Aldolase DNA polymorphism in subterranean mole-rats: genetic differentiation and environmental correlates

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We analysed the genetic diversity and environmental correlates of the aldolase A and B genes by means of restriction endonucleases (DNA RFLP analysis), in the four chromosomal species ($2n = 52$, 54, 58 and 60) of the actively speciating subterranean mole-rats of the *Spalax ehrenbergi* superspecies in Israel. The results indicated that: (i) both aldolase genes are highly polymorphic; (ii) Fragment frequencies and fragment profiles display geographical patterns and significant ecological correlates; (iii) discriminant analysis largely succeeded in separating the four chromosomal species on the basis of variation of aldolase RFLPs.

INTRODUCTION

The glycolytic enzyme fructose-1, 6-bisphosphate aldolase (aldolase; EC 4.1.2.13) is a tetrameric protein composed of a specific combination of different subunits A (muscle type), B (liver type) and C (brain type) (Penhoet *et al.*, 1967). These isozymes have the same molecular size ($M_r = 40,000$ each), differ in antigenic properties and in net charges, and have been identified and characterized in many mammals (Horecker *et al.*, 1972; Schapira *et al.*, 1975; Lebherz and Rutter, 1969). The genes encoding these subunits appear to be located separately on the chromosomes. Nevertheless, they seem to be closely related structurally: hence they are thought to have originated by gene duplication during evolution (Penhoet *et al.*, 1967; Benfield *et al.*, 1979, Lai, 1975).

The structure, genomic organization, and expression of the rat aldolase isozyme genes have been the subject of intensive studies in tissue specific expression, regulation, and evolution (reviewed in Hori *et al.*, 1987; see also Joh *et al.*, 1985, 1986; Sakakibara *et al.*, 1985*a*, *b*; Mukai *et al.*, 1984, 1986; Tsutsumi *et al.*, 1983, 1984, 1985 and gene mapping in humans in Kukita *et al.*, 1987). However, genetic differentiation and environmental correlates of aldolases in natural populations are largely unknown. Our objective in this research was to study geographic variation of aldolase in the four chromosomal species of mole-rats of the *Spalax ehrenbergi* complex across a transect of aridity stress in Israel.

Subterranean mole-rats of the *Spalax ehrenbergi* superspecies in Israel represent a case of active speciation and adaptive radiation suitable for investigating aldolase polymorphisms. Several reviews highlighting different perspectives describe the multidisciplinary studies on the *S. ehrenbergi* complex both in terms of adaptation and speciation (Nevo 1979, 1982, 1985*a*, *b*, 1986*a*, *b*, 1988*a*, 1989, 1990). The complex comprises four chromosomal species ($2n = 52$, 54, 58, 60) displaying progressive final stages of chromosomal speciation. Hybrid zones, progressively becoming narrower northward, separate the chromosomal species (Nevo and Bar-El 1976), and along with differential interspecific assortative mating, this provides a measure of the stage of speciation of each species (Nevo 1985*a*).

The adaptive radiation of the *S. ehrenbergi* superspecies in Israel, from the early Pleistocene to recent times, is closely associated with fossoriality, increasing aridity, progressive deforestation and savannization. It is associated with distinct climatic diversity in both the Mediterranean and steppic climatic regimes: $2n = 52$ in the cool-humid north; $2n = 54$ in the cool-dry north-

Table 1 The ecogeographical background of 13 populations of mole-rats, *Spalax ehrenbergi*, in Israel, and sample size of aldolase DNA study

