Genetic Variation in Constant Environments

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Summary. Allozymic variation in proteins encoded by 32 gene loci was analyzed electrophoretically in 64 specimens from 6 localities representing 2 species of the spadefoot toads *Pelobates syriacus* and *P. cultripes* from Israel and Portugal, respectively. Out of the 32 loci examined, *Esterase-1* was the only locus that proved strongly polymorphic in all 6 localities and in the 2 species. The pattern of genetic variation in *Pelobates* is best explained by the environmental variability model. Selection for homozygosity as an adaptive strategy seems to operate in the relatively constant and narrow subterranean niche.

A major problem in evolutionary theory is the relative contribution of deterministic and stochastic processes in the genetic structure and differentiation of populations. A corollary of this problem is whether protein polymorphisms are adaptively selected or adaptively neutral[2]. The problem may be approached by testing the niche-variation model[3]. If protein polymorphisms are adaptively selected, the degree of genetic variation in a population should be correlated with an index of environmental heterogeneity, corroborating the niche-variation model. The present study is a test of this hypothesis in the subterranean spadefoot toads, *Pelobates syriacus* and *Pelobates cultripes*, both narrow habitat specialists[4], of the narrow underground niche, living mostly in a relatively constant environment.

Materials and methods. Allozymic variation in proteins encoded by 32 loci was analyzed electrophoretically in 64 specimens representing 2 species sampled at 6 localities (Table). All 1971–1973 specimens were kept at −80 °C and processed in September, 1973; the remainder in February, 1975. For processing of blood and tissues and for full names of proteins see NEVO et al.[5]. Standard gel electrophoresis was conducted according to SELANDER et al.[6]. Following are the abbreviations of the 32 loci tested *Mdh-1, Mdh-2, aGpd, Ldh-1, Ldh-2, 6Pgd, Gdh, Sdh, Adh, Odh, Xdh, Ipo, Fum, Ao, Idh-2, Pgm-1, Pgm-2, Pgm-3, Pgi, Got-1, Pept-1, Pept-2, Acph, Aph, Est-1, Est-2, Hb, Alb, Tf, Hp, Prot-2, Prot-3.* Alleles were designated alphabetically in order of decreasing mobility of their allozymes.

Results and discussion. The results, based primarily on the two population samples of *P. syriacus* from Dalton and Hadera, are truly remarkable. Of the 32 loci examined, 29 were monomorphic in both populations and fixed for the same allele. The polymorphic loci are given in the Table. *Est-1* was the only locus that proved strongly polymorphic in all 6 localities and in the 2 species. The 3 specimens of *P. cultripes* from Portugal proved as monomorphic as *P. syriacus*, yet they displayed an extensive amount of alternative fixations. Out of 30 loci (*Pept-1* and *Est-2* did not show up in *P. cultriples*) 15 loci were fixed for alternative alleles ($Mdh\text{-}1^b$, $Ldh\text{-}1^b$, $Ldh\text{-}2^a$, $6Pgd^b$, $Pgm\text{-}1^b$, $Pgm\text{-}2^b$, $Pgm\text{-}3^b$, $Pept\text{-}2^b$, Ipo^b, $Got\text{-}1^a$, Odh^b, Xdh^a, $Acph^b$, Adh^b, Aph^a). The genetic distance between both populations of *P. syriacus* and the sample of *P. cultripes*, derived after NEI[7], was $I = 0.54$, indicating early separation. By contrast, the genetic distance between the two populations of *P. syriacus*, Dalton and Hadera, separated by 90 km, was $I = 0.97$. Genetic estimates of mean alleles per locus, $\overline{A}$, mean proportion of loci polymorphic per population, $\overline{P}$, (1% criterion of

[1] Prof. J. M. CEI kindly sent me from Portugal the specimens of *P. cultripes*. Mr. M. AVRAHAMI and Mrs. HANA BAR-EL assisted in field and laboratory work. Dr. S. H. YANG assisted in the electrophoretic work. Mr. S. MENDLINGER critically commented the manuscript. This work was supported by U.S. Israel Binational Science Foundation Grant No. 330 to E. NEVO and R. C. LEWONTIN. I am grateful to all.

