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Genetic Diversity and Ecological Relationships of Marsh Frog Populations in Israel

E. Nevo
Institute of Evolution, University of Haifa, Haifa (Israel)

S. Y. Yang
Department of Biology, Inha University, Incheon (Korea)

Summary. Allozymic variation in proteins encoded by 28 loci was analyzed electrophoretically in 340 mostly adult specimens representing 11 populations, 8 central and 3 isolated, of aquatic marsh frogs, *Rana ridibunda* in Israel, along a north-south transect of generally increasing aridity. In addition, geographic variation in 3 morphological variables of 144 frogs and in vertebral stripe color polymorphism of 262 frogs were also studied. The results indicate that. (a) Of the 28 loci examined, 12 (=43%) are largely monomorphic in all populations; out of the remaining loci, 6 were locally and weakly polymorphic and 10 regionally and strongly polymorphic. (b) No fixation of alternative alleles was found in any of the 28 loci and 11 populations studied. The commonest allele predominated across all populations, central as well as isolates. (c) Clinal patterns associated with increasing aridity southwards and eastwards occurred in polymorphism, P; heterozygosity, H; and in allele frequencies of Esterase-1, Xanthine dehydrogenase, Aldehyde oxidase and Albumin. (d) In the 3 estimates of genic variation, mean number of alleles per locus, A, mean proportion of polymorphic loci per population, P, and heterozygous loci per individual, H, marsh frogs displayed average estimates of genetic variation. The 3 estimates were: A=1.14 (range, 1.18–1.57); P=0.33 (range, 0.14–0.54): H=0.069 (range, 0.032–0.094). (e) Central populations harbored distinctly more genic variation than isolated populations. (f) Genic similarity between populations was high. (g) Significant deviations from Hardy-Weinberg equilibrium were found in 8 out of 11 populations involving 8 loci. (h) P, H, and allozymic variation in several gene loci were significantly correlated and predictable by environmental variables, primarily those related to water and temperature. (i) A significant amount of morphological variation was found between localities for body length, foot length, and weight in both sexes. Body weight in females was negatively correlated with temperature; and all three morphological variables in females were predicted significantly by a combination of temperature and humidity. (j) The three vertebral stripe color phenotypes, gray, green and red occurred in the following frequencies: 0.59, 0.24, 0.17, respectively. The red morph increased clinally southwards and was significantly correlated with most temperature and water variables. The geographic variation in both the green and red morphs was predicted significantly by climatic variables, both colors blending with local substrates.

The spatial patterns and environmental correlates of genetic and morphological variation in *Rana ridibunda* in Israel suggest that (i) protein polymorphisms are at least partly adaptive and that part is moulded by natural selection rather than by stochastic processes or neutrality; (ii) the environmental variation model seems to be a good predictor of genetic variation in marsh frogs; (iii) body size varies adaptively, presumably determined primarily through thermoregulation; (iv) the spatial pattern of the color polymorphism seems to be adaptively selected by at least two factors: visual predation and climatic determinants.

Key words: Allozymic variation – Protein polymorphism – Spatial pattern – *Rana ridibunda* – Marsh frog

Introduction

The relative importance in the genetic structure and differentiation of populations of various deterministic versus random processes remains a major unresolved problem of evolutionary biology. In particular, attempts are currently being made to assess how much and what kind of protein polymorphisms in natural populations provide the basis for adaptive evolution. One of the possible tests for the adaptive theory of

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allozymic variation is to unveil potential relationships between ecological and genetic profiles. If genetic structures, on both the single and multilocus levels, are indeed associated with, and predictable by ecological determinants, the adaptive nature of variation is reinforced. This study is part of a general one concerning the environmental correlates of the genetic structure of toads and frogs in Israel (Nevo, 1976a). The general study was aimed at testing the environmental variation model as a predictor of genetic variation in four anuran species in Israel whose range extends from relative ecological constancy to environments that vary greatly in both space and time.

The four species in Israel occupy increasingly varied and unpredictable environments in this order: the spadefoot toad, *Pelobates syriacus,* a subterranean narrow-habitat specialist (Nevo 1976a, b); the marsh frog, *Rana ridibunda,* an aquatic species, intermediate in its habitat-range (Nevo 1976a, and the subject of the present study); the tree frog, *Hyla arborea,* an arboreal species also intermediate in its habitat range (Nevo 1976a; Nevo and Yang 1979) and the green-toad, *Bufo viridis,* a terrestrial broad-habitat generalist species (Dessauer et al. 1975; Nevo et al. 1975; Nevo 1976a). The environmental variability hypothesis seems the best explanatory model of the genetic variation displayed by the four anuran species. Heterozygosity was found to be positively correlated with environmental heterogeneity and uncertainty. Consequently, it was concluded that protein polymorphisms in these anurans is adaptively important and is maintained in these species primarily by natural selection.

The present study is a specific test of the ecological-genetic relationships in the aquatic habitat-intermediate marsh frog, *Rana ridibunda,* in Israel. The results suggest that in marsh frogs (a) protein polymorphisms are at least partly associated with climatic factors, hence adaptive, and (b) the environmental variation hypothesis is a good explanatory model of genetic variation.

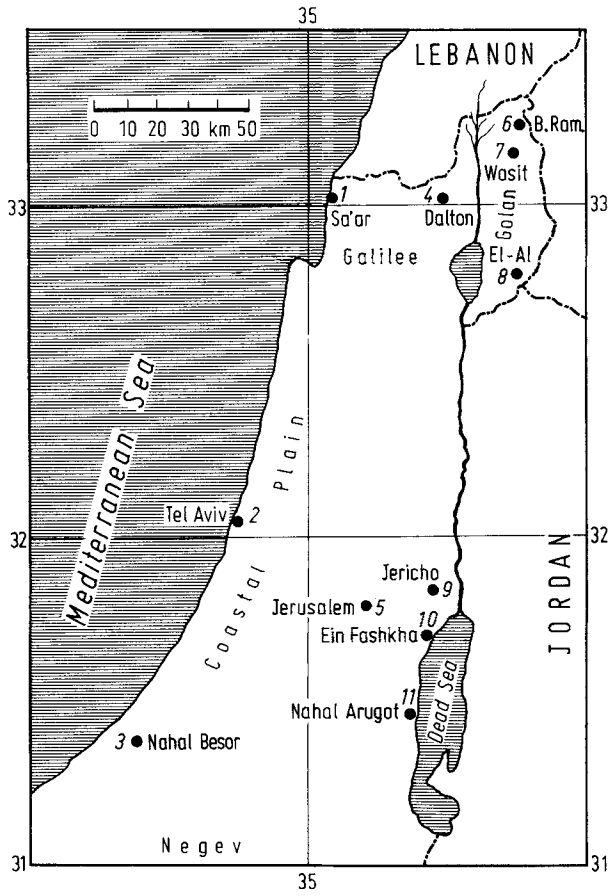

Fig. 1. Geographic distribution of sampling localities of *Rana ridibunda* in Israel

Materials and Methods

1 Systematics and Ecology of the European Aquatic Frogs of the Rana esculenta Complex

Aquatic frogs of the *Rana esculenta* complex inhabit nearly all of Europe, Southwest Asia, and parts of North Africa (Kauri 1959). Aquatic frogs of Central Europe occur in three distinct morphological forms: *Rana lessonae* Cam; *Rana esculenta* L.; and *Rana ridibunda* Pall. Many investigations (see Berger 1976 and his references) support the hypothesis that both *R. lessonae* and *R. ridibunda* are good biological species while the third one, *Rana esculenta,* is their hybrid. This view, based on ecological compatibility between the species (Berger 1973) and crossing experiments (Berger, 1976), was also supported by electrophoretical analysis (Tunner, 1970; Uzzel and Berger, 1975). In Europe, *Rana ridibunda* lives mostly in large water basins such as lakes and rivers, and is always restricted to the proximity of water. This pattern is in contrast to that of *Rana lessonae* which lives mainly in small ponds and in periodically drying water basins. Furthermore, while *lessonae* always hibernates on land, *ridibunda* always hibernates in water (Berger 1973). Their hybrid, *Rana esculenta,* occurs in practically every type of water body with either *lessonae* or *ridibunda,* or rarely, with both.

