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Genetic Variation and Climatic Selection in the Lizard *Agama stellio* in Israel and Sinai

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Summary. Allozymic variation in proteins encoded by 25 loci was analyzed electrophoretically in 242 adult specimens representing nine populations of the Levantine lizard, *Agama stellio*, comprising two subspecies: the Mediterranean *A. stellio* subsp., and the desert-inhabiting *A. stellio brachydactyla* from the Negev and Sinai. Likewise, four body traits were measured in the same populations. The nine populations were sampled along a general southward transect of increasing aridity. *Agama stellio* is above average in both polymorphism, P, and heterozygosity, H, as compared to other reptiles and vertebrates in general, displaying levels of genetic variation characterizing habitat generalist vertebrates. In the populations studied no fixation of alternative alleles was found in any of the 25 loci: rather the commonest allele was either fixed or predominated in 23 of 25 loci examined. Eleven loci (44%) were monomorphic in all nine populations. However, of the remaining 14 polymorphic loci, eight were strongly polymorphic displaying distinct genetic differentiation between populations. Genetic diversity (indexed by P and H) displayed geographic variation and was slightly higher in *A.s. brachydactyla* than in *A. stellio* subsp. Nevertheless, genic similarity between populations was high. A statistically significant amount of morphological variation between localities was found for all body characters. In general, body size increased southwards and eastwards with aridity.

Selection at some loci is suggested by significant deviation from Hardy-Weinberg expectations and possibly by excess heterogeneity of effective inbreeding coefficients, F_e. Furthermore, allozymic variation at seven loci (Ldh-1, Idh-1, 6-Pgd-1, Aat-1, Pgm-2, Pept-1, and Trf) and geographic variation in body size and weight were significantly correlated with, and predictable by, climatic variables, primarily by water availability and secondarily by temperature. Finally, allozymic and morphological variations were partly correlated.

The spatial patterns and ecological correlates of genic and morphological variations in *Agama stellio* in Israel and Sinai suggest that at least some proteins and body size differentiate geographically and appear to be adaptive, presumably with respect to factors affecting the availability of water.

Key words: *Agama stellio* – Levantine lizard – Climatic selection – Genetic variability – Geographic variation – Polymorphism

Introduction

Genetic polymorphisms are central to evolutionary theory yet their nature is still controversial, particularly at the molecular level. The neutral theory holds that most intraspecific molecular variability is caused not by selection but by random drift of selectively equivalent mutant genes (Kimura 1979). One promising way to assess whether allozyme variants are adaptive or neutral is to search for potential associations with the environment. We have explored potential environmental correlates of allozymes and morphological traits in the most conspicuous reptile of Israel, *Agama stellio*, as a part of the study of four Israeli agamid species which vary in their ecogeographical ranges (Atlas of Israel 1970) and thermal biology (Hertz et al. 1981).

The old world family *Agamidae* includes 36 genera and approximately 320 species (Duellman 1979) which are much more abundant in the tropics than in temperate areas. The large genus *Agama*, which contains about 60 species, ranges from South Africa to central India and northwards to extreme southeastern Europe: its species diversity seems to be highest in Africa. The Levantine lizard *Agama stellio* (or hardun) ranges from southeastern Europe to West Asia and eastern North Africa (Mertens and Wermuth 1960; Daan 1967). It is widely and abundantly distributed around the eastern end of the Mediterranean, including Israel. Within this range *A. stellio* is a generalist species inhabiting rocks, stone fences, ruins, tree-trunks, alluvial badlands, etc., and displays considerable morphological variation in size, body proportions, colour, and scale ar-

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rangement. The intraspecific taxonomy of this species has remained obscure and controversial (Daan 1967 and his references). We have studied two subspecies in Israel: *Agama stellio* subsp. (Hoofien 1972), and *Agama stellio brachydactyla* (Haas 1951).

In Israel *A. stellio* subsp. ranges over the Mediterranean biogeographical region in large populations penetrating only marginally into the steppe and desert of the northern Negev region where it is replaced by *A. stellio brachydactyla*. Both subspecies range from below sea level to above 2000 meters on Mount Hermon and the mountains of southern Sinai, respectively. Harduns are diurnal, largely insectivorous, partly torpid during December through February, and fully active in spring and summer. Eggs hatch in July.

The present paper describes the pattern of genetic variation in nine populations of *Agama stellio* across the entire ecogeographical ranges of the two subspecies in Israel and the Sinai Peninsula. The evidence presented suggests that *Agama stellio* in Israel and Sinai displays levels of genetic variation characterizing habitat generalist vertebrates, and that the patterns of the genic and morphological variations in natural populations are in part predictable ecologically, primarily by factors affecting the availability of water.

Materials and Methods

Sampling

A total of 242 specimens representing nine populations and two subspecies of *Agama stellio* (*A. stellio* subsp., populations 1-7; *A. stellio brachydactyla*, populations 8,9) in Israel and the Sinai Peninsula were collected from May through October 1976. Data on localities, collecting dates and ecogeographical parameters are given in Table 1; distribution of localities is shown in Fig. 1. Each of the nine samples was collected in 1-3 days, in an area of about one square kilometer.

Electrophoresis

Living specimens were processed in the laboratory for blood and tissue samples (kidney, liver, heart, muscle) and measured morphologically. Blood samples were separated into plasma and hemolysed red cells. Blood fractions, tissue samples and bodies were stored in a −80°C freezer at the Institute of Evolution, University of Haifa.

Allozymic variation of 22 enzymes and three other proteins encoded by 25 loci was studied by standard horizontal starch gel electrophoresis (Selander et al. 1971). Having found no difference in initial comparisons between isozymes of kidney, liver, heart and muscle, all tissues were homogenized together. Alleles were designated alphabetically in order of decreasing mobilities of their allozymes.

