B	T	0.06	0.28	16	Hanken, 1983
T. sp C	T	0.055	0.094	16	Hanken, 1983
T. sp D	T	0.055	0.125	16	Hanken, 1983
T. sp E	T	0.13	0.50	16	Hanken, 1983
T. sp F	T	0.13	0.50	16	Hanken, 1983
T. sp G	T	0.11	0.50	16	Hanken, 1983
Triturus alpestris	T	0.156	0.554	21	Nevo et al., 1984
T. cristatus	T	0.035	0.241	30	Nevo et al., 1984
T. marmoratus	T		0.032	50	Nevo et al., 1984
T. vulgaris	T	0.090	0.401	16	Nevo et al., 1984

[1] **This is a combined value of *Dicamptodon ensatus* and *D. aterrimus*, because the latter has not been in common usage.**

[t] **These are currently being described as three species of *Rhyacotriton*, and so are considered as three species (Good, pers. comm.).**

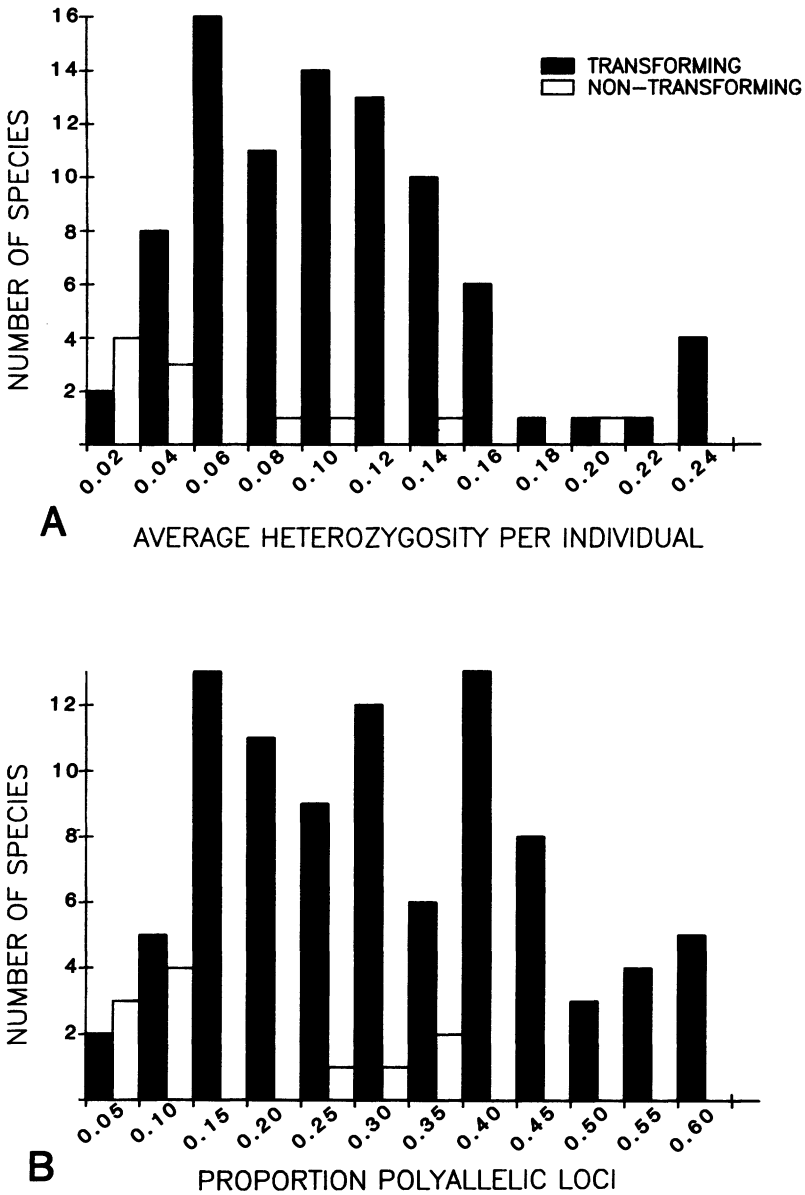

Fig. 1. Frequency histograms of average heterozygosity (H) and proportion polyallelic loci (P) for 102 species of transforming and non-transforming salamanders. For the identity of individual taxa, see Table 1.

separately, allowing us to average variability values across populations. In virtually all cases, a species is represented by at least five individuals, and generally by 18–35 loci (range, 10–50). For studies before 1980, we have relied on the values provided by Nevo et al. (1984) (37 species); we compiled later studies directly from the literature. In all, our sample contains 11 species of non-transforming salamanders from four families; the total data set of 102 species represents about a third of the living salamander species (Duellman and Trueb, 1986).