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Allele frequencies at the polymorphic loci of *Pelobates syriacus* and *Pelobates cultripes*[a]

			Israel *Pelobates syriacus*					Portugal *Pelobates cultripes*
Locus	Allele	Localities	Dalton	Hadera	Wasit	Nesher	Holon	Porto de Mos
		Collecting Date	April '71	January '75	April '73	January '75	January '75	March '71
		(N =)	(31)	(25)	(1)	(3)	(1)	(3)
Idh-2	a		1.00	0.94	1.00	1.00	1.00	1.00
	b		—	0.06	—	—	—	—
Est-1	a		0.48	0.48	0.50	0.50	0.50	—
	b		0.52	0.52	0.50	0.50	0.50	0.83
	c		—	—	—	—	—	0.17
Est-2	a		0.90	1.00	0.50	1.00	1.00	?
	b		0.10	—	0.50	—	—	

[a] All other 29 loci mentioned in the text were monomorphic, but 15 loci were alternatively fixed in *P. cultripes* as compared with *P. syriacus*.

polymorphism) and mean proportion of loci heterozygous per individual, $\overline{H}$, for Dalton and Hadera populations respectively were: $\overline{A}$ = 1.06, 1.06; $\overline{P}$ = 0.062, 0.062; $\overline{H}$ = 0.021, 0.028. Mean $\overline{H}$ for *P. syriacus* was 0.024. Heterozygosity in *P. cultripes*, based on the 3 specimens analyzed, was 0.011.

The pattern of genetic variation in *Pelobates* is best explained by the environmental variability model. Selection for homozygosity as an adaptive strategy seems to operate in the relatively narrow and constant subterranean niche[5,8,9]. LEVINS'[10] theory of fitness suggests that the amount of genetic variation is adapted to environmental heterogeneity and uncertainty. Therefore, homozygous patterns are expected to be adaptively selected in species occupying relatively narrow and constant niches, as is also postulated by the niche-variation model[3]. The subterranean niche is ecologically narrower, more constant and predictable than surface environments in terms of annual and daily fluctuations of temperature and relative humidity[11].

The evolutionary history of the pelobatids is very long, dating back to Cretaceous times[12], and that of the modern species of *Pelobates* to the Miocene[13]. Throughout its long history *Pelobates* evolved burrowing habits that made it a narrow habitat specialist living in a relatively constant and highly predictable underground environment, and thus insulated from short term environmental perturbations. In Israel, adult spadefoots are mainly seen above ground at night during the short winter breeding season, spending the greater part of their existence, during the dry and hot summer, underground (NEVO, unpublished). Significantly lower genetic variation was found in *Pelobates* when compared to the habitat intermediate species *Rana ridibunda* and *Hyla arborea*, and the habitat generalist *Bufo viridis*, when all 4 species were sampled at the same sites[9]. It therefore appears plausible that the relative narrowness and constancy of the subterranean niche are the major selective determinants of high homozygosity in spadefoots in accord with the prediction of the niche-variation model[3] and the theory of adaptive strategies[10].

Alternative explanations to the extreme homozygous patterns of *Pelobates* can be ruled out. First, genetic drift seems an unlikely agent of homozygosity since both the Dalton and Hadera populations of *P. syriacus* are quite large, involving hundreds, if not thousands, of breeding adults. Second, neutrality also appears unlikely. The evolutionary history of the modern species of *Pelobates* is certainly long enough to result in total fixation if neutrality indeed operated[14]. Nor can migration explain the esterase polymorphism, since the Hadera and Dalton populations are probably geographically disjunct and the two *Pelobates* species are certainly geographically and reproductively isolated.

Relatively low heterozygosity characterizes most subterranean and fossorial mammals yet studied[5], and also subterranean mole crickets, genus *Gryllotalpa*[15]. Similar reduced genetic variation associated with increasingly homogeneous environments has been demonstrated in marine bivalves[16], in bees[17], and was experimentally generated in *Drosophila* by POWELL[18] and MCDONALD and AYALA[19]. Despite some evidence to the contrary[20], a growing body of evidence indicates an overall positive correlation between protein polymorphisms and environmental heterogeneity[9,21,22]. It therefore appears plausible that allelic variation of protein polymorphisms is at least partly adaptive and is regulated in natural populations by selection in accord with an index of environmental diversity.

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Reactivation by Glycerol and Ethylene Glycol of Inactivated δ-Aminolevulinic Acid Synthetase of *Rhodopseudomonas spheroides*

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Summary. Reactivation effects by glycerol and ethylene glycol of inactivated ALA synthetase of *R. spheroides* were observed. Accompanying the reactivation of the inactivated enzyme, K_m value for PLP decreased to levels similar to those of the freshly prepared enzyme.

2-Mercaptoethanol is the most popularly used and potent stabilizer of δ-aminolevulinic acid (ALA)synthetase (E.C. 2.3.1.37), a regulatory enzyme of the tetrapyrrole biosynthetic pathway, of some bacteria and of animal tissues, but the effect becomes less remarkable as the purification of the enzyme proceeds and finally it is inhibitory on the activity of the highly purified ALA synthetase[1,2]. As another stabilizer of this enzyme, some workers recently applied glycerol which is well-known as a cryoprotectant; they reported that 10%[3,4] and 30%[5]

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