1. Enzymatic proteins in homogenate: malate dehydrogenases (E.C.1.1.1.37) 2 loci (Mdh-1,2); malic enzyme (E.C. 1.1.1.40), (Me); α glycerophosphate dehydrogenase (E.C.1.1.1.8), (α Gpd); lactate dehydrogenases (E.C.1.1.1.27), 2 loci (Ldh-1,2); isocitrate dehydrogenases (E.C.1.1.1.42), 2 loci (Idh-1,2); glucose-6-phosphate dehydrogenase (E.C.1.1.1.49), (G-6Pd); 6-phosphogluconate dehydrogenases (E.C.1.1.1.44), 2 loci (6-Pgd-1,2); phosphoglucomutases (E.C.2.7.51), 2 loci (Pgm-1,2); glucosephosphate isomerase (E.C.5.3.1.9), (Pgi); aspartate aminotransferase (E.C.2.6.1.1), (Aat-1, previously Got-1); peptidases (E.C.3.4.13.11) 2 loci (Pept-1,2), substrate: L-Leucyl-L-alanine; tetrazolium oxidase = superoxide dismutase (E.C.1.10.3.1), 2 loci (To-1,2); alcohol dehydrogenase (E.C.1.1.1.1), (Adh); glutamate dehydrogenase (E.C.1.4.1.2) (Gdh); leucine amino peptidase (E.C.3.4.1.1), (Lap);

2. Nonenzymatic proteins: (a) Hemolysate: erythrocytic protein (Prot-1); (b) Plasma: albumin (Alb); transferrin (Trf).

Morphological Measurements

To demonstrate the range of morphological variation in *A. stellio*, four measurements were made on each lizard; body weight, body length (snout-vent), forefoot length, and hindfoot length (measured from wrist and ankle, respectively, to tip of the longest digit).

Table 1. Ecogeographical data for nine populations of *Agama stellio* in Israel

Population	Collecting sample		Longitude	Latitude	Altitude	Mean temp.			Annual		
	Month in 1976	Size (N)	Degrees (Decimal)		(m)	Annual	Jan. (°C)	Aug.	Rainfall (mm)	Humidity at 14:00 (%)	Evaporation (cm)
Agama stellio subsp.											
1. Gesher Haziv	6	27	35.10	33.05	25	18.8	13	25	584	59	174
2. Dalton	7	30	35.48	33.02	660	17.1	7	25	625	50	220
3. Bet-Oren	6	28	35.00	32.72	450	20.5	12	26	686	59	190
4. Yifat	5	33	35.22	32.68	130	21.4	12	28	495	47	200
5. Sede Eliyyahu	10	30	35.50	32.45	−180	22.7	13	30	277	44	238
6. Jerusalem	5	29	35.23	31.78	700	18.5	10	25	486	45	220
7. Lahav	6	11	34.87	31.38	400	20.6	13	26	303	43	209
A.s.brachydactyla											
8. Avedat	10	30	34.77	30.82	525	19.7	13	25	116	32	290
9. Santa Katharina	10	24	33.92	28.58	2200	10.8	1	18	64	26	320

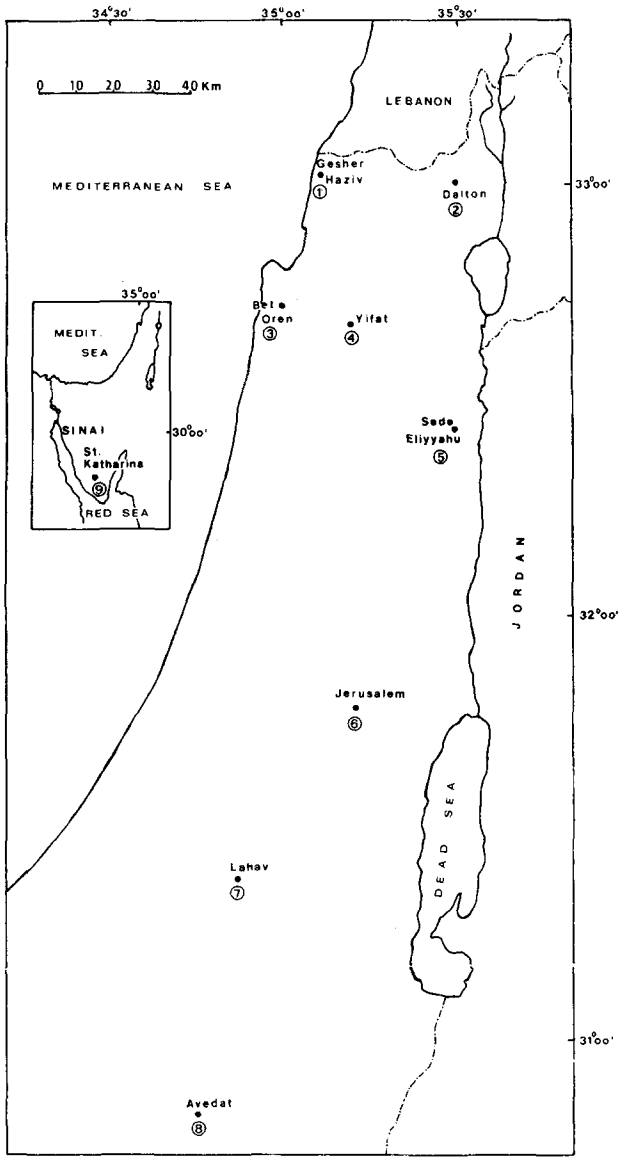

Fig. 1. Geographic distribution of sampling localities of *Agama stellio* in Israel and Sinai. Populations at Avedat (8) and St. Katharina (9) are *A. s. brachydactyla*, the subspecific status of the others is not known

The ratio of forefoot length divided by snout-vent length and hindfoot length divided by snout-vent length were calculated. Measurements and ratios of measurements were averaged separately for each sex.