Chromosomal species (2n)	Population	Sample size (N)	Ecogeographical																					Demographic	
			Lon	Lat	Alt	Tm	Ta	Tj	Td	Tdd	Rn	Rd	Hu 14	Hu an	Dw	Sh	Th	Trd	Ev	So	Pl	Cov	Den	Ter
52	Maalot	4	35·27	33·00	500	16·8	23·2	8·1	15·0	8·8	785	53	53	64	58	63	20	0	152	1	3	85	31	64
	Kerem Ben Zimra	4	35·47	33·03	700	16·5	23·3	7·0	16·3	8·5	650	59	49	60	47	85	0	0	152	$	3	88	40	64
	Qiryat Shemona	4	35·57	33·22	187	19·0	26·0	9·5	16·5	10·3	655	60	49	61	42	65	0	50	151	$	5	90	23	54
54	El Al	4	35·75	32·80	370	18·7	26·0	9·6	16·4	11·1	464	52	45	57	50	24	−30	130	170	3	4	100	39	55
	Quneitra	3	35·83	33·12	950	14·9	22·7	6·1	16·6	9·6	857	65	49	61	60	71	—	21	150	3	3	40	22	53
	Mt. Hermon	4	35·73	33·30	1300	12·4	20·1	3·0	17·1	5·0	1450	65	60	61	60	80	—	0	150	1	1	80	32	58
58	Kabri	4	35·15	33·02	100	20·0	26·0	10·5	13·5	10·1	600	50	58	66	62	35	−8	0	145	$	5	75	30	61
	Zippori	4	35·28	32·74	250	18·5	26·0	10·4	15·4	12·0	500	53	48	63	70	50	−12	120	160	2	4	75	26	51
	Afiq	2	35·70	32·77	325	18·8	26·7	10·5	16·2	11·0	460	51	44	57	50	24	−30	130	170	3	4	70	17	57
60	Anza	4	35·22	32·35	400	18·0	24·5	9·6	14·9	9·6	630	46	47	62	55	65	0	15	161	2	3	65	22	83
	Lahav	4	34·87	31·38	400	18·8	26·0	11·0	14·6	12·0	303	33	45	58	55	60	−45	100	168	5	2	20	15	104
	Jerusalem	3	35·23	31·78	700	17·5	23·9	8·7	15·2	9·0	500	42	51	62	42	104	−15	0	158	1	3	45	24	121
	Sede Boqer	2	34·78	30·87	450	19·0	26·0	9·0	15·0	13·0	91	15	36	53	70	—	−50	100	168	5	6	—	—	—
Total		46																						

Climatic values were taken from the Atlas of Israel (1970) and from multiple-year records of the Meteorological Service of Israel
Symbols of variables:

(i) Geographical:　Lon = Longitude, in decimals
　　　　　　　　　Lat = Latitude, in decimals
　　　　　　　　　Alt = Altitude, in metres

(ii) Temperature:　Tm = Mean annual temperature
　　　　　　　　　Ta = Mean August temperature
　　　　　　　　　Tj = Mean January temperature
　　　　　　　　　Td = Mean seasonal temperature difference
　　　　　　　　　Tdd = Mean day-night temperature difference
　　　　　　　　　Trd = Mean number of tropical days
　　　　　　　　　Sh = Mean number of Sharav days, i.e., hot and dry days
　　　　　　　　　Ev = Mean annual evaporation

(iii) Water availability: Dw = Mean number of dew nights in summer
　　　　　　　　　Rn = Mean annual rainfall, in mm
　　　　　　　　　Rd = Mean number of rainy days
　　　　　　　　　Hu14 = Mean humidity at 14:00
　　　　　　　　　Huan = Mean annual humidity
　　　　　　　　　Th = Thornthwaite's moisture index

(iv) Edaphic:　　So = Soil type:　1 = Terra Rosa
　　　　　　　　　　　　　　　　2 = Rendzina
　　　　　　　　　　　　　　　　3 = Basalt
　　　　　　　　　　　　　　　　4 = Alluvium
　　　　　　　　　　　　　　　　5 = Loess

(v) Biotic:　　Pl = Climax plant community (mole-rats live only in open
　　　　　　　　　　　spaces or secondary habitats:
　　　　　　　　　　　1 = Tragacantic batha
　　　　　　　　　　　2 = Marginal batha
　　　　　　　　　　　3 = Liveoak maquies of *Quercus calliprinos* and
　　　　　　　　　　　　　Pistacia palaestina
　　　　　　　　　　　4 = *Quercus ithaburensis-Styrax officinalis* Part Forest
　　　　　　　　　　　5 = *Ceratonia siliqua-Pistacia lentiscus* Park Forest
　　　　　　　　　　　6 = *Hammada scoparia-Atriplex halimus* Desert Vegetation
　　　　　　　Cov = Plant cover (percentage)
　　　　　　　Den = Mole-rat population density (animals per 10,000 m)
　　　　　　　Ter = Territory size (square metres)

†Soil types in: Karem Ben Zimra −1, 3; Qiryat Shemona −1, 4; Kabri −1, 2, 4.

east; $2n = 58$ in the warm-humid centre; finally, $2n = 60$ in the warm-dry south. Noteworthy, aridity increases primarily southward ($2n = 52 \rightarrow 58 \rightarrow 60$), but also, though less so, eastward ($2n = 52 \rightarrow 54$). The biology of mole rats is correlated at all levels of organization with the climatic trends of aridity stress.