2 Range and Ecology of Rana ridibunda ridibunda

The marsh frog, *Rana ridibudna ridibunda,* which ranges from Central Europe to West Asia and North Africa (Mertens and Wermuth 1960), is primarily an aquatic species. It has been reported to migrate between ponds separated by a distance of 2.5 km or more (Berger, 1973). In Israel, *R. r. ridibunda* inhabits lakes, ponds, rivers, and creeks. It abounds in the Mediterranean region in large continuous populations but also occurs in a few isolated populations in desert oases (localities 9–11, Table 1). It is restricted to a variety of permanent and semi-permanent bodies of water which serve as buffers to climatic perturbations of both humidity and temperature. These habitats may guarantee in permanent bodies of water almost constant moisture throughout the year thus serving as effective safeguards against drought, desiccation, and freezing temperatures. Nevertheless, numerous smaller ponds dry up in the Mediterranean summer and migration of frogs takes place between them (migration was observed but has not been studied). Notably, in Israel aridity increases southwards and southeastwards (Atlas of Israel 1970), therefore, migration risks increase accordingly. Breeding takes place from February to September, varying geographically. *Rana ridibunda* may

Table 1. Geographical and climatological data for 11 populations of *Rana ridibunda* in Israel, followed by the Pearsonian correlation (r) between some of the variables

Locality	Sample size (N)	Longitude and latitude (Decimal) (Ln)	(Lt)	Altitude (m) (Al)	Mean temperature (°C) Annual (Tm)	Jan. (Tj)	Aug. (Ta)	Rainfall annual (mm) (Rn)	Humidity at 14:00 annual (%) (Hu)	Evaporation annual (cm) (Ev)	Moisture index (Thornthwaite 1948) (Mi)
1 Saar	30	35.08	33.03	20	20.1	13	25	584	59	174	C_1 −20 to 0
2 Tel Aviv	29	34.78	32.07	20	19.7	13	25	539	63	180	D −40 to −20 C_1 −20 to 0
3 Nahal Besor	30	34.45	31.38	75	21.1	14	26	284	52	190	E −60 to −40
4 Dalton	35	35.50	33.02	800	17.1	7	25	625	50	220	C_2 0 to 20
5 Jerusalem	30	35.23	31.78	700	18.6	10	25	486	45	220	C_1 − D − 30 to −10
6 Berekhat Ram	33	35.75	33.23	990	13.9	5	23	1296	58	220	C_2 0 to 20
7 Wasit	19	35.73	33.13	850	16.4	6	25	822	49	220	C_2 0 to 20
8 El-Al	30	35.75	32.60	370	20.5	11	27	455	45	240	D −40 to −20
9 Jericho	32	35.45	31.85	−230	24.7	15	33	144	35	330	E −60 to −40
10 Ein Fashkha	32	35.45	31.72	−375	24.4	16	31	84	37	340	E −60 to −40
11 Nahal Arugot	40	35.37	31.45	−300	24.4	16	31	84	37	340	E −60 to −40
r											
Lt		0.29	1.00	0.69	−0.78	−0.80	−0.62	0.82	0.51	−0.47	0.90
Al		0.35	0.69	1.00	−0.94	−0.98	−0.78	0.85	0.42	−0.54	0.86
Rn		0.42	0.82	0.85	−0.95	−0.90	−0.82	1.00	0.68	−0.60	0.69
Hu		0.22	0.51	0.42	−0.66	−0.45	−0.85	0.68	1.00	−0.90	0.61
Ev		−0.09	−0.47	−0.54	0.68	0.50	0.90	−0.60	−0.90	1.00	−0.60
Mi		0.28	0.90	0.86	−0.92	−0.92	−0.80	0.89	0.61	−0.60	1.00

represent therefore a relatively habitat-intermediate species living in a moderately buffered habitat ranging ecologically between the constant habitat specialist *Pelobates syriacus* (Nevo 1976b) and the habitat-generalist *Bufo viridis* which lives in ecologically heterogeneous and fluctuating environments (Nevo 1976a). This is also expressed at the southern border in the distribution pattern of *R. ridibunda* in Israel (Atlas of Israel 1970; Nevo 1976a, Fig. 1) which extends more into the xeric environments than *Pelobates* but much less so than *Bufo*.

2.1 Ecological Background

The climatological variables in this study (Table 1) provide the major comparative data with the genetic diversity found. All climatological values for localities 1–5 and 9–11 are from the Atlas of Israel (1970), and for localities 6–8 from the Israel Meteorological Service (various publications and unpublished data). The former are multiple year averages, mostly based on 30 or more years of recordings, whereas the latter are based on 3–10 years of recordings only. All records are derived from the closest station of the Meteorological Service to the site of the frog population studied. In general, the distance between the recording station and the frog population studied did not exceed 15 km. Finally, The Atlas of Israel (1970) includes a short and precise description of the climatic regions of Israel, and the Thornthwaite (1948) climatic classification.

The bodies of water from which frogs were collected were relatively big (mostly more than several hundred square meters), at least 1/2 m deep, and included (localities in parentheses as numbered in Table 1): the Yarkon-river (2); a small lake (6); creeks (1, 3, 11; both 1 and 3 in the Coastal Plain dry up during the summer when frogs migrate to nearby pools and ponds; locality 11, though in the Judean Desert, consists of a continuous water body throughout the year deriving from permanent springs); ponds (4, 7, 8); a pool (5); the water bodies of 5 and 8 dry up during the summer; and springs with their outgoing irrigation canals (9, 10). Most localities consisted largeley of freshwater, though salinity levels varied both spatially and temporally, climaxing in the Dead sea saline springs of Ein Fashkha (1650 mg/l, CL^-). Vegetation varied spatially but a substantial element of hydrophylic vegetation predominated in both the Mediterranean (1–8) and desert (9–11) localities reflecting the relative uniformity of the aquatic habitat. In general, gene exchange may be involved in the Mediterranean region, due to a relative continuum of populations, in contrast to the partial isolation of population 9 (Jericho oasis which may be partly connected with the Jordan river) and the total isolation of populations 10 and 11, in the Judean desert. This assessment is intuitive rather than measured.

We did not attempt to measure ecological data, either physical (climatic, hydrological etc.) or biotic (vegetation, predators, competitors etc.) locally, but used only long-term

Table 2. Allele frequencies at 28 gene loci in 11 populations of *Rana ridibunda* in Israel[a]