Statistical Analysis

Stepwise multiple regression (Draper and Smith 1966) was used to determine whether environmental factors influence or are associated with the transformed allele frequencies of allozymes and the variation of morphological traits. In addition, Pearson's correlation coefficients were computed between allozymic and morphological variation. The variance within and between populations in morphology was tested by Kruskal-Wallis one-way analysis of variance.

Results

Pattern of Variation

Of the 25 loci examined, 11 (44%) were monomorphic in all nine populations studied (Mhd-1,2, Ldh-2, Idh-2, 6-Pdg-2, Pgm-1, To-1,2, Gdh, Lap, Alb). The allelic frequencies of the 14 polymorphic loci are given in Table 2. One locus (4%) was locally (≤ 2 populations) and weakly (frequency of rare allele $< 5\%$) polymorphic (Pgi); three loci (12%) were locally and strongly polymorphic (Me, Pept-1, Adh); three loci (12%) were regionally and in most cases weakly polymorphic (α Gpd, Ldh-1, Prot-1). At 23 loci, including 5 of the 7 regionally and strongly polymorphic ones, the same allele was either fixed or predominant in all nine populations. No fixation of alternative alleles was found either within or between the subspecies studied. However, eight loci (Me, Idh-1, 6-Pgd, Pgm-2, Pept-1,2, Adh, and Trf) displayed distinct genetic differentiation between populations. The general genetic pattern of the two subspecies was similar. Three unique alleles were found in at least one population of *A. stellio brachydactyla*: Me^a (only in Sinai); Pept-1^c (only in Sinai) and Adh^a (only in the Negev).

To assess the various kinds of allele distributions we followed the classification of Brown (1978). Each of the 51 alleles found in the 9 populations and two subspecies of *A. stellio* in Israel and Sinai was placed into one of the following classes (the site of each of the nine populations is considered an ecological region for this classification):

(i) *Common* (= at least one sample with frequency $\geq$ 10%): (a) widespread, occurrence in more than 2 regions (34 alleles or 34.6%); (b) sporadic, occurrence in 2 regions (1 allele or 3.8%); (c) localized, occurrence in only one region (1 allele or 3.8%).

(ii) *Rare* (= never occurs with frequency $\geq$ 10%); (d) widespread in more than one region (10 alleles or 38.5%); (e) localized in only one region (5 alleles or 19.2%).

The percentage of the variants was computed by subtracting the number of loci studied both from the number of alleles in class (i) (a), and from the total number of alleles. This adjustment standardized any differences in the number of invariant loci recorded. Note that about 27% of the variant alleles were either localized (or sporadic) but not widespread. This figure suggests that despite the predominance of the major alleles at 23 loci across the range, populations of *Agama stellio* in Israel and Sinai differed in their allelic content. It will be shown later that most loci including the localized fraction of alleles were associated with climate.

A summary of the genetic data on the nine populations of *Agama stellio* is given in Table 3. The following features were indicated: (a) Polymorphism, P, and heterozygosity H, were above the mean for 17 reptilians: (P = 0.22, s.d.

Table 2. Allele frequencies at 14 polymorphic loci of nine populations of *Agama stellio* in Israel and Sinai

Locus	Allele	Populations (Code number as in Table 1)									Mean ($\bar{P}$) (242)	Effective inbreeding coefficient (F_e)
		1 N = 27	2 30	3 28	4 33	5 30	6 29	7 11	8 30	9 24		
Me	a	0.00	0.00	0.00	0.00	0.00	0.00	–	0.00	0.02	0.00	0.02
	b	0.00	0.00	0.00	0.00	0.15	0.00	–	0.00	0.08	0.03	0.11
	c	1.00	1.00	1.00	1.00	0.85	1.00	–	1.00	0.90	0.97	0.12
aGpd	a	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.04
	b	1.00	1.00	0.93	0.97	0.90	1.00	1.00	1.00	0.92	0.97	0.05
	c	0.00	0.00	0.04	0.00	0.07	0.00	0.00	0.00	0.04	0.02	0.04
	d	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.02
Ldh-1	a	0.02	0.00	0.04	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.02
	b	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
	c	0.98	0.95	0.96	1.00	0.97	1.00	1.00	1.00	0.94	0.98	0.03
	d	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.06	0.01	0.04
Idh-1	a	0.20	0.03	0.09	0.09	0.09	0.02	0.00	0.03	0.00	0.07	0.07
	b	0.80	0.97	0.91	0.91	0.91	0.98	1.00	0.97	1.00	0.93	0.07
G6pd	a	0.22	0.10	0.39	0.27	0.19	0.28	–	0.37	0.31	0.26	0.05
	b	0.72	0.90	0.61	0.72	0.79	0.66	–	0.63	0.69	0.72	0.04
	c	0.06	0.00	0.00	0.02	0.02	0.06	–	0.00	0.00	0.02	0.03
6Pgd-1	a	0.44	0.38	0.29	0.00	0.28	0.00	0.00	0.02	0.00	0.17	0.26
	b	0.56	0.62	0.71	0.26	0.72	0.45	0.18	0.17	0.42	0.47	0.19
	c	0.00	0.00	0.00	0.74	0.00	0.55	0.82	0.82	0.58	0.36	0.63
Pgm-2	a	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03
	b	0.50	0.53	0.43	0.29	0.28	0.25	0.27	0.30	0.50	0.38	0.06
	c	0.50	0.47	0.53	0.57	0.72	0.69	0.68	0.70	0.50	0.59	0.04
	d	0.00	0.00	0.04	0.14	0.00	0.03	0.05	0.00	0.00	0.03	0.07
Pgi	a	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	b	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	0.02
Aat-1	a	0.04	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00	0.02	0.03
	b	0.96	1.00	1.00	0.95	0.92	0.95	1.00	0.97	0.94	0.96	0.03
	c	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.03	0.06	0.02	0.05
Pept-1	a	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.17	0.08	0.03	0.11
	b	1.00	1.00	0.98	1.00	1.00	1.00	1.00	0.83	0.88	0.97	0.12
	c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.05
Pept-2	a	0.24	0.20	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.06	0.18
	b	0.76	0.80	1.00	0.97	0.98	0.98	0.91	1.00	1.00	0.94	0.14
	c	0.00	0.00	0.00	0.00	0.02	0.02	0.09	0.00	0.00	0.01	0.11
Adh	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.01	0.11
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.99	0.11
Prot-1	a	0.92	1.00	0.96	0.98	0.98	0.96	1.00	0.97	0.96	0.97	0.02
	b	0.08	0.00	0.04	0.02	0.02	0.04	0.00	0.03	0.04	0.03	0.02
Trf	a	1.00	0.88	1.00	0.86	0.78	0.89	0.91	0.97	1.00	0.92	0.08
	b	0.00	0.12	0.00	0.14	0.22	0.11	0.09	0.03	0.00	0.08	0.08