Allozymic variation has been studied in this superspecies at 30 gene loci (Nevo and Shaw, 1972; Nevo and Cleve, 1978; Nevo, Filippucci and Holmes, in preparation). These studies revealed low levels of allozyme polymorphism and heterozygosity.

Interestingly, all three aldolase isozymes tested electrophoretically, at the protein level proved monomorphic throughout Israel. Here we present evidence indicating that at the DNA level two of the aldolase genes, A (muscle type) and B (liver type) appear very polymorphic. Furthermore, these aldolase DNA polymorphisms appear to be correlated with, and predictable by, the environment, especially aridity stress.

MATERIAL AND METHODS

Sampling

We tested aldolase DNA polymorphisms in 46 mole-rats comprising 13 populations and four chromosomal species of the *Spalax ehrenbergi* superspecies in Israel. A list of these and their ecogeographical background appears in table 1, and distribution in fig. 1. Sede Boqer is a desert isolate involving only about 100 individuals (Nevo, 1989), hence we analysed only two individuals. The analysis of two DNAs out of the four Afiq animals failed, hence the remaining $N = 2$. Live animals were brought from their natural populations and dissected in the laboratory. The tissues were immediately frozen in liquid nitrogen and then stored at $-80°C$.

Southern Blot Analysis

Chromosomal DNA was isolated from kidney tissue of the different species, using standard methods (Holland, 1983). Each of the DNAs (10 μg) was digested separately with two restriction enzymes, *Eco*RI and *Hind*III, electrophoresed on a 1 per cent agarose gel, and blotted onto GeneScreen membrane (NEN) according to the protocol of the supplier. Prehybridization and hybridization were performed in a solution containing $4 \times SET$ (0.6 M NaCl, 4 mM EDTA, 80 mM Tris-HCl, pH 7.8), $1 \times$ Denhardt's solution (0.02
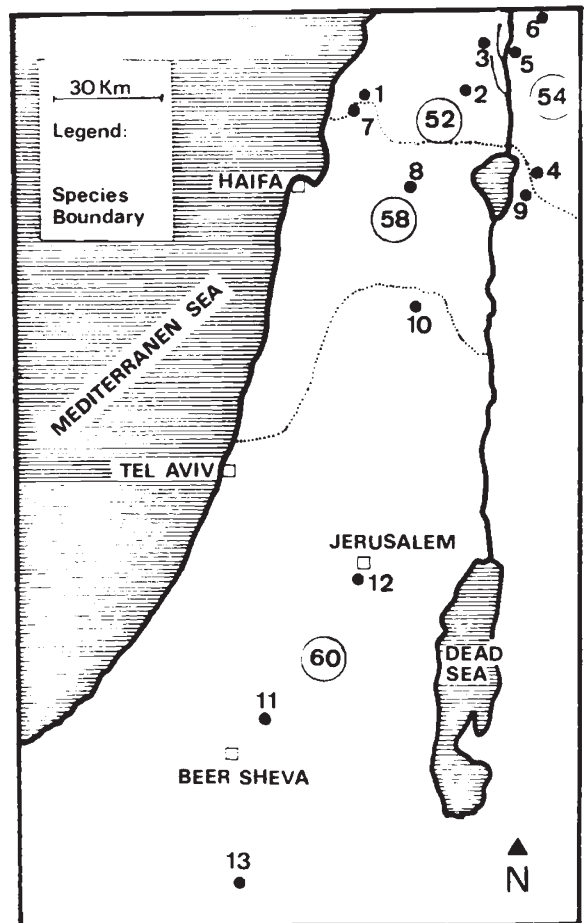

Figure 1 Geographic distribution of the chromosome species and the tested populations of the *Spalax ehrenbergi* superspecies in Israel. $2n = 52$; (1) Maalot, (2) Kerem Ben Zimra, (3) Qiryat Shemona; $2n = 54$: (4) El-Al; (5) Quneitra, (6) Hermon; $2n = 58$: (7) Kabri, (8) Zippori, (9) Afiq; $2n = 60$: (10) Anza, (11) Lahav, (12) Jerusalem, (13) Sede Boqer (a very small desert isolate in the northern Negev desert).