Locus	Allele	Locality												
		1	2	3	4	5	6	7	8	9	10	11	Mean	Effective inbreeding coefficient F_e
	N =	30	29	30	35	30	33	19	30	32	32	40	340	
αGpd	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.0	0.02
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	0.02
Ldh-1	1	0.78	0.62	0.87	0.24	0.63	0.41	0.58	0.55	0.56	0.95	0.40	0.59	0.18
	2	0.22	0.38	0.13	0.73	0.37	0.55	0.39	0.45	0.44	0.05	0.60	0.40	0.17
	3	0.0	0.0	0.0	0.03	0.0	0.05	0.03	0.0	0.0	0.0	0.0	0.01	0.03
Ldh-2	1	0.98	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.04
	2	0.02	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.04
6Pgd	1	0.95	0.91	0.85	0.97	0.90	0.82	0.87	0.90	1.00	1.00	1.00	0.93	0.06
	2	0.05	0.09	0.15	0.03	0.10	0.18	0.13	0.10	0.0	0.0	0.0	0.07	0.06
Pgm-1	1	0.08	0.0	0.0	0.0	0.07	0.06	0.19	0.08	0.03	0.0	0.0	0.04	0.09
	2	0.92	1.00	1.00	1.00	0.93	0.94	0.81	0.92	0.97	1.00	1.00	0.96	0.09
Pgm-2	1	0.03	0.0	0.0	0.0	0.0	0.09	0.0	0.02	0.0	0.0	0.0	0.01	0.06
	2	0.97	1.00	1.00	1.00	1.00	0.91	1.00	0.98	1.00	1.00	1.00	0.99	0.06
Pgm-3	1	1.00	0.98	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	0.04
	2	0.0	0.02	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.04
Sdh	1	0.03	0.0	0.04	0.0	0.0	0.0	0.06	0.0	0.0	0.0	0.0	0.01	0.05
	2	0.97	1.00	0.96	1.00	1.00	1.00	0.94	1.00	1.00	0.95	0.94	0.98	0.03
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.06	0.01	0.04
Est-1	1	0.03	0.38	0.33	0.34	0.16	0.03	0.0	0.03	0.0	0.0	0.15	0.14	0.19
	2	0.57	0.33	0.07	0.40	0.27	0.53	0.95	0.38	0.16	0.27	0.0	0.34	0.31
	3	0.40	0.29	0.60	0.26	0.55	0.44	0.05	0.59	0.84	0.73	0.85	0.53	0.25
	4	0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02
Est-2	1	0.0	0.0	0.35	0.17	0.55	0.38	0.16	0.0	0.03	0.0	0.41	0.20	0.26
	2	0.63	0.20	0.50	0.83	0.45	0.59	0.76	0.68	0.93	1.00	0.39	0.62	0.25
	3	0.37	0.80	0.15	0.0	0.0	0.03	0.08	0.32	0.03	0.0	0.20	0.18	0.40
Est-3	1	0.0	0.0	0.0	0.13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.11
	2	0.50	0.64	0.45	0.20	0.48	0.37	0.21	0.48	0.84	0.30	0.0	0.40	0.22
	3	0.50	0.36	0.55	0.67	0.52	0.63	0.79	0.52	0.16	0.70	1.00	0.59	0.20
Est-4	1	0.02	0.05	0.0	0.0	0.07	0.09	0.13	0.0	0.14	0.0	0.0	0.04	0.08
	2	0.0	0.0	0.0	0.0	0.0	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.03
	3	0.98	0.95	1.00	1.00	0.93	0.80	0.87	1.00	0.86	1.00	1.00	0.95	0.10
	4	0.0	0.0	0.0	0.0	0.0	0.08	0.0	0.0	0.0	0.0	0.0	0.01	0.07
Aat-1	1	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.02
(*Got-1*)	2	0.0	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02
Aat-2	1	0.07	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.07
(*Got-2*)	2	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.07
Odh	1	0.98	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.04
	2	0.02	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.04
Xdh	1	0.20	0.21	0.40	0.0	0.15	0.0	0.11	0.0	0.02	0.0	0.0	0.09	0.21
	2	0.80	0.76	0.60	1.00	0.83	0.98	0.89	1.00	0.83	1.00	1.00	0.89	0.17
	3	0.0	0.03	0.0	0.0	0.02	0.02	0.0	0.0	0.16	0.0	0.0	0.02	0.11
Ao	1	0.14	0.12	0.41	0.03	0.0	0.0	0.13	0.0	0.02	0.05	0.0	0.07	0.23
	2	0.86	0.82	0.59	0.97	1.00	1.00	0.87	0.92	0.55	0.95	1.00	0.88	0.23
	3	0.0	0.06	0.0	0.0	0.0	0.0	0.0	0.08	0.44	0.0	0.0	0.06	0.33
Alb	1	0.0	0.05	0.0	0.66	0.13	0.18	0.08	0.03	0.0	0.0	0.0	0.11	0.39
	2	0.0	0.0	0.0	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03
	3	1.00	0.95	1.00	0.31	0.87	0.82	0.92	0.97	1.00	1.00	1.00	0.89	0.41
Trf	1	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03
	2	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.03
Summary statistiscs														
A		1.57	1.50	1.39	1.39	1.46	1.54	1.50	1.39	1.39	1.18	1.18	1.41	
P		0.54	0.39	0.32	0.25	0.36	0.36	0.43	0.36	0.29	0.18	0.14	0.33	
H		0.094	0.078	0.076	0.066	0.083	0.085	0.076	0.071	0.066	0.032	0.045	0.069	

A = Mean no. of alleles per locus; P = Mean proportion of polymorphism per population; H = Mean proportion of heterozygosity-per individual

[a] The following 9 loci were monomorphic in all 11 populations: *Pgi, Gdh, Pept-1, Pept-2, Sod, Hb, Prot-1, Prot-1, Hpt.*

multiple year climatological data (moisture and temperature variables) which directly or indirectly affect frog biology. The rationale is that regional and gross population genetic structure is more influenced by past climatological history than by present conditions on the collecting date.

3 Sampling and Analysis

3.1 Sampling

A total of 340 adult marsh frogs representing eleven populations of *Rana ridibunda ridibunda* in Israel were collected during May-June 1973. Data on localities, geography and climate are given in Table 1, distribution is shown in Fig. 1. The 11 populations comprise 8 central populations in the Mediterranean region (1–8), and 3 in the Judean Desert (9–11). The central populations consist of 3 in the Coastal Plain (1–3); 2 in the Galilee and Judean Mountains (4, 5), and 3 in the Golan Heights (6–8). The eleven populations are distributed along a general north-south transect of increasing aridity. Similar trends of increasing aridity are displayed along the north-south transects in the Coastal Plain, Mountain Ridge and Golan Heights. All populations were collected during the breeding season when frogs congregate in ponds; each sample was collected in an area of about 1 square kilometer.

3.2 Electrophoresis

Live specimens were processed in the laboratory for blood and tissue samples (kidney, liver, muscle) and then stored in −80 °C at the Institute of Evolution, University of Haifa. From each specimen blood was separated into plasma and hemolyzed red cells. Blood fractions and tissue samples were stored in a −80 °C freezer.

Allozymic variation of enzymes and other proteins encoded by 28 gene loci was studied by standard horizontal starch gel electrophoresis. The procedures were those described by S. H. Yang (in Selander et al., 1971). The 28 loci coding for soluble proteins studied are given below. Having found no difference in pilot tests between isozymes of kidney, liver and muscle, all tissues were homogenized and run together.

Alleles were designated numerically in order of decreasing mobilities of their allozymes. I. Enzymatic proteins in homogenate: alpha glycerophosphate dehydrogenase (E.C. 1.1.1.8), (*αGpd*); lactate dehydrogenases (E.C. 1.1.1.27), 2 loci (*Ldh-1, Ldh-2*); 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) (*6-Pgd*); phosphoglucomutase (E.C. 2.7.5.1), 3 loci (*Pgm-1, Pgm-2, Pgm-3*); phsophoglucose isomerase (E.C. 5.3.1.9) (*Pgi*); glutamate dehydrogenase (E.C. 1.4.1.2) (*Gdh*); sorbitol dehydrogenase (E.C. 1.1.1.14) (*Sdh*); peptidases (E.C. 3.4.13.11), 2 loci (*Pept-1 : Pept-2*); esterases (E.C. 3.1.12), substrate: α–β naphtyl acetate, 4 loci (*Est-1, Est-2, Est-3, Est-4*), super oxide dismutase (E.C. 1.10.3.1) (*Sod*); aspartate amino transferase, (E.C. 2.6.1.1), 2 loci (*Aat-1, Aat-2,* previously *Got-1,2*); octanol dehydrogenase (E.C. 1.1.1.1) (*Odh*); xanthine dehydrogenase (E.C. 1.2.3.2) (*Xdh*); acetaldehyde oxidase (E.C. 1.2.1.3) (*Ao*); II. Nonenzymatic proteins (a) Hemolysate: hemoglobin (*Hb*); erythrocytic proteins, 2 loci (*Prot-1, Prot-2*); (b) Plasma: albumin (Alb); transferrin (Trf); haptoglobin (Hpt).