Table 3. Genetic variation based on 25 loci in nine populations of *Agama stellio* in Israel

Population	Sample size (N)	Mean no. of alleles per locus (A)	Mean proportion of loci		
			Polymorphic per population (P)	Heterozygous per individual (H) Mean	S.E
1. Gesher Haziv	27	1.360	0.320	0.077	0.030
2. Dalton	30	1.320	0.280	0.056	0.025
3. Bet Oren	28	1.400	0.320	0.057	0.024
4. Yifat	33	1.480	0.400	0.055	0.025
5. Sede Eliyyahu	30	1.560	0.440	0.087	0.027
6. Jerusalem	29	1.440	0.320	0.053	0.027
7. Lahav[a]	11	1.217	0.174	0.043	0.031
8. Avedat	30	1.400	0.360	0.070	0.027
9. St. Katharina	24	1.480	0.360	0.070	0.026
	242				
Mean		1.406	0.330	0.065	0.024
Range		1.217-1.560	0.174-0.440	0.043-0.087	

[a] the low values of A and P at Lahav may relate to the small sample size

Table 4. Coefficients of genetic similarity (I, Upper figure), and genetic distance (D, Middle figure) with standard error (Lower figure) between 9 populations of *Agama stellio* in Israel

Population	1	2	3	4	5	6	7	8	9
Agama stellio subsp.									
1. Gesher Haziv		0.996	0.994	0.977	0.990	0.982	0.936	0.970	0.982
		0.004	0.006	0.024	0.010	0.018	0.066	0.031	0.019
		0.002	0.002	0.003	0.003	0.003	0.006	0.004	0.003
2. Dalton			0.993	0.977	0.993	0.983	0.934	0.969	0.983
			0.007	0.023	0.007	0.017	0.069	0.032	0.018
			0.003	0.003	0.003	0.003	0.006	0.004	0.003
3. Bet-Oren				0.979	0.994	0.987	0.941	0.974	0.987
				0.021	0.006	0.013	0.061	0.026	0.013
				0.003	0.002	0.003	0.006	0.003	0.003
4. Yifat					0.979	0.997	0.963	0.996	0.995
					0.022	0.003	0.037	0.004	0.005
					0.003	0.002	0.002	0.002	0.002
5. Sede Eliyyahu						0.987	0.943	0.971	0.983
						0.013	0.059	0.029	0.017
						0.003	0.006	0.004	0.004
6. Jerusalem							0.963	0.994	0.996
							0.038	0.006	0.004
							0.003	0.002	0.002
7. Lahav								0.964	0.963
								0.037	0.038
								0.002	0.004
A.s. brachydactyla									
8. Avedat									0.994
									0.006
									0.003
9. St. Katharina									

I: mean = 0.978 range = 0.934 – 0.997; D: mean = 0.022 range = 0.003 – 0.069

0.13 and H = 0.047, s.d. 0.023; Nevo, 1978: 143). Furthermore, P for eight of the nine samples was above the mean (0.248, s.d. 0.15) and H for two populations was above the mean (0.071, s.d. 0.04) for generalist vertebrates (Nevo 1978: 151). All population values were above the means of P and H of vertebrate specialists (0.15 and 0.037, respectively). P and H were significantly correlated ($r = 0.72$, $p < 0.05$). (b) All three genetic indices, mean number of alleles per locus, A; the proportion of polymorphic loci per population, P; and mean heterozygous loci per individual, H displayed geographic variation increasing, although non-significantly, with aridity. The genetic variation in *A. stellio brachydactyla* indexed by A (1.44), P (0.36) and H (0.07) was slightly higher than in *A. stellio* subsp. (A, P and H were 1.40, 0.32, and 0.06, respectively). This pattern was true also within the seven populations of *A. stellio* subsp. The steppe-inhabiting population of Sede Eliyyahu had the highest levels of genetic variation.