per cent BSA, 0.02 per cent polyvinylpyrrolidone, 0.02 per cent Ficol), 1 per cent SDS, 200 μg/ml denatured salmon sperm DNA at 62°C for 12 to 16 h and for 48 h, respectively. Two kinds of aldolase cDNA were used as probes: a mixture of pRAAM83 and pRAAM103, cDNA clones of rat aldolase A mRNA (Joh *et al.* 1985), and pRAB1710, a cDNA of rat aldolase B mRNA (Tsutsumi *et al.*, 1984). They were labelled to 1×10^9 cpm/μg by the procedure of Feinberg and Vogelstein (1983), heat-denatured, and used at a concentration of 10^6 cpm/ml. After hybridization, filters were rinsed four times with $2 \times SET$ containing 1 per cent SDS at room temperature for 5 min,

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Table 2 RFLPs and fragment profiles of aldolase A and B in four chromosomal species of *Spalax ehrenbergii* digested by two restriction enzymes *Eco*RI and *Hind*III

Population	Animal no	Phenotype of aldolase A	Phenotype of aldolase B	Probe A *Eco*RI	Probe A *Hind*III	Probe B *Eco*RI	Probe B *Hind*III
2n = 52							
Maalot	47	a	w	+ + + + + + + + + + + + +	+ + + + + + + + + + +	+ + + − − + +	+ + + + + +
	48	a	x	+ + + − + + + + + + + + +	+ + + + + + + + + + +	+ + + − + + +	+ − + + + +
	49	o-p?		+ + − − + + + + + + + +	+ + + + + + + + + +	+ − − + +	− + − + +
	50	p	j	+ + − − + + + + + + + + +	+ + + + + + + + + + +	+ + + − + +	− + − + + +
Kerem Ben Zimra	43	a	k	+ + + + + + + + + + + +	+ + + + + + + + + +	+ + + − + + +	+ + + + + +
	44	a	u	+ + + + + + + + + + + +	+ + + + + + + + + +	+ + + − + + +	+ + + − + +
	45	b	v	+ + + + + + + + + + + + +	− + + + + + + + + + +	+ + + − + +	+ + + + + +
	46	n	k	− − + + + + + + + + + +	− − + + + + + + + +	+ + + − + +	+ + + − + +
Qiryat Shemona	39	a	z	+ + + + + + + + + + + +	+ + + + + + + + + +	− + + − + +	+ + + + + +
	40	a	d	+ + + + + + + + + + + +	+ + + + + + + + + +	− + + − + +	+ + + + + +
	41	m	t	− + + + + + + + + + + +	− + + + + + + + + +	− + + − + +	+ + + − + +
	42	n	t	− − + + + + + + + + + +	− − + + + + + + + +	− + + − + +	+ + + − − +
2n = 54								
Hermon	27	a-b?	j	+ + + + + + + + + + + +	? + + + + + + + + +	+ + + − + +	− + + + + +
	28	a	k	+ + + + + + + + + + + +	+ + + + + + + + + +	+ + + − + +	+ + + + + +
	29	m	j	− − + + + + + + + + + +	− + + + + + + + + +	+ + + − + +	− + + + + +
	30	n	j	− − + + + + + + + + + +	− + + + + + + + + +	+ + + − + +	− + + + + +
Quneitra	31	a	q	+ + + + + + + + + + + +	+ + + + + + + + + +	+ + + − + +	+ + + + + +
	32	o	k	+ − + + + + + + + + + +	− + + + + + + + + +	− + + − + +	+ + + − + +
	33	o	k	+ − + + + + + + + + + +	− + + + + + + + + +	+ + + − + +	+ + + + + +
El Al	35	p	r	+ + − + + + + + + + + +	+ + + + + + + + + +	+ + + − + +	+ + + + + +
	36	p	s	+ + − + + + + + + + + +	+ + + + + + + + +	− + + − + +	− + + − + +
	37	q	m	− − + + + + + + + + + +	+ + + + + + + + + +	− + + − + +	− + + − + −
	38	n	y	− − − + + + + + + + + +	− + + + + + + + +	− + + − + +	− + + − − −

Table (rotated in original; reconstructed below). Genotypes are listed as rows (population, number, and the two allele codes); fragments are the columns, with their sizes given in the bottom "Fragments size (KB):" row. Symbols: + = present, − = absent, ? = unscored fragment.