3.3 Morphological Measurements

To demonstrate the range of phenotypic variation in *Rana ridibunda,* 3 measurements were taken on each frog along with the phenotypic recording of the dorsal stripe color (green, red, or gray).

3.4 Statistical Analysis

Pearsonian correlations as well as stepwise multiple regression (Draper and Smith, 1966) and principal components analysis (Morrison, 1967) were used to determine whether environmental factors influence, or are associated with, gene frequencies of allozymes and phenotypic variation of morphological variation. The variance within and between populations in morphology was tested by analysis of variance, ANOVA.

Results

(i) Pattern of Variation

1. Of the 28 loci examined, 12 (=43%) were largely monomorphic in all populations (*αGpd, Pgi, Gdh, Pept-1, Pept-2, Sod, Aat-1, Hb, Prot-1, 2, Trf, Hpt*); the remaining 16 loci were either locally (in $\leqq$ 2 populations) and weakly (allele frequency $\leqq$5%) polymorphic (*Ldh-2, Pgm-3, Odh*) or regionally (in $\geqq$3 populations) and strongly (=allele frequency at least in one population >5%) polymorphic (*Ldh-1, 6Pgd, Pgm-1, Pgm-2, Sdh, Est-1, 2, 3, 4, Aat-2, Xdh, Ao, Alb,* excepting *Aat-2* which occurred only in one population). At 23 loci, the same allele was either fixed or predominated in all 11 populations, including central and isolated populations. Even in the 5 most polymorphic loci (*Ldh-1, Est-1, Est-2, Est-3, Alb*), the same allele predominated in most populations. Most remarkably, no fixation of alternative alleles was found in any of the 28 loci and 11 populations studied. The commonest allele occurred in all populations, central as well as isolates (Table 2).

2. Generally, "clinal" patterns of geographic variation occurred in polymorphism, P, heterozygosity, H, and in allele frequencies of *6Pgd, Est-1, Xdh,* and *Alb.* P and H decreased progressively with increasing aridity both southwards and eastwards. P decreased southwards from 0.54 at Saar to 0.14 at N. Arugot; and eastwards from 0.39 at Tel Aviv to 0.14 at N. Arugot. H decreased southwards from 0.094 at Saar to 0.032 at Ein Fashkha; and eastward from 0.078 at Tel-Aviv and 0.083 at Jerusalem to 0.032 at Ein Fashkha. $6Pgd^1$ increased southward from 0.82 at B. Ram to 1.00 at N. Arugot. $Est\text{-}1^3$ increased southward from 0.26 at Dalton to 0.85 at N. Arugot, and eastward from 0.29 at Tel-Aviv to 0.85 at N. Arugot. Xdh^2 decreased southward in frequency in the coastal Plain from 0.80 at Saar to 0.60 at N. Besor, but it increased eastward from 0.76 at Tel-Aviv to 1.00 at N. Arugot. Finally, Alb^3 increased in frequency southward from 0.31 at Dalton to 1.00 at N. Arugot (Table 2).

3. A summary of the genetic data on the 11 populations of *R. ridibunda* is given in Table 2. The main features are: (a) In all 3 genetic indices: mean number of alleles per locus (A=1.14), mean proportion of polymorphism per population (P=0.33) and mean proportion of heterozygosity per individual (H=0.069),

R. ridibunda approximates average estimates of genetic variation (Nevo, 1978); this is less distinct in P than in A and H. (b) All 3 genetic estimates decreased (sometimes progressively) southward. (c) Central populations harbored more genic variation than 2 of the isolated populations in the Judean desert. (d) The Jericho population was distinctly more variable in both P and H than the Ein Fashkha isolate which is only 16 km from it.

4. The proportions of A, P and H have each been recalculated in two different ways. The first, following Gillespie and Kojima (1968), included 3 groups of proteins:
- Group I, Glucose metabolizing enzymes (8 loci),
- Group II, Other enzymes (14 loci),
- Group III, Nonenzymatic proteins (6 loci).

The results for the 3 groups, respectively, were: A = 1.50, 1.86, 1.17; P = 0.37, 0.40, 0.11; and H = 0.08, 0.09, 0.01. The second grouping, following Johnson (1974) included 3 groups of enzymes:
- Group I, Variable substrate enzymes (8 loci),
- Group II, Regulatory enzymes (6 loci),
- Group III, Nonregulatory enzymes (7 loci).

The results for the 3 groups, respectively, were: A = 1.88, 2.00, 1.45; P = 0.44, 0.39, 0.38; and H = 0.12, 0.06, 0.08. The proportions of A, P and H varied nonsignificantly between the three Gillespie-Kojima groups (Group II exhibited more variation than either I or III, as expected by the hypothesis). The proportions of A, P and H varied only partly in accord with the Johnson hypothesis: Group I was more variable than the other two groups, but group II did not harbour more variation than Group III, as expected. Thus, relatively nonspecific enzymes exhibited more variation than presumably more specific ones.

5. Coefficients of genetic similarity, I, and genetic distance were calculated for paired combinations of all 11 populations based on the normalized identity of genes between each pair of populations (Nei, 1972).

The mean I was 0.973, the range 0.945–0.994; whereas mean genetic distance was 0.028, range 0.006–0.056. The standard errors ranged from 0.002 to 0.006, mean 0.004. These estimates indicate very high genic similarity, or very low genetic distance between populations. Even widely separated populations, such as Saar and N. Arugot which are separated by 165 km, display I = 0.976.

6. Significant linkage (gametic phase) disequilibria (Lewontin and Kojima, 1960) were found in 6 out of 378 tests in the following populations: Nahal Besor, between *Est-3³–Ao²* (D = 0.124; P < 0.001); and *Xdh²–Ao²* (D = 0.138; P < 0.001); Jerusalem, between *Est-3³–Pgm-1²* (D = –0.032; P < 0.05) and *Alb³–Pgm-1²* (D = –0.24; P < 0.05); Wasit, between *Est-1³–Est-3³* (D = –0.042; P < 0.01); El-Al, *Alb³–Pgm-1²* (D = 0.014; P < 0.05). However, these results may well be spurious.

Table 3. Correlation matrix of P, H, the first principle component of *Est-1* and *Est-2* and several gene loci with the environmental variables[a]

Environmental variable	P	H	*Ldh-1¹*	*Ldh-1²*	*Est-1* FPC	*Est-1¹*	*Est-1²*	*Est-1³*	*Est-2* FPC	*Est-2³*	Est-3²	*Xdh²*	*Ao²*	*Alb¹*
Ln	–0.021	–0.022	–0.302	0.279	–0.344	0.070	0.308	–0.356	0.170	0.236	0.009	0.218	0.026	0.102
Lt	0.568	0.506	–0.413	0.372	–0.832**	–0.271	0.851**	–0.725*	–0.132	–0.022	–0.107	0.349	0.342	0.462
Al	0.376	0.554	–0.503	0.462	–0.684*	0.037	0.627*	–0.688*	–0.146	–0.281	–0.191	0.177	0.393	0.595
Tm	–0.498	–0.614*	0.405	–0.358	0.785**	–0.082	–0.712**	0.800**	–0.033	0.071	0.216	–0.114	–0.408	–0.521
Tj	–0.384	–0.514	0.533	–0.487	0.765**	0.033	–0.723**	0.742**	0.169	0.250	0.209	–0.254	–0.392	–0.602*
Ta	–0.649*	–0.751**	0.169	–0.135	0.693*	–0.314	–0.566	0.789**	–0.330	–0.204	0.089	0.184	–0.323	–0.390
Rn	0.549	0.652*	–0.388	0.341	–0.736**	–0.075	0.708*	–0.698*	0.061	–0.013	–0.068	0.128	0.344	0.374
Hu	0.712*	0.743**	–0.018	–0.006	–0.575	0.409	0.435	–0.700*	0.599	0.570	0.152	–0.383	0.056	0.177
Ev	–0.794**	–0.850**	–0.033	0.052	0.598	–0.432	–0.448	0.734**	–0.485	–0.425	–0.189	0.510	0.028	–0.223
Moisture index	0.525	0.568	–0.494	0.451	–0.870**	0.017	0.809**	–0.862**	–0.015	–0.036	–0.206	0.230	0.444	0.616*