Coefficients of genetic similarity, I, and distance, D, the accumulated allele differences per locus with standard errors, were calculated for paired combinations of all nine populations based on the normalized identity of alleles between each two populations (Nei 1972). The results are given in Table 4. Mean I was 0.978, with a range of 0.934-0.997; mean D was 0.022, range 0.003-0.069. These estimates indicate high genetic similarity between populations. Even the most distant populations, belonging to the two different subspecies, such as Gesher-Haziv (*A. stellio* subsp.) in the northern coastal plain of Israel and St. Katharina (*A. stellio brachydactyla*) in southern Sinai, separated by 530 km, had an I of 0.982. The highest genetic distance D = 0.069 was between two populations: Dalton and Lahav of *A. stellio* subsp., rather than between the two subspecies.

Deviations from Hardy-Weinberg equilibrium owing to heterozygote paucity were found in the following populations and loci: Gesher-Haziv, Pept-2*; Dalton, Pgm-2**, Trf**; Yifat, Pgm-2**, Trf*; Sede Eliyyahu, Trf**; Jerusalem, Trf*; where * = $p < 0.05$ and ** = $p < 0.01$. In addition, Pept-2 in Yifat and Lahav, and Trf in Lahav appeared as single homozygotes of a rare allele, an unlikely event. In sum, Trf showed consistent paucity of heterozygotes in all 6 populations where it was polymorphic; Pept-2 in 3 populations, and Pgm-2 in 2 populations. Thus, out of 36 polymorphic cases (excepting the rare alleles) four deviations were significant at $p < 0.01$, and three deviations at $p < 0.05$.

The pattern of genetic variation within and between populations may be used to test alternative theories of selection and neutrality (Lewontin and Krakauer 1973). While natural selection operates differentially on each allele, or linked sequence of alleles, the breeding structure involving random genetic drift, inbreeding and migration, affects all alleles similarly. Differential genic variation among polymorphisms in space and time may thus provide evidence of selection in natural populations. We have tested the differential gene frequencies in *Agama stellio* as an indicator of natural selection. Significant heterogeneity between loci in their effective inbreeding coefficients, $F_e = S^2_p / \bar{p}(1 - \bar{p})$ was found for 35 alleles at 14 polymorphic loci (mean 0.087, range 0.02-0.63; Table 2). The F statistics for the ratio of observed and theoretical variances of F_e (0.0122/0.00189) was $F(25, \infty) = 6.41$, $p < 0.001$. This excess heterogeneity of F_e values can be taken as supportive evidence for selection, but as shown by Robertson (1975) and by Nei and Maruyama (1975) historical factors in the founding of populations can also lead to a significant heterogeneity among genes in the Lewontin-Krakauer test.

The means of morphometrics of body characters for the nine populations examined for allozymic variation are given separately in Table 5 for males and females. A statistically significant amount of variation between localities (in most cases $p < 0.01$) was found for all body characters. In general, both size and weight in females increase southwards. This trend was obvious when the four northern populations (no. 1-4) were compared with all other populations. Likewise, the southern subspecies, *Agama stellio brachydactyla*, was conspicuously longer and heavier than the northern subspecies, *A. stellio* subsp. (Kruskal-Wallis test: $p < 0.001$ for both weight and snout-vent length in males, and $p < 0.05$ for snout-vent length in females, for the comparisons between the subspecies).

Environmental Predictors of Genic Variation

A test for the best predictors of P,H, 12 polymorphic loci and 4 body measurements was conducted by stepwise multiple regression analysis (Nie et al. 1975), employing the above mentioned variables as dependent variables, and geographic and climatic factors as independent variables. The results are given in Table 6, first for geographic, then for climatic variables. Relative humidity (Hu) or evaporation (Ev) proved to be the first climatic variable explaining significantly the variation in some allozymes (i.e., Idh-1^b, 6-Pgd-$1^{a,c}$, Pept-$1^{a,b}$) as well as in some body characters (weight, snout-vent length, and the ratio of hindfoot to snout-vent length). In some cases (i.e., 6-Pgd-1^a) when a second climatic variable was added progressively the explanation increased as follows: HuEv, $R^2 = 0.72$, $p < 0.05$; and in Pgm-2^c a two-variable combination of temperature and humidity (TmHu) accounted significantly, ($p < 0.05$) for 77% of the variation, whereas the single variable Tm was not significant.

Loci and alleles may be classified into those affected by water factors including relative humidity, evaporation and rainfall (i.e., Ldh-1^a, Idh-1^b, 6-Pgd-1^a, Aat-1^c, Pept-$1^{a,b}$); those affected by temperature (i.e., Ldh-1^d, Trf^a); and finally, those affected by a combination of tempera-

Table 5. Morphometrics of 9 populations of *Agama stellio* in Israel

Males

Population	Sample Size (N)	Weight (gm)		Length (mm)						Relationships of length			
				Snout vent		Hind foot		Fore foot		HF/SV[a]		FF/SV[b]	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
1. Gesher Haziv	13	33.7	21.5	83.8	24.9	26.9	6.2	17.4	4.5	0.329	0.040	0.211	0.029
2. Dalton	20	60.5	13.1	105.1	8.5	31.0	2.3	19.8	1.7	0.296	0.016	0.188	0.011
3. Bet Oren	17	57.5	22.7	104.3	14.9	31.7	4.3	19.6	2.3	0.306	0.035	0.189	0.019
4. Yifat	17	48.5	11.7	93.3	11.4	30.2	3.1	18.7	2.2	0.326	0.031	0.201	0.018
5. Sede Eliyyahu	15	56.1	13.5	105.6	10.0	31.3	2.1	19.7	0.7	0.298	0.022	0.188	0.016
6. Jerusalem	17	61.3	10.9	106.9	6.7	34.4	1.8	21.7	1.3	0.322	0.022	0.204	0.015
7. Lahav	7	50.4	15.6	107.4	11.6	30.6	3.1	20.3	2.1	0.287	0.039	0.190	0.017
8. Avedat	17	73.0	25.6	114.5	14.8	29.0	2.2	18.1	2.0	0.257	0.034	0.160	0.020
9. Santa Katharina	15	67.4	22.6	117.7	14.8	31.5	2.7	19.9	1.9	0.270	0.023	0.171	0.013
Total	138												
Mean		57.5	18.2	104.4	13.6	30.8	3.2	19.5	2.2	0.299	0.029	0.189	0.018
Range		33.7-73.0		83.8-117.7		26.9-34.4		17.3-21.7		0.257-0.329		0.160-0.211	
H[c]		33.579***		44.755***		39.583***		36.174***		56.946***		58.559***	