2n = 58

Population	No.	code 1	code 2
Kabri	15	a	k
	16	a	l
	17	k	m
	18	i–r?	f
Zippori	19	l	c
	20	k	n
	21	j–k?	o
	22	l–s?	b
Afiq	23		
	24		
	25	j–k?	p
	26	o–p?	q

2n = 60

Population	No.	code 1	code 2
Anza	1	a	a
	2	o–p?	b
	3	b	c
	4	o–p?	d
Jerusalem	5	c	e
	6		
	7	d	f
	8	e	g
Lahav	9	f	h
	10	g	d
	11	h	i
	12	i	j
Sede Boqer	13	a	k
	14	j	j

Fragments size (KB):

? = Unscored fragment.

washed twice with the same solution at 56°C for 1 h, and autoradiographed at −70°C.

Statistical analysis

We used SPSS-x (1986) statistical programs for uni- and multivariate analyses. We designted fragments by referring first to the gene probe (A or B), then to the restriction enzyme (E for *Eco*RI and H for *Hind*III) followed by the kilobase of the fragment; for example AE 2·1 = Probe A, digested by *Eco*RI and 2·1 kilobase in size.

RESULTS

Patterns of variation

The results appear in tables 2–6 and in fig. 2. We have found scorable polymorphisms with both probes (*Ald*-A and B). For *Ald*-A we found 12 fragments with both *Eco*RI and *Hind*III. For *Ald*-B we found six and seven fragments with *Eco*RI and *Hind*III, respectively. The fragment distribution appears in table 2. Several patterns are indicated, for each probe and for each restriction enzyme. For probe A, digested with *Eco*RI, seven of 12 fragments were polymorphic, producing 13 different"fragment profiles" (which are essentially different phenotypes of the aldolase A genotypes). In probe A, digested with *Hind*III, only one of 12 fragments was polymorphic, producing two different fragment profiles. For probe B, digested by *Eco*RI, four of six fragments were polymorphic, producing eight different fragment profiles; and with *Hind*III three of seven fragments were polymorphic, producing eight different fragment profiles.

Fragment frequencies of the 15 polymorphic fragments across the four chromosomal species appear in table 3. Several geographic patterns are apparent. The first pattern displayed high frequency or fixation in the two northern species ($2n = 52, 54$) (*e.g.*, Probe-A, *Eco*RI, 2·1 kb; Probe B, *Eco*RI, 4·9 kb). The second pattern displayed high frequency or fixation in the southern species ($2n = 58, 60$) (*e.g.*, Probe-A *Eco*RI, 20 kb). The third pattern displayed highest frequency in $2n = 58$, decreasing both southward and northward (*e.g.*, Probe B, *Eco*RI, 3·3 kb). The fourth pattern displayed highest frequency in the two mesic species ($2n = 52, 58$) and the lowest frequency in the two xeric species ($2n = 54, 60$) (*e.g.*, Probe B, *Hind*III, 6·2 kb; an opposite trend is displayed by the 5·0 kb fragment). The fifth pattern displayed a decrease in frequency in the southern species, $2n =$

60 (*e.g.*, Probe A, *Eco*RI, 4·2, 2·3, 1·0 kb). The sixth pattern was opposed to the fifth pattern and displayed decrease in frequency in $2n = 52$ (*e.g.*, Probe B, *Eco*RI, 1·7 kb). Patterns 4–6 are not significant, but similar patterns in other molecular systems were significant (Nevo, 1990), hence we suspect that they may display biological reality. However, their confirmation clearly needs additional testing.

Geographical trends of phenotypic fragment profile diversity

The profiles generated for each of the aldolase probes by the two restriction enzymes have been designated alphabetically from profile A to S in aldolase A, and A to Z in aldolase B (see alphabetical designations in table 2). Overall, we defined 17–19 phenotypic profiles in aldolase A, and 26 in aldolase B. We calculated for each probe in each of the four chromosomal species, the average number of animals for each of the profiles in table 2. Consequently, we obtained a profile diversity measure for each species, representing the average number of profiles per ten animals. The profile diversities for each of the four chromosomal species were 6·2, 6·0, 7·5 and 8·5 for $2n = 52, 54, 58$ and 60 respectively. The two southern species ($2n = 58, 60$) displayed higher aldolase DNA diversities than the two northern ones ($2n = 52, 54$).