The results are presented in Table 1, and summarized graphically in Figure 1. To assess the relationship between genetic variation and metamorphic pattern, we conducted a Mann-Whitney U test on the ranks of each species for both H and P. Both are significantly correlated

with metamorphosis (for **H:** U = 704, z = 2.63, $P < 0.0044$; for P: U = 806, z = 3.29, $P < 0.0007$; both I-tailed tests). Salamander species which fail to metamorphose are, on average, far less genetically variable than those with terrestrial adults.

In calculating these values, we did not include the values for *Pseudobranchus, Amphiuma,* or *Necturus* mentioned in Merkle et al. (1977), because only passing reference was made to these species, and the results have never been reported in detail. However, all three are larval reproducers, and were reported as nearly or completely monoallelic. Thus, their inclusion would only strengthen the relationship reported here. (We did include *Necturus maculosus* from the survey of Ashton and Braswell [I 980], who reported **H** = 0.006; P = 0.012.) We also did not include populations of ambystomatid salamanders from Shaffer (1984) which are polymorphic for metamorphosis, because the population dynamics and stability of life history pattern of these animals are unknown. For species in which some populations metamorphose and others do not *(Ambystoma tigrinum,* Shaffer, 1984; *Notophthalmus viridescens,* Merritt et al., 1984), separate values were computed for both types of populations.

A possible objection to this analysis is that we are not justified in treating each species as an independent sample, because degree of phylogenetic relationship may influence patterns of metamorphosis and genetic variation (Felsenstein, 1985). Within the Ambystomatidae, Shaffer (1984) argued that most, and perhaps all, of the Mexican non-transforming populations were independently derived from transforming or polymorphic progenitors. This suggests that larval reproduction is an extremely labile character, at least in ambystomatids, and it is reasonable to consider each occurrence as an independent evolutionary event. However, it may be inappropriate to consider all species as independent sampling units for the three species of *Nectarus* reported by Ashton and Braswell (1980), because the validity of at least one species, *N. lewisi,* has been questioned. If all three species of *Necturus* are averaged into a single value, the relationship between **H** ($P < 0.029$) and P ($P < 0.0039$) and metamorphic pattern still holds.

Within families, the association of larval reproduction with reduced genetic variation may hold, although differences among families in overall levels of variability and the relatively small number of available comparisons restrict the analysis to an examination of trends. In *Dicamptodon,* there are two species; *D. ensatus* metamorphoses (H = 0.012, P = 0.032), while *D. copei* does not **(H** = 0.00278, **P** = 0.0097; Daugherty et al., 1983). Similarly, for the salamandrid *Notophthalmus viridescens,* Merritt et al. (1984) report values for metamorphosing **(H** = 0.157, **P** = 0.420) and non-transforming **(H** = 0.120, **P** = 0.330) populations. In both of these cases, the non-transforming species is less variable than its transforming congener. In the Mexican ambystomatids, (Shaffer, 1984), the pattern is less clear. Within *A. tigrinum* (which is itself a heterogeneous assemblage of different populations), the transforming samples are less variable than the non-transforming ones (transforming H = 0.055, P = 0.086; non-transforming H = 0.18, P = 0.32). Among all species of Mexican ambystomatids, the mean rank of five non-transforming taxa for both measures is lower than for seven transforming taxa, although this mean rank difference is not statistically significant (mean rank for larval reproducers is 5.8; mean rank for transforming taxa is 7.0, $P < 0.3$). Thus, when comparisons can be made among closely related congeners, a non-transforming taxon is usually less genetically variable than its transforming relative.

Why might this relationship exist? Two explanations seem plausible. First, it may be that there is some causal, mechanistic relationship between life history pattern and levels of genetic variation. Merkle et al. (1977) suggested, and Pierce and Mitton (1980) further developed, the idea that non-transforming taxa may have greater amounts of DNA than transforming ones. They further argued that these higher (repetitive) DNA levels free non-transforming taxa from the adaptive necessity of having high levels of structural genie variation that are detectable electrophoretically. This argument has been severely criticized (Larson, 1981) both on statistical and mechanistic grounds. Our interpretation is that while there may be a relationship among development time, heterozygosity, and genome size (Pierce and Mitton, 1980; Mitton and Grant, 1984), there are insufficient populations for which all three values are known to examine a causal relationship at this time.