Females

Population	Sample Size (N)	Weight (gm)		Length (mm)						Relationships of length			
				Snout vent		Hind foot		Fore foot		HF/SV[a]		FF/SV	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
1. Gesher-Haziv	13	29.9	15.9	85.3	17.1	26.0	3.9	17.6	3.1	0.303	0.021	0.208	0.020
2. Dalton	10	38.3	16.4	95.6	16.7	26.7	3.8	18.3	2.6	0.282	0.027	0.194	0.020
3. Bet Oren	11	37.7	19.0	91.9	18.6	27.4	3.6	17.6	2.5	0.303	0.034	0.194	0.018
4. Yifat	12	35.9	12.6	87.3	12.3	27.3	2.9	16.1	1.9	0.315	0.024	0.186	0.023
5. Sede Eliyyahu	15	41.1	9.6	101.2	10.4	27.8	2.2	17.7	1.2	0.277	0.030	0.177	0.017
6. Jerusalem	12	51.8	14.3	105.5	8.2	32.2	2.3	20.6	1.2	0.306	0.026	0.196	0.018
7. Lahav	3	62.9	37.6	104.0	19.7	31.3	6.4	20.3	2.9	0.301	0.015	0.197	0.012
8. Avedat	13	47.3	24.3	100.4	17.3	25.8	4.0	16.0	2.9	0.258	0.017	0.159	0.013
9. Santa Katharina	9	51.3	14.8	111.4	10.5	29.2	1.9	19.1	2.1	0.263	0.014	0.171	0.009
Total	98												
Mean		42.1	17.1	97.2	14.5	27.8	3.3	17.9	2.3	0.290	0.025	0.186	0.018
Range		29.9-62.9		85.3-111.4		25.8-32.2		16.0-20.6		0.258-0.315		0.159-0.208	
H[c]		17.515*		26.379***		27.234***		32.653***		43.770***		43.116***	

* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$

[a] Hind-foot/snout-vent; [b]Fore-foot/snout-vent; [c]Statistic of Kruskal-Wallis one way analysis of variance; distributed as $\chi^2_{(8)}$

Table 6. Coefficients of multiple determination (R^2) employing as dependent variables *P*,*H*, the frequencies[a] of alleles at 12 polymorphic loci and as independent variables (A) geographic and (B) climatic factors[b]

Variable	Allele	Stepwise model					
		A. Geographic			B. Climatic		
P		Al	AlLt	AlLtLn	Ev	EvTa	EvTaTm
		0.01	0.04	0.07	0.08	0.25	0.38
H		Al	AlLt		Rn	RnHu	RnHuEv
		0.01	0.10		0.10	0.32	0.55
Me[d]	(c)[3]	Lt	LtAl		Rn	RnEv	RnEvHu
		0.06	0.22		0.12	0.56	0.58
αGpd	(b)	Ln			Tj	TjTm	TjTmTa
		0.05			0.07	0.32	0.37
	(c)	Lt			Tj	TjTm	TjTmRn
		0.06			0.06	0.21	0.26
Ldh-1	(a)	Lt	LtLn	LtLnAl	Hu	HuRn	HuRnEv
		0.21	0.36	0.40	0.42	0.64*	0.78*
	(c)	Al	AlLt	AlLtLn	Tj	TjHu	TjHuRn
		0.13	0.34	0.35	0.32	0.54	0.74
	(d)	Al	AlLn	AlLnLt	Tj	TjTa	TjTaRn
		0.40	0.46	0.50	0.66**	0.76*	0.84*
Idh-1	(b)	Lt	LtLn	LtLnAl	Hu	HuEv	HuEvRn
		0.55*	0.63	0.67	0.52*	0.60	0.67
G6pd[d]	(a)	Ln	LnLt	LnLtAl	Ev	EvHu	EvHuRn
		0.04	0.05	0.06	0.09	0.40	0.43
	(b)	Ln	LnAl	LnAlLt	Tj	TjTa	TjTaTm
		0.06	0.25	0.28	0.08	0.24	0.43
	(c)	Ln	LnAl		Ev	EvRn	EvRnTm
		0.18	0.19		0.21	0.24	0.26
6Pgd-1	(a)	Lt	LtAl	LtAlLn	Hu	HuEv	HuEvRn
		0.41	0.45	0.47	0.45*	0.72*	0.75
	(b)	Lt	LtAl	LtAlLn	Hu	HuTj	HuTjTa
		0.18	0.34	0.36	0.29	0.48	0.59
	(c)	Lt	LtAl	LtAlLn	Hu	HuEv	HuEvTj
		0.35	0.45	0.46	0.45*	0.63	0.71
Pgm-2	(b)	Al	AlLt	AlLtLn	Tm	TmHu	TmHuEv
		0.15	0.50	0.65	0.37	0.71*	0.81*
	(c)	Al	AlLt	AlLtLn	Tm	TmHu	TmHuTj
		0.13	0.66*	0.85*	0.31	0.77*	0.78*
	(d)	Lt	LtLn	LtLnAl	Ev	EvHu	EvHuRn
		0.06	0.08	0.09	0.22	0.46	0.52
Aat-1	(a)	Lt	LtLn		Ev	EvHu	EvHuRn
		0.11	0.12		0.22	0.29	0.32
	(b)	Lt	LtAl	LtAlLn	Rn	RnTa	RnTaTm
		0.08	0.20	0.24	0.21	0.23	0.35
	(c)	Lt	LtLn	LtLnAl	Rn	RnHu	RnHuEv
		0.33	0.46	0.52	0.62*	0.64*	0.73

Table 6. (Continued).