Discriminant analysis

We conducted stepwise discriminant analysis (SPSS-x, 1986) based on individual variation across all fragments, and the results are given in table 4 and fig. 3. The program succeeded in separating the four chromosomal species, based on nine chosen fragments (table 4(a)) by two significant discriminant functions (table 4(b)). Pairwise comparison indicates significant differences between the four species (table 4(c)), except between $2n = 52$ and $2n = 54$. Thirty-two of the 43 individuals (excluding the Sede Boqer isolate) analysed (74·4 per cent) were correctly classified in their respective species (table 4(d)).

Spearman correlations with environment

Spearman rank correlations were conducted among the frequency of the DNA fragments, and between the polymorphic fragments and environment (table 5). First, Spearman intercorrelations between fragments are relatively low, mostly non-
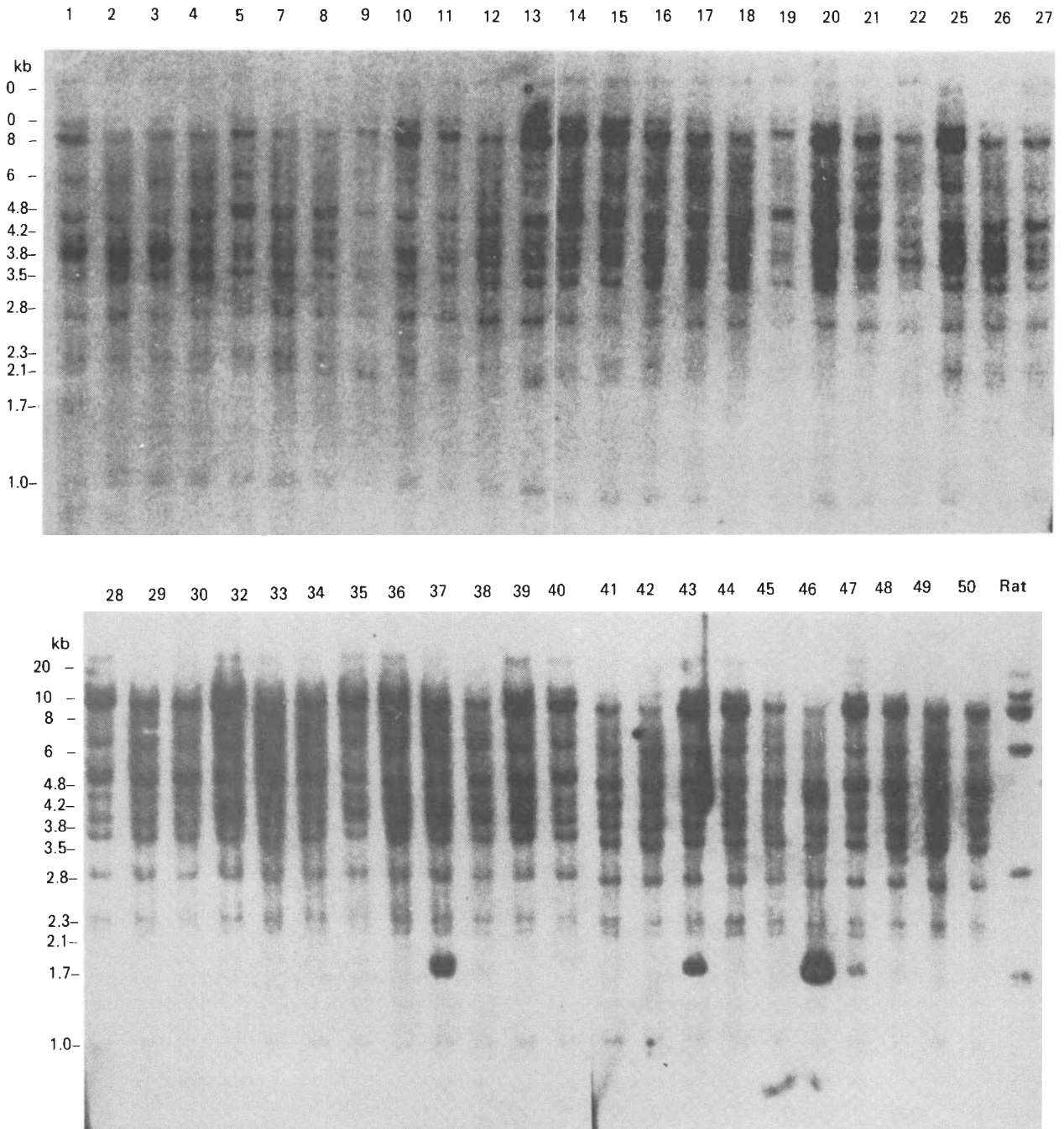
Figure 2 Allelic fragments of aldolase A and B genes resulting from digestion with *Eco*RI.