[a] Abbreviations for environmental variables as in Table 4
* P < 0.05; ** P < 0.01

7. Deviations from Hardy-Weinberg equilibrium owing to heterozygote paucity were found in the following populations and loci: (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) Saar, *Est-2****; Tel-Aviv, *Ldh-1***, *Est-2**; Nahal Besor, *Est-3****, Ao** *Sdh**; Dalton, *Est-1****, *Alb***; Jerusalem, *Pgm-1**, *Est-1** *Est-3**; B. Ram, *Pgm-1***, *Est-1***, *Est-3**; Wasit, *Est-2****, *Est-3**; El-Al, *Est-1**, *Alb**, Jericho, *Ao***, Nahal Arugot, *Est-1**. In sum, the polymorphic loci showed distinct nonrandom differences of Hardy-Weinberg equilibria. Significant paucity of heterozygotes varied among the 12 polymorphic loci in the following decreasing order (the ratio in parentheses): *Est-1* (5 cases of heterozygote paucity out of 11 polymorphic populations); *Est-3* (4 of 10); *Pgm-1* and *Alb* (2 of 6); *Est-2* (3 of 10); *Ao* (2 of 8); *Sdh* (1 of 5). In *Est-4, 6Pgd, Xdh,* and *Pgm-2* no significant heterozygote paucity was found. Out of 89 polymorphic cases 22 cases showed significant heterozygote paucity; 5 deviations were significant at $P < 0.001$; 5 deviations at $P < 0.01$; 10 deviations at $P < 0.05$; 22 cases of heterozygote paucity were nonsignificant. In contrast, 45 cases showed good fit to Hardy-Weinberg expectations and only 2 cases showed nonsignificant excess of heterozygotes (*Ldh-1* in Jericho, and *Xdh* in Saar). The above patern makes inbreeding an unlikely explanatory model for the deviations from Hardy-Weinberg equilibrium.

In general *Ldh-1* and *6Pgd* showed consistent good fit to Hardy-Weinberg expectation (in 17 of 19 polymorphic cases) while the esterases and *Alb* showed consistent paucities in 35 cases out of 44 polymorphic cases.

(ii) Environmental Correlates of Allozymic Variation

Polymorphism and heterozygosity as well as allozymic variation in several gene loci are significantly correlated with environmental variables, primarily those of water and temperature. The following are the main conclusions first based on Pearsonian correlations, then on multiple regression analysis:

1. Some of the geographical and climatological variables employed in this study to characterize the ecogeographical background of marsh frogs are intercorrelated (Table 1). While no correlation is apparent between longitude and climatological variables, latitude and altitude are highly correlated with temperature, rainfall, and the moisture index. Water variables (rainfall, humidity, moisture index) are negatively correlated with temperature variables and evaporation is positively correlated with temperature variables but negatively correlated with water factors. In other words, temperature increases, and rainfall and moisture indices decrease southwards towards the Negev desert.

2. The correlation matrix of P, H, and 7 strongly polymorphic loci with the geographical and climatological background variables are given in Table 3. Significant correlations are evident for some of the genetic variables with several ecogeographical factors. Thus, P and H are primarily negatively and significantly correlated with evaporation (*Ev*) and temperature, and positively with humidity factors. *Ldh-1, Est-2, Est-3, Xdh, Ao* show no significant correlations but *Est-1,* and *Alb* show significant corelations with single temperature and humidity variables, respectively (Table 3).

3. A test for the best predictors of P, H, and the 7 strongly polymorphic loci was conducted using a stepwise multiple regression analysis, MR (BMD program 02R; Dixon 1971), employing the above mentioned as dependent variables and geographic and climatic factors as independent variables. Likewise, to represent allelic variation within a locus we computed the first principal component (FPC) of allelic frequencies for *Est-1* and *Est-2.* These FPC's were employed as dependent variables in the MR along with the frequency of each individual allele. The results are given in Table 4, first for geographic, then for climatic variables. Evaporation (*Ev*) proved the first and best variable explaining the variation of P and H, ($R^2 =$ 0.630 ($P < 0.01$); 0.722 ($P < 0.01$), respectively. Most remarkably, the 3 variable combination of evaporation, rainfall and mean August temperature explained 0.78 ($P < 0.05$) and 0.84 ($P < 0.01$) of the variation in P and H, respectively. No combination of geographic variables explained the variation in P, and H; climatic factors, primarily of water and temperature appear to best account for the variation in polymorphism and heterozygosity. The variation in individual loci was best explained by temperature related variables, including evaporation (*Est-1, Ao, Xdh*) and by a combination of water and temperature variables (*Est-2, Alb.*).

(iii) Morphometrics and Color Polymorphism

1. Morphometrics. Geographic variation in morphometrics was also studied in 144 marsh-frogs, across the range of *R. ridibunda* in Israel, in the same populations tested for allozymic variation. The means of body-length (BL), foot length (FL), and body weight (W) are given in Table 5 for each sex in each of the 11 populations. Analysis of variance (ANOVA) of these data indicates that statistically significant amount of variation ($P < 0.001$) was present between localities for all three morphological variables in both sexes. The proportion of body length to foot length (BL/FL) did not show extensive variation between localities. Body weight in females was negatively correlated with temperature ($r = -0.60$, $P < 0.05$, Table 5), and all three female morphological variables (body length, foot

Table 4. Coefficients of Multiple Regression (R^2) employing as dependent variables: $\bar{P}$, $\bar{H}$, the frequencies of common alleles of 7 strongely polymorphic loci, the first principle component of *Est-1* and *Est-2*; morphological variation and color morphs, and as independent variables (A) geographical and (B) climatic factors[a]

		Stepwise			Model		
		A) Geographic variables			B) Climatic variables		
	$\bar{P}$	Lt 0.323	LtLn 0.360		Ev 0.630**	EvTa 0.653*	EvTaRn 0.780*
	$\bar{H}$	Al 0.307	AlLn 0.359	AlLnLt 0.394	Ev 0.722**	EvRn 0.753**	EvRnTa 0.839**
Ldh-1	allele						
	1	Al 0.253	AlLn 0.272	AlLnLt 0.278	Tj 0.285	TjTm 0.415	TjTmHu 0.437
Est-1	First Principle Component (FPC)	Lt 0.692**	LtAl 0.714**	LtAlLn 0.721*	Tm 0.616**	TmEv 0.624*	TmEvTa 0.700*
	alleles						
	1	Lt 0.073	LtAl 0.171	LtAlLn 0.179	Ev 0.187	EvRn 0.364	RnTaHu 0.498
	2	Lt 0.724*	LtAl 0.728*	LtAlLn 0.730*	Tj 0.523*	TjRn 0.540	TjRnEv 0.543
	3	Lt 0.526*	LtAl 0.592*	LtAlLn 0.602	Tm 0.640**	TmEv 0.710**	TmEvTa 0.748*
EST-2	FPC	Ln 0.029	LnAl 0.077	LnAlLt 0.080	Hu 0.359	HuTj 0.599*	HuTjRn 0.605
	alleles						
	1	Al 0.145	AlLt 0.645*	AlLtLn 0.742*	Tm 0.067	TmHu 0.167	TmHuTj 0.387
	2	Lt 0.053	LtAl 0.124	LtAlLn 0.130	Hu 0.268	HuTj 0.364	TjTaEv 0.482
	3	Al 0.079	AlLn 0.206	AlLnLt 0.253	Hu 0.325	HuTm 0.677*	HuTmTj 0.687*
Est-3	allele						
	3	Al 0.019	AlLn 0.022	AlLnLt 0.025	Ev 0.047	EvTa 0.385	EvTaRn 0.557
Xdh	allele						
	2	Lt 0.122	LtLn 0.137	LtLnAl 0.151	Ev 0.260	EvTa 0.661*	EvTaTj 0.678*
Ao	allele						
	2	Al 0.154	AlLn 0.168	AlLnLt 0.179	Tm 0.167	TmEv 0.336	TmEvTa 0.766*
Alb	allele						
	3	Al 0.343	AlLn 0.356	AlLnLt 0.362	Tj 0.351*	TjRn 0.499	TjRnHu 0.558
Body length (Bl)	Female	Lt 0.271	LtLn 0.303	LtLnAl 0.309	Tj 0.309	TjTa 0.493	TjTaHu 0.664*
	Male	Lt 0.126	LtLn 0.164	LtLnAl 0.166	Rn 0.151	RnEv 0.375	RnEvHu 0.480
Foot length (Fl)	Female	Lt 0.348	LtLn 0.373	LtLnAl 0.376	Tj 0.345	TjTa 0.399	TjTaHu 0.701*
	Male	Lt 0.076	LtLn 0.097		Rn 0.150	RnTa 0.351	RnTaHu 0.389
Weight (W)	Female	Lt 0.378*	LtLn 0.393	LtLnAl 0.395	Tj 0.355	TjTa 0.520	TjTaHu 0.691*
	Male	Lt 0.087	LtLn 0.116	LtLnAl 0.121	Rn 0.091	RnTa 0.396	RnTaTm 0.409