Variable	Allele	Stepwise model					
		A. Geographic			B. Climatic		
Pept-1	(a)	Ln 0.47*	LnLt 0.48	LnLtAl 0.51	Ev 0.70**	EvHu 0.82**	EvHuTj 0.83*
	(b)	Ln 0.56*	LnLt 0.59	LnLtAl 0.60	Ev 0.74**	EvHu 0.83**	EvHuTa 0.86*
Pept-2	(a)	Lt 0.29	LtAl 0.45	LtAlLn 0.48	Rn 0.31	RnTm 0.39	RnTmTa 0.45
	(b)	Lt 0.32	LtAl 0.35		Ev 0.35	EvTm 0.42	EvTmTa 0.48
	(c)	Al 0.04	AlLt 0.22	AlLtLn 0.31	Tm 0.11	TmRn 0.21	TmRnEv 0.52
Prot-1	(a)	Ln 0.07	LnLt 0.17	LnLtAl 0.20	Ta 0.05	TaTj 0.18	TaTjTm 0.24
Trf	(a)	Ln 0.44	LnLt 0.83**	LnLtAl 0.83*	Ta 0.43	TaTj 0.67*	TaTjHu 0.81*
Weight males (gm)		Lt 0.36	LtLn 0.52	LtLnAl 0.58	Ev 0.67	EvRn 0.79**	EvRnTm 0.83*
Snout-vent length males (mm)		Lt 0.55*	LtLn 0.70*	LtLnAl 0.72	Ev 0.65**	EvTj 0.66*	EvTjTm 0.71
Hindfoot length males (mm)		Al 0.11	AlLn 0.42	AlLnLt 0.51	Tj 0.12	TjTm 0.40	TjTmTa 0.51
Forefoot length males (mm)		Al 0.11	AlLn 0.38	AlLnLt 0.50	Tj 0.11	TjTm 0.28	TjTmTa 0.35
Hindfoot/Snout-vent (males)		Lt 0.49*	LtAl 0.53	LtAlLn 0.53	Ev 0.73**	EvTj 0.75*	EvTjTa 0.77*
Forefoot/Snout-vent (males)		Lt 0.46	LtAl 0.48	LtAlLn 0.48	Ev 0.80**	EvHu 0.84**	EvHuTm 0.86*
Weight females (gm)		Lt 0.40	LtLn 0.52	LtLnAl 0.53	Hu 0.38	HuEv 0.55	HuEvTj 0.56
Snout-vent length (females)		Lt 0.64*	LtLn 0.86**	LtLnAl 0.87*	Hu 0.62*	HuTj 0.64*	HuTjEv 0.65
Hindfoot length females (mm)		Lt 0.11	LtLn 0.22	LtLnAl 0.24	Hu 0.05	HuEv 0.49	HuEvTa 0.53
Forefoot length females (mm)		Al 0.13	AlLn 0.20	AlLnLt 0.23	Tm 0.10	TmEv 0.21	TmEvHu 0.41
Hindfoot/Snout-vent (females)		Lt 0.35	LtLn 0.42		Ev 0.79**	EvHu 0.88**	EvHuRn 0.89**
Forefoot/Snout-vent (females)		Lt 0.34	LtLn 0.45	LtLnAl 0.51	Ev 0.78**	EvTa 0.93**	EvTaRn 0.94**

[a] Angular transformations were conducted on all gene frequencies.

[b] Abbreviations of environmental variables: Ln = longitude, Lt = latitude, Al = altitude, Tm = mean annual temperature, Tj = mean January temperature, Ta = mean August temperature, Rn = mean annual rainfall, Hu = mean humidity at 14:00, Ev = mean annual evaporation.

[c] The small letters appearing in parenthesis following the name codes of the enzymes, represent specific alleles in order of mobility.

[d] Data for Lahav are missing.

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

Table 7. Correlation coefficients (r) of morphological variables with *P,H* and allozymic variables of *Agama stellio*

		P	*H*	*Idh-1*^{b}	*G-6pd*^{b}	*6Pgd*^{a}	*Pgm-2*^{c}	*Pept-2*^{b}	*Trf*^{a}
Males	W	0.16	−0.03	0.77*	0.12	−0.47	−0.25	0.64(*)	0.03
	SV	−0.04	−0.09	0.87**	−0.17	−0.53	0.31	0.63(*)	0.04
	FF	−0.26	−0.46	0.67*	−0.22	−0.38	0.29	0.37	−0.33
	HF	0.01	−0.37	0.62(*)	0.01	−0.39	0.27	0.54	−0.34
Females	W	−0.50	−0.45	0.83**	−0.73*	−0.72*	0.51	0.40	−0.03
	SV	−0.12	−0.05	0.82**	−0.28	−0.57	0.37	0.49	−0.06
	FF	−0.60	−0.42	0.47	−0.45	−0.20	0.11	−0.06	−0.03
	HF	−0.42	−0.52	0.57	−0.55	−0.56	0.38	0.29	−0.19

For symbolism see text and Table 5
(*)P = 0.05; *P < 0.05; **P < 0.01

ture and humidity (i.e., Pgm-2^{a,c}). Noteworthy, Ldh-1^a was affected by water factors whereas Ldh-1^d was affected by temperature, although their frequencies were very low and larger samples are needed to substantiate this presumable differentiation of alleles within a locus. No combination of geographic or ecologic variables explained significantly the variation in either P or H. However, more than half of the geographic variation in H was explained by factors affecting water availability similar to the pattern found in the aforementioned five loci. Thus, climatic factors, primarily of water availability and secondarily of the temperature regime, proved the best predictors of allozyme variants at seven polymorphic loci as well as of body characters.