significant, and ranging between 0·01 to 0·48. Second, Spearman rank correlations were calculated between the population means of fragment profile frequencies defined as phenotypes in table 2 with representative ecogeographical variables that appear in table 1. Six fragment profiles out of the 15 analysed showed no correlation at all with any of the environmental variables. Six fragments (AE 2·3, AE 2·1, AE 20·0, BE 8·0, BE 4·9 and BE 3·3) displayed many correlations with temperature
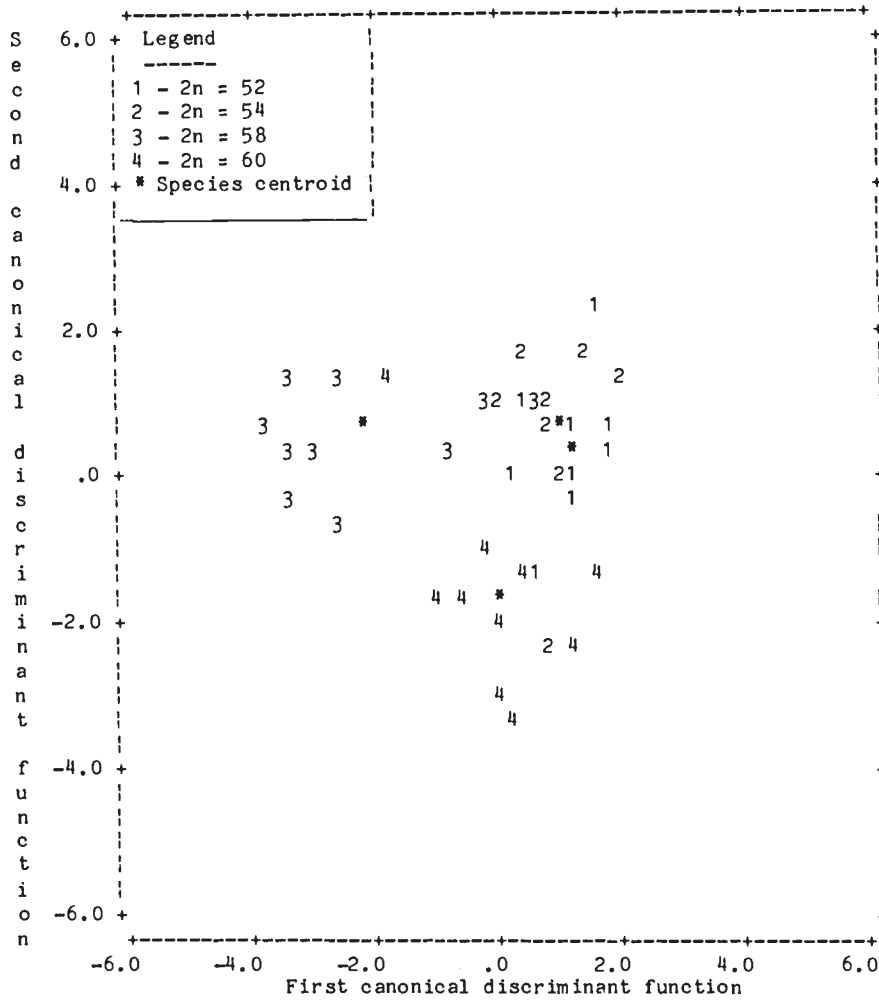
Figure 3 Discriminant analysis of the four chromosomal species of *S. ehrenbergi* based on nine fragments of aldolase A and B.

and rainfall variables (see table 5 for details). These significant correlations, with a high proportion of the climatic variables comprising 40 per cent of the aldolase polymorphisms, are above those expected by chance.