Table 4. (continued)

		Stepwise		Model		
		A) Geographic variables		B) Climatic variables		
Color Morphs (proportions)	Gray	Lt 0.223	LtAl 0.258	Rn 0.380	RnTm 0.519	RnTmEv 0.669
	Green	Al 0.060	AlLt 0.117	Tj 0.034	TjRn 0.594*	TjRnHu 0.845**
	Red	Al 0.489*	AlLt 0.509	Ev 0.525*	EvTj 0.652*	EvTjTm 0.867**

[a] Abbreviations for environmental variables: Al = Altitude; Ln = Longitude; Lt = Latitude; Tj = mean January temperature; Ta = mean August temperature; Tm = mean annual temperature; Hu = mean humidity at 14:00 hr; Rn = annual rainfall; Ev = annual evaporation

* $P < 0.05$; ** $P < 0.01$

length and body weight) were accounted for significantly ($P < 0.05$) by a three-variable combination of temperature and humidity (Table 4). Finally, out of the 7 strongly polymorphic loci *Ldh-1* was correlated ($P < 0.01$) with all body characters, and *Alb* only with body length.

2. Color Polymorphism. Geographic variation of the vertebral stripe color polymorphism (Moriya 1952; Nevo 1973b) was studied in 262 marsh frogs representing 10 out of the 11 populations studied for allozymic variation. The phenotypic proportions of the dorsal stripe color in each population is given in Table 5. The mean frequencies of the three color phenotypes, gray, green and red were 0.59, 0.24 and 0.17, respectively. Note that the gray morph predominated all populations except in 7, 10 and 11. The green morph predominated in population 7, whereas the red morph predominated in two of the desert isolates, 10 and 11. Therefore, the red morph increased clinally southwards from the Golan Heights to the Judean Desert, and was significantly correlated with most temperature and water variables ($P < 0.05$, Table 5). The geographic variation in both the green and red morph was explained significantly by two ($P < 0.05$) and three ($P < 0.01$) climatic variables (Table 4).

Discussion

The spatial patterns and environmental correlates of genetic variation in *Rana ridibunda* in Israel suggest that (i) the levels of genic diversity (P and H), and some allozymic variation, color polymorphism and morphological variation are largely adaptive and moulded primarily by natural selection rather than by stochastic processes, and (ii) the environmental variation model accounts best for genetic variation in marsh frogs as well as in the other three genera of anurans in Israel studied previously (Dessauer et al. 1975; Nevo et al. 1975; Nevo 1976a, b; Nevo and Yang 1979).

(i) The Adaptive Nature of Genetic Variation in Marsh Frogs

Genic diversity, allozyme and color polymorphisms, as well as morphological variation, are at least partly adaptive in marsh frogs, i.e., some of the genetic patterns observed seem to suggest the operation of natural selection as an important determinant of the genetic structure of populations. This conclusion is based on the following findings: (a) The commonest allozymic allele predominated across all populations including the desert isolates (Table 2); consequently, mean genic similarity, I, between populations was very high. This is particularly striking in view of the fact that populations were derived from both mesic and xeric environments. (b) "Clinal" patterns of genetic differentiation in P, H, *Est-l, Xdh, Ao, Alb,* red color morph and female weight were associated with increasing aridity and temperature (Tables 2, 5). (c) Relatively nonspecific enzymes exhibited more variation than presumably more specific ones. (d) Significant deviations from Hardy-Weinberg equilibrium occurrred in allozymes of several populations. (e) Climatic determinants were significantly associated with the geographic variation in P, H, *Est-l, Xdh, Alb,* red and green color morphs, and all 3 morphological variables in females (Tables 3–5).

The correlation between climatic means and allele frequencies is straightforward, but the correlation between climatic means and genic diversity needs clarification. Theory predicts that genetic diversity is associated with environmental variability in space and time (Dobzhansky 1951; Levene 1953; Levins 1968; Hedrick et al. 1976). This prediction for P and H was generally

Table 5. Morphometrics and color morph proportions of 11 populations of *Rana ridibunda* in Israel[a]

Locality	Females						Males						Vertebral stripe (phenotypic proportions)			
	N	Body length (mm)		Foot length (mm)		Body weight (g)	N	Body length (mm)		Foot length (mm)		Body weight (g)	N	Gray	Green	Red
		Mean	s. d.	Mean	s. d.	Mean		Mean	s. d.	Mean	s. d.	Mean	s. d.				
1 Saar	11	75.1	8.0	59.3	9.2	50.9	18	65.4	7.6	49.6	4.5	27.7	10.7	28	0.715	0.173	0.107
2 Tel-Aviv	23	78.5	14.6	61.1	9.5	48.1	3	72.7	5.9	54.7	1.5	34.4	4.7	26	0.615	0.269	0.115
3 N. Besor	9	74.8	6.0	55.9	4.8	37.5	16	57.4	5.5	43.9	3.9	17.3	5.7	25	0.640	0.240	0.120
4 Dalton	17	100.4	11.0	69.8	7.8	88.6	18	75.5	7.6	53.8	5.3	39.5	10.1			No information	
5 Jerusalem	13	68.5	8.3	52.1	5.0	30.4	14	61.6	4.9	47.9	3.4	21.8	5.5	27	0.556	0.407	0.037
6 B. Ram	13	88.3	7.9	68.8	3.0	71.1	20	79.4	8.0	59.8	6.5	46.3	12.8	33	1.000	0.000	0.000
7 Wasit	10	95.5	15.0	66.9	8.1	90.2	6	74.2	6.8	53.8	5.5	41.6	11.9	16	0.375	0.563	0.063
8 El Al	6	70.0	12.5	53.0	8.2	36.4	17	60.7	7.0	44.6	4.1	22.5	9.0	23	0.652	0.304	0.043
9 Jericho	10	87.1	6.1	62.8	9.1	64.9	23	71.0	6.0	53.7	6.8	39.7	10.1	33	0.727	0.152	0.121
10 Ein Fashkha	15	70.5	7.4	49.9	4.9	30.5	11	59.5	3.9	43.5	1.5	18.6	2.1	26	0.308	0.231	0.462
11 N. Arugot	17	82.6	18.3	62.1	11.5	60.7	8	81.3	8.2	60.6	3.7	53.0	16.9	25	0.120	0.240	0.640
Total	144						154							262			
Mean		81.6	11.8	60.7	8.0	56.2	21.3	68.4	6.7	51.1	5.0	32.4	9.9		0.595	0.237	0.168
Range		68.5 – 100.4		49.9 – 69.8		30.4 – 90.2		57.4 – 81.3		43.5 – 60.6		17.3 – 53.0			0.120 – 1.000	0.0 – 0.563	0.0 – 0.640