An alternative interpretation is that the reduced genetic variation in non-transforming species simply reflects their population structure. Non-transforming salamanders are by definition aquatic, living in habitats ranging from

streams and small rivers to ponds, sloughs, and lakes. While these habitats, and the salamanders inhabiting them comprise a variety of ecological conditions, aquatic environments are presumably less stable than terrestrial ones, making populations subject to local extinctions when aquatic habitats either dry or change radically in stream flow (Schlosser, 1987). Whether such habitats are colonized by larval reproducers during wetter periods (as presumably happens in obligate non-transforming taxa like *Cryptobranchus* or *Necturus)* or by metamorphosing individuals carrying the genetic potential for larval reproduction (Shaffer, 1984), a pattern of extinction and recolonization by a small number of individuals will lead to a reduced effective population size, and therefore reduced genetic variation, in the species as a whole (Nei et al., 1975; Maruyama and Kimura, 1980).

Unfortunately, both of these hypotheses make similar predictions concerning the relationship between metamorphic failure and genetic variation. If either interpretation is correct, they predict that other non-transforming salamanders should have relatively low levels of genetic variation; we are currently testing this prediction in a group of populations of *A. tigrinum* in eastern Puebla, Mexico. To distinguish among these hypotheses requires additional data on larval growth rates and.levels of repetitive DNA that are not currently available. However, an interpretation similar to that suggested here has been used to explain the relationship between movement patterns and electrophoretically detectable variation for anurans (Inger et al., 1974) and large mammals (Sage and Wolff, 1986), and may underlie the variation seen in many taxa with different patterns of migration and mating structures (Maruyama and Kimura, 1980).

The traditional view of variation in metamorphic completion is that larval reproduction represents an evolutionary response to harsh terrestrial environmental conditions: metamorphic failure represents one way in which salamanders may remain in predator free aquatic habitats and avoid the physiological rigors of xeric terrestrial conditions (Wilbur and Collins, 1973; Sprules, 1974). Our results suggest that this response to proximate, ecological conditions has, on average, the population genetic consequences of reduced genetic variation due to frequent extinction and recolonization by small founding groups. Because the relationship is a statistical association rather than a causal mechanistic one, we expect that not all populations will show reduced genetic variation when larval reproduction becomes fixed. However, on average, the relationship should hold, and this may genetically constrain the evolutionary potential of non-transforming populations.

ACKNOWLEDGMENTS

We thank C. Peterson, **R.** Wilkinson, and **P.** Mayer for sharing their knowledge ofhellbenders and helping with the field work. D. McCauley and **M.** Griswold read the manuscript, and S. Sessions, **R.** Sage and **M.** Wade provided thoughtful discussions. This study was supported by National Science Foundation grant **BSR** 8407465 to HBS.

LITERATURE CITED

ASHTON,JR., R. E., AND A. L. BRASWELL. 1980. Electrophoretic analysis of three species of *Necturus* (Amphibia: Proteidae), and the taxonomic status of *Necturus lewisi* (Brimley). Brimleyana 4:439-446.

DAUGHERTY, C.H., F. W. ALLENDORF, **W.W.** DUNLAP AND **K.** L. KNUDSEN. 1983. Systematic implications of geographic patterns of genetic variation in the genus *Dicamptodon.* Copeia 1983:679-691.

DUELLMAN, **W.** E., AND L. TRUEB. 1986. Biology of amphibians. McGraw Hill Book Co., Inc., New York, New York.

FELSENSTEIN, J. 1985. Phylogenies and the comparative method. Amer. Nat. 125:1-15.

GooD, D. A., G. Z. WuRST AND D. B. WAKE. 1987. Patterns of geographic variation in allozymes of the Olympic Salamander, *Rhyacotriton olympicus* (Caudata, Dicamptodontidae). Fieldiana Zool. 32:1-15.

HANKEN, J. 1983. Genetic variation in a dwarfed lineage, the Mexican salamander genus *Thorius* (Amphibia: Plethodontidae): taxonomic, ecologic and evolutionary implications. Copeia 1983:1051-1073.

---, AND D. B. WAKE. 1982. Genetic differentiation among plethodontid salamanders (genus *Bolitoglossa)* in Central and South America: Implications for the South American invasion. Herpetologica 38:272-287.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam, The Netherlands.

HIGHTON, **R.** 1983. A new species of woodland salamander of the *Plethodon glutinosus* group from the southern Appalachian mountains. Brimleyana 9:1-20.

---, ANDJ. **R.** MACGREGOR. 1983. *Plethodon kentucki* Mittleman: a valid species of Cumberland Plateau woodland salamander. Herpetologica 39:189-200.