Finally, significant correlations were found between some allozymic frequencies (i.e., Idh-1^b, G-6Pd^b, 6-Pgd-1, Pept.-2^b) and body variables (i.e., weight and snout-vent lengths) (Table 7).

Discussion

The pattern of genetic and morphological variation in *A. stellio* in Israel and Sinai appears to be influenced by climate. The most important factors affecting the genetic variation of *A. stellio* appear to be those related to water availability followed by temperature. Thus, both allozymic and morphological variation, which are to some extent intercorrelated, appear to be at least partly adaptive. Although *A. stellio* is a reptile, water availability seems to influence population differentiation more than temperature.

The Adaptive Nature of Genic Variation in A. stellio

Climate appears to be a major determinant of population genetic and morphological structures in *A. stellio* in Israel and Sinai on the following grounds: (a) geographic variation in Ldh-1, Idh-1, 6-Pgd-1, Aat-1, Pgm-2, Pept-1, Trf, body weight and size can be predicted by climatic factors, primarily water availability and secondarily by temperature (Table 6); (b) significant deviations from Hardy-Weinberg expectations involve the following loci: G-6Pd, 6-Pgd-1, Pept-2, and Trf; this observation suggests selection; (c) significant excess heterogeneity of effective inbreeding coefficient, F_e, can be taken as supportive evidence of selection; both uniform and diversifying selections were suggested by the low and high F_e values observed in the data (Table 2; Lewontin and Krakauer test 1973). Although the test has been challenged (Ewens and Feldman 1976; Nei and Maruyama 1975; Robertson 1975), the heterogeneous pattern of F_e values is in accord with other lines of evidence suggesting selection. (d) A correlation was found between allozymes and morphological variables (Table 7), both sets of variables being affected by climate.

The genetic structure found in *A. stellio* in this study includes two patterns: (a) some allozymes and morphological variables differentiate sharply between populations and are also associated with climate, and (b) some of the polymorphic loci displayed substantial similarity between populations regardless of climate. Both patterns may be explained simultaneously by selection, diversifying and unifying, respectively. In contrast, gene flow alone can not be invoked to explain both patterns. If gene flow was effective, it could cause similarity but not differentiation, which in that case would necessitate strong selection. If gene flow was insignificant, then the regional similarity probably resulted from unifying selection. Finally, since the effective breeding size of *A. stellio* population seems to be large (hundreds to thousands of individuals in appropriate habitats), the genetic pattern found rules out random factors as a likely explanation for population differentiation. Climatic selection of allozymes is therefore indicated at least for part of the allozyme polymorphisms observed and thus negates neutral theory in these cases.

The values of A, P, and H found in *Agama stellio* are certainly an underestimate. The omission of the commonly variable esterases and phosphatases, because of difficulties in reliable scoring, resulted in relatively lower than the real values of the genetic indices. The habitat generalist *A. stellio* may therefore potentially harbor higher levels of P and H than those found. The latter were shown to be relatively high for reptiles in general and as expected for habitat-generalist vertebrates (Nevo 1978), presumably as an adaptation to ecologically heterogeneous environments.

Adaptive Variation in Size of Agama stellio

Despite the apparent adaptability of thermal and desiccation sensitivities of reptiles, little evidence documents their intraspecific genetic and size variations over moisture and temperature gradients in natural populations (Hertz 1977, 1980; Hertz et al. 1979 and included references). Evidence is still largely lacking as to whether moisture, temperature or both are causing differentiation of population genetic structure in reptiles, and whether these operate in concert.

The evidence presented in this paper suggests that moisture rather than temperature may be the prime selective factor influencing in concert both allelic as well as morphological variation in *A. stellio* in the Near East (Table 6). Second, genic and morphological variation are, at least partly, significantly correlated (Table 7). Notably, size increased with and was predictable by aridity (Tables 5 and 6). Size increase with aridity is presumably an adaptation to arid climates, as is also true for frogs (Nevo 1973) and was demonstrated for toads along the same southwards transect of increasing aridity in Israel (Nevo 1972). The larger size of *A. s. brachydactyla* as compared to *A. stellio* subsp., appears to be a watersaving adaptation to arid environments. Since evaporative water loss is a function of surface to volume ratio in frogs (Farell and MacMahon 1969 and their references; Nevo 1973) as well as in lizards (Hertz et al. 1979, Hertz 1981) larger individuals lose relatively less water and their vital time limit is significantly larger than that of small ones. Large lizards appear, therefore, superior in dry habitats, hot or cold, because of their relatively low surface to volume ratio and correspondingly low rates of water loss.

The rich thermoregulatory behaviours of *A. stellio* involves individual selection of habitat, posture, retreat, basking frequency and time of activity (Hertz et al. 1981). This apparently minimizes the importance of temperature as a selective factor among *A. stellio* populations in the Near East and elevates desiccation as a prime selective agent of population genetic and morphological differentiation.

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