[a] Significant correlations: a) Female body weight is correlated with mean temperature of January, *Tj*, (r = – 0.60*). b) The red color morph is correlated with: mean annual temperature, *Tm*, (r = 0.66*), mean temperature of January, *Tj* (0.67*), mean temperature of August, *Ta* (r = 0.67*) rainfall, *Rn* (r = 0.65*), and evaporation. Ev (0.72*)
* $P < 0.05$

verified in nature (Nevo 1978). In general, climatologic variability increases from marine to continental, from lowlands to highlands, and from mesic to xeric climates. In Israel, mean values of rainfall and humidity decrease and their variances increase southward and eastward towards the deserts. Temperature variables, however, increase both in means and variance along the same directions (see climatic maps in Atlas of Israel 1970). If genic diversity is largely determined by environmental uncertainty then P and H are expected to increase towards the desert. This expectation was indeed fulfilled in desert landsnails (Nevo et al., 1981), a lizard (Nevo, 1981), and in subterranean mole rats (Nevo and Cleve, 1978) in Israel. The genetic pattern found in *Rana* showed an opposed trend, particularly in the desert isolates, which, as explained below, may be primarily related to both the buffering effect of the aquatic habitat and the potential risks frogs face during inter-pond migration.

The Mediterranean climate is sharply divided into cool and rainy winter (October–February) and hot and dry summer (May–September). Therefore, many semi-permanent bodies of water are established during the winter but shrink and may dry-up during the summer. Thus the run-off evaporation ratio determines the availability and the variation in size of most water bodies which provide a suitable frog habitat. Drying-up of ponds leads to migration of frogs to the more enduring bodies of water, thus increasing the risks involved through inter-pond migration by leaving the relatively predictable aquatic habitat for a highly unpredictable terrestrial one. Migration is caused largely by the development of summer xeric conditions which are intimately related to mean August temperature. The latter is correlated with mean annual temperature and evaporation (r = 0.90 and 0.68, respectively). Consequently, the climatic means of temperature, humidity, rainfall and evaporation are associated with the habitat change and the potential risks *Rana ridibunda* experiences in the Mediterranean climate.

The three desert isolates of *R. ridibunda* appear to be relicts of a previously more humid environment. Progressive desiccation has been the major climatic trend during the late Pleistocene and Holocene of Israel (Tchernov 1975) including the Dead-Sea basin (Neev and Emery 1967). Desertification presumably resulted in disjunct marsh frog populations that survived only in the big desert springs of Jericho, Ein Fashkha, and Nahal Arugot. These permanent springs form a relatively constant homogeneous environment for marsh frogs in contrast to the surrounding unpredictable dry and hot desert conditions. Obviously, the harsh desert is uncrossable for *Rana*. The frogs in the desert isolates are restricted to the buffered aquatic habitat and do not experience migratory risks. Although the terrestrial desert climate is by far more fluctuating than the Mediterranean climate, for the frog it appears more stable due to the permanent water bodies of the isolates. Therefore, the higher level of genic diversity in northern *R. ridibunda* populations may have been se-

lected in accord with their higher environmental heterogeneity and unpredictability. In contrast, the lower genic diversity in the Ein Fashkha and Nahal Arugot populations may relate to their relative habitat constancy and predictability. The higher genic diversity of the Jericho isolated population may be an exception on two counts: (i) the Jericho marsh frogs were collected from a variety of small pools and irrigation canals within the oasis rather than from the major spring flow, and (ii) in very humid years Jericho may receive imigrants from the nearby Jordan river in contrast to the apparently total isolation of Ein Fashkha and Nahal Arugot populations. although inbreeding may also be involved in the increased homozygosity of the isolated desert populations, it appears unlikely because of the large population size involving probably thousands of frogs, and the strong polymorphism in some loci (*Ldh-1* in population 11, and *Est-3* in population 10). Nevertheless, our data can not rule out inbreeding as affecting P and H in the isolates. We conclude that climatic factors, chiefly related to water availability, seem to be primary determinants of the genetic variation in *R. ridibunda.* They also presumably determine the southern range of the species in Israel which does not penetrate the Northern Negev and Sinai deserts as does that of *Bufo viridis* (Atlas of Israel 1970; Nevo 1976 a). The hypothesis that heterozygosity varies between *R. ridibunda* populations in accord with an index of environmental heterogeneity and unpredictability is testable, both macro- and microgeographically, in northern Israel, as well as within the Jericho oasis, respectively.

The pattern of allozymic variation (points a–e mentioned above) and the consideration of genic diversity, support natural selection as an important determinant of population genetic structure in *R. ridibunda* and make random factors and neutrality alone unlikely explanatory models. In general, effective breeding size in central populations of *R. ridibunda* (localities 1–8, Table 1, Fig. 1) is large; many ponds involve hundreds to thousands of breeding individuals. Consequently, effects of small population size are excluded as major determinants of differentiation. Likewise, the expected outcome of genetic drift, i.e. fixation of many alternative alleles in small isolated populations, has not been found. In contrast to this expectation, the same alleles in polymorphic loci predominate in the central as well as in the smaller isolated populations in the Judean Desert (localities 9–11, Table 1, Fig. 1). In particular, populations 10 and 11 are separated from the nearest central population by several dozen kilometers uncrossable by frogs. If these isolates date back to late Pleistocene times (for the geological and climatic histories of the Dead Sea basin see Neev and Emery 1967) their genetic pattern involving uniformity of alleles and the continuation of clinal pattern in levels of genic diversity, make the drift and/or neutrality hypotheses unlikely, and suggest selection as a major factor of genetic differentiation of populations.

Size Differentiation

The geographic differentiation in size in *R. ridibunda* primarily reflects its relatively uniform aquatic habitat which safeguards marsh frogs from death due to catastrophic desiccation, but possibly not from thermal death. Body size, at least in females, can be accounted for chiefly by a combination of temperature and rainfall (Table 4), but does not regress significantly on either rainfall or evaporation. In females, body weight decreases significantly ($r = -0.596$, $P < 0.05$) with increasing temperature and increases nonsignificantly with rainfall ($r = 0.413$; Table 5). This pattern contrasts sharply with that of "terrestrial" anurans in which body size increases with aridity as in terrestrial *Bufo viridis* (Nevo, 1972), and semi-aquatic *Acris crepitans* (Nevo, 1973 a). In these two cases, body size is causally related to humidity, large size being an adaptation to arid climates. In aquatic *R. ridibunda* where size decreases with temperature, thermoregulation, rather than desiccation, may be a prime determinant of adaptive size differentiation in accord with both Bergmann's and Allen's rules, as was also demonstrated for other poikilotherms (Ray, 1960). Diminishing food resources southward may also be involved.

Color Polymorphism

The geographic variation and environmental correlates of the vertebral-stripe color polymorphism in *R. ridibunda* suggest its maintenance by balancing natural selection similar, but not identical, to that found in cricket frogs (Nevo, 1973 b). The climatic correlates of both the green and red morphs (Tables 4, 5) suggest that their spatial pattern is adaptive and may be related to at least two factors: (i) visual predation, and (ii) climatic selection. The latter may contribute indirectly by causing geographic changes in substrate color, owing to the varying humidity index. In Israel, transects southward and eastward are largely, but not invariably, associated with increasing aridity and hence with decreasing green and increasing yellow-colored substrates. Therefore, the southward clinal increase in frequencies of the red morph may match this regional change in substrate color. Nevertheless, local variation in rock and soil colors contribute their share in generating substrate color mosaicism. The later may sometimes be responsible for local variation in color morph frequencies, regardless of the aforementioned regional geographic change in substrate color.