HOWARD, J. H., AND R. L. WALLACE. 1981. Microgeographical variation of electrophoretic loci in

populations of *Ambystoma macrodactylum columbianum* (Caudata: Ambystomatidae). Copeia 1981:466-471.

--, --AND J. H. LARSEN, JR. 1983. Genetic variation and population divergence in the Larch Mountain salamander *(Plethodon larselli).* Herpetologica 39:41-47.

INGER, R. F., H. K. VORIS AND H. H. VORIS. 1974. Genetic variation and population ecology of some southeast Asian frogs of the genera *Bufo* and *Rana.* Biochem. Genet. 12:121-145.

INTERNATIONAL UNION OF BIOCHEMISTRY, NOMENCLATURE COMMITTEE. 1984. Enzyme nomenclature, 1984. Academic Press, Orlando, Florida.

LARSON, A. 1981. A reevaluation of the relationship between genome size and genetic variation. Amer. Nat. 118:119-125.

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

LYNCH, J. F., D. B. WAKE AND S. Y. YANG. 1983. Genie and morphological differentiation in Mexican *Pseudoeurycea* (Caudata: Plethodontidae), with a description of a new species. Copeia 1983:884-894.

MARUYAMA, T., AND M. KIMURA. 1980. Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. Proc. Natl. Acad. Sci. 77:6710-6714.

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.

MERRITT, R. B., W. H. KROON, D. A. WIENSKI AND K. A. VINCENT. 1984. Genetic structure of natural populations of the red-spotted newt, *Notophtalmus viridescens.* Biochem. Genet. 22:669-686.

MITTON, J. B., AND M. C. GRANT. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Ann. Rev. Ecol. Syst. 15: 479-499.

NEI, M., T. MARUYAMA AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.

NEVO, E., A. BEILES AND R. BEN-SHLOMO. 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. Lecture Notes in Biomathematics 53:13-213.

PAPENFUSS, T. J., D. B. WAKE AND K. ADLER. 1983. Salamanders of the genus *Bolitoglossa* from the Sierra Madre del Sur of Southern Mexico. J. Herpetol. 17:295-307.

PETERSON, C. L., R. F. WILKINSON, JR., M. S. TOPPING AND D. E. METTER. 1983. Age and growth of the Ozark hellbender *(Cryptobranchus alleganiensis bishopi).* Copeia 1983:225-231.

PIERCE, B. A., AND J. B. MITTON. 1980. The relationship between genome size and genetic variation. Amer. Nat. 116:850-861.

———, AND ———. 1982. Allozyme heterozygosity and growth in the tiger salamander, *Ambystoma tigrinum.* J. Heredity 73:250-253.

SAGE, R. D., AND J. O. WOLFF. 1986. Pleistocene glaciations, fluctuating ranges, and low genetic variability in a large mammal *(Ovis dalli).* Evolution 40: 1092-1095.

SCHLOSSER, I. J. 1987. A conceptual framework for fish communities in small warmwater streams, p. 17-24. *In:* Community and evolutionary ecology of North American stream fishes. W.J. Mathews and D. C. Heins (eds.). University of Oklahoma Press. Norman, Oklahoma.

SHAFFER, H. B. 1983. Biosystematics of *Ambystoma rosaceum* and *A. tigrinum* in Northwestern Mexico. Copeia 1983:67-78.

———. 1984. Evolution in a paedomorphic lineage. I. An electrophoretic analysis of the Mexican ambystomatid salamanders. Evolution 38:1194-1206.

SOULE, M. 1976. Allozyme variation: Its determinants in space and time, p. 60-77. *In:* Molecular evolution. F.J. Ayala (ed.). Sinauer Associates, Inc., Sunderland, Massachusetts.

SPRULES, W. G. 1974. The adaptive significance of paedogenesis in North American species of *Ambystoma* (Amphibia: Caudata): an hypothesis. Can. J. Zool. 52:393-400.

WAKE, D. B., AND J. F. LYNCH. 1982. Evolutionary relationships among Central American salamanders of the *Bolitoglossa franklini* group, with a description of a new species from Guatemala. Herpetologica 38:257-272.

———, AND K. P. YANEV. 1986. Geographic variation in allozymes in a "ring species," the plethodontid salamander *Ensatina eschscholtzii* of western North America. Evolution 40:702-715.

WILBUR, H. M., AND J. P. COLLINS. 1973. Ecological aspects of amphibian metamorphosis. Science 182: 1305-1314.

(HBS) DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA 95616 AND (FB) DIVISION OF BIOLOGICAL SCIENCES, UNIVERSITY OF MISSOURI, COLUMBIA, MISSOURI 65211. Accepted 11 Feb. 1989.