We hypothesize that the color polymorphism in green frogs may be at least partly maintained by visual selective predation which eliminates disharmonious morphs and preserves those that best blend with the local substrate. The substrate polymorphism hypothesis is supported by qualitative observations at the sampling sites. The B. Ram lake, in which the gray morph was fixed, consisted largely of gray tuffaceous substrate with very little green along its water bank. In contrast, the Wasit and Jerusalem sites, where the green morph was highest in frequency, consisted of a large green component in their substrate color involving green swampy vegetation in the former and green algae in the latter. Even the Ein Fashkha and Nahal Arugot desert isolates involved green algae and reeds. The substrate color polymorphism hypothesis is testable regionally and locally, as well as temporally, and may provide a rewarding ecological-genetic study.

(ii) The Environmental Variation Model as a Good Predictor of Genetic Variation in Marsh Frogs

The environmental variation model, also referred to as the environmental amplitude, or niche variation hypothesis, suggests that the amount of genetic variation may be regarded as an adaptive strategy for increasing population fitness in a spatio-temporally heterogeneous and uncertain environment (Dobzhansky 1951; Levene 1953; Levins 1968; Nevo 1978). It predicts that uncertain environments will give rise to broad-niche or habitat generalist species rich in polymorphism and heterozygosity in contrast to the nearly invariant patterns expected in narrow-niche or habitat specialist species. The predictions of the environmental variation model have been reasonably confirmed for several specific individual tests such as in edaphically restricted and widespread plant species (Babbel and Selander 1975), evolving species of mole rats, *Spalax* (Nevo and Shaw 1972), but refuted by others, i.e., in a test of marine fish (Somero and Soulé 1974).

The environmental variation model seems a good predictor of the differential genetic variation between the four anuran species studied in Israel, where increased environmental heterogeneity and uncertainty at the same sites is associated with increased heterozygosity (Nevo 1976a). The narrow habitat specialist, *Pelobates syriacus* (Nevo 1976a, b) living in a relatively constant and predictable underground environment, sheltered from drastic environmental perturbations, has significantly lower genetic variation than the habitat intermediates *Hyla arborea* (Nevo 1976a; Nevo and Yang 1979) and *Rana ridibunda* (Nevo 1976a, and this study). In contrast, the habitat generalist, *Bufo viridis,* living in distinctly varying environments in space and time, exhibits the highest degree of genetic variation (Dessauer et al. 1975; Nevo et al. 1975; Nevo 1976a). Genetic variation increased in the following order: *Pelobates* < *Rana* < *Hyla* < *Bufo.* The estimates of A, P, and H, based on 14 shared loci, for *Pelobates, Rana, Hyla,* and *Bufo,* respectively, were: A = 1.13, 1.55, 1.86, 1.84; P = 0.095, 0.44, 0.50, 0.56; and H = 0.029, 0.088, 0.088, and 0.169. Genetic variation seems to be positively correlated with environmental heterogeneity and uncertainty, as was also shown by comparing central and isolated populations of *R. ridibunda* in this study. This result strongly supports the hypothesis that environmental variation seems to select for the level of protein polymorphism in natural populations. Moreover, the predominance of the same alleles across all central and even in isolated marsh frog populations where gene flow is cut off, suggests that the nature of allozymic variation seems to be adapted to the aquatic environment which varies apparently less in space and time than the terrestrial one.

The genetic profiles of the aforementioned four anurans in Israel are correlated with the apparent differences between subterranean and surface environments, and, in the latter between aquatic and terrestrial habitats. The subterranean environment (where *Pelobates* spends most of its life) is ecologically more uniform and stable than surface environments (where *Rana, Hyla* and *Bufo* live) in terms of temperature and relative humidity (McNab 1966; Nevo 1979 and their references), both factors being crucial for anurans. Among surface environments, the aquatic habitat (where *Rana* lives) is less fluctuating in terms of temperature and water, than terrestial habitats (where *Bufo* lives). The aquatic habitat is therefore intermediate in terms of environmental fluctuations in space and time between the relatively constant underground and highly variable terrestrial habitats. Nevertheless, inter-pond migration may introduce some intermittent terrestrialism into the biology of *Rana.* The environmental variation model of genetic variation predicting a moderate amount of genetic variation in *Rana,* as compared to the extreme specialist *Pelobates,* and the extreme generalist *Bufo,* was confirmed. Similar results were obtained by comparing the heterozygosity, H, of 13 vertebrate species living in a combined terrestrial and freshwater habitat with 31 vertebrate species living solely in freshwater (H = 0.079 vs 0.056, respectively; $P < 0.05$; Nevo 1978, Table VA).

The environmental variation model predicting lower genetic variation in relatively more predictable environments was confirmed also in a variety of unrelated subterranean taxa such as fossorial mammals, amphisbaenians and lizards, skinks, frogs, subterranean mole crickets, *Gryllotalpa,* landsnail species of *Buliminus* and *Sphincterochila,* and habitat specialist species in general (Nevo 1978, 1979 and references therein).

It is instructive to compare and contrast estimates of genetic variation in terrestrial versus aquatic anurans in general. The terrestrial genus *Bufo* is highly variable in North and South America as well as in the Old World.

The estimates of H for *Bufo americanus* (Guttman 1975), *Bufo arenarum* (Matthews 1975) and *Bufo viridis* in Israel (Dessauer et al. 1975; Nevo et al. 1975) are 0.116, 0.163, and 0.133, respectively. Noteworthy, the isolate of *Bufo viridis* on Vis Island, in the Adriatic Sea, has a very low H, 0.029, similar to the low H's of the Israeli isolates of *Rana ridibunda,* where H = 0.032 and 0.045. In contrast to the terrestrial species, the cricket frog *Acris crepitans* is a semi-aquatic species always associated with water. Genetic variation in *A. crepitans* was not estimated for H, but the range of the P values is on the lower side, P = 0.14–0.23. Most of the variation occurs between the three geographical regions, Appalachians, Central Plains and Mississippi Gulf rather than within populations (Dessauer and Nevo 1969; Salthe and Nevo 1969). Finally, low H was reported for 7 American species of aquatic *Rana* (Case 1978).

An attempt to relate the amount of genic variation, based on 10–12 gene loci, to the ecology and population structure for 7 tropical anuran species, 3 bufonids and 4 ranids, from South East Asia has been reported by Inger et al. (1974). In these 7 anurans, ecological differentiation cuts within, rather than between genera. Thus, within the 3 bufonids the amount of genic variation agreed with theoretical predictions based on ecology and population structure: the open-living bufonid has a greater amount of genic variation than the two forest-restricted species. Predictability was less satisfactory among the ranid species, where the ecological knowledge may have been insufficient.

In general, the environmental variation model is supported by a recent review of 243 plant and animal species in which 14 or more loci were studied (Nevo 1978). The amounts of genetic polymorphism, P, and heterozygosity, H, vary nonrandomly between loci, populations, species, habitats, and life zones, and are correlated with ecological heterogeneity. Cosmopolitans, terrestrial-freshwater vertebrates (amphibians) and habitat generalists have on the average high levels of genetic variation as reflected by both P and H values. The estimates found in the above mentioned study for habitat specialist species (N = 120) and for habitat generalist species (N = 117) were P = 0.19 and 0.35 and H = 0.046 and 0.106, respectively. The estimates of *Rana ridibunda* reported in this study are P = 0.33 and H = 0.069. While *Rana ridibunda* approaches the mean for generalist species in P it exhibits an intermediate estimate between specialist and generalist species for H.

The evidence of genetic structure of populations of *Rana ridibunda* presented herein and compared to the other 3 anuran species in Israel suggests that genetic variation based on protein polymorphisms is correlated with ecological heterogeneity and uncertainty. Several forms of selection (uniform and diversifying, balancing and directional) may be the determinants of genetic population structure and differentiation in marsh frogs. However, in marsh frogs, as elsewhere, direct experimental evidence is desirable on the microgeographical scale to critically test the environmental variation model and unequivocally exclude alternative hypotheses (Hedrick et al. 1976).

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Prof. Dr. E. Nevo
Institute of Evolution
University of Haifa, Haifa (Israel)

Dr. Shu Y. Yang
Department of Biology
Inha University, Incheon (Korea)