Multiple regression analysis

In order to explain the geographical patterning of the aldolase polymorphisms we used 12 population means of the fragment profile frequencies or phenotypes (see table 2) as dependent variables, and ecogeographical variables as independent ones, in a stepwise multiple regression analysis (MR). Two or three primarily climatic combina-

tions plus plant coverage, explain a substantial amount of the aldolase RFLPs, in both genes (table 6). For example, 79 per cent of the variance of AE 4·2 is significantly explained by a three-variable combination involving number of rainy days, altitude and mean annual temperature; 74 per cent of the variance of BE 4·9, is significantly explained by daily temperature difference (Tdd), number of dew nights in summer (Dw) and evaporation (Ev). Likewise, 62 per cent of the variance of AE 20·0, is significantly explained by a two-variable combination of seasonal temperature difference (Td) and plant cover (Cov). In general, temperature and water availability variables played a major role in explaining (directly or indirectly) the variance in fragment frequencies of both aldolase loci A and B.

Table 3 Frequencies of aldolase RFLPs of four chromosomal species of *Spalax ehrenbergi* digested by two restriction enzymes *Eco*RI and *Hind*III

| Probe | KB | $2n =$ | Frequency | | | | Significance | Comments | |
			52	54	58	60		Highest	Lowest
*Eco*RI A	20·0		0·75	0·64	1·00	1·00	*	South	
	10·0		0·67	0·45	0·60	0·69			$2n = 54$
	6·0		1·00	1·00	0·80	0·77		North	
	4·2		1·00	1·00	1·00	0·85			$2n = 60$
	2·3		1·00	1·00	1·00	0·85			$2n = 60$
	2·1		1·00	1·00	0·30	0·85	***	North	
	1·0		1·00	1·00	1·00	0·92			$2n = 60$
*Hind*III A	24·0		0·64	0·50	0·60	0·64			$2n = 54$
*Eco*RI B	8·0		0·64	0·73	0·30	0·31	†	North	
	4·9		1·00	0·73	0·40	0·62	*	North	
	3·3		0·0	0·18	0·60	0·15	**	$2n = 58$	
	1·7		0·91	1·00	1·00	1·00			$2n = 52$
*Hind*III B	6·2		0·82	0·55	0·80	0·46		Mesic	
	5·0		0·55	0·73	0·60	0·69		Xeric	
	2·4		0·64	1·00	0·70	0·62		$2n = 54$	

Significance by Kruskal–Wallis one-way ANOVA.
  * $P < 0.05$.
 ** $P < 0.01$.
*** $P < 0.001$.
  † $P < 0.10$.

DISCUSSION

The glycolytic enzyme fructose-1,6-bisphosphate aldolase, widely distributed in almost all organisms, catalyzes the following reversible reaction, yielding two different triose phosphates: fructose-1,6-bisphosphate = dihydroxyacetone phosphate + D-glyceraldehyde-3-phosphate. Variation in aldolase activity is achieved by various mechanisms. *First*, there are three isozymic forms, aldolase A (muscle type), B (liver type) and C (brain type), that evolved through gene duplication (Scandalios, 1983), displaying tissue and developmental stage specificities (Lebherz and Rutter, 1969; Horecker *et al.*, 1972). Dramatic changes in the expression of aldolase A occur during ontogenesis and carcinogenesis of rat liver (Gracy *et al.*, 1970; Horecker *et al.*, 1972). *Second*, it has been found recently that three aldolase A mRNA species with different molecular sizes expressed tissue-specifically in rat (Joh *et al.*, 1985; Mukai *et al.*, 1986). Furthermore, additional study of aldolase A indicated that a single gene of rat aldolase A has multiple promoters allowing the tissue-specific generation of three species of mRNA by alternative usage of two exons encoding the 5' noncoding regions of the mRNAs (Joh *et al.*, 1986). This is in contrast to the pattern of rat and chicken mRNA is transcribed (Tsutsumi *et al.*, 1985; Burgess and Penhoet, 1985). This distinction between aldolase A multiple promoters generating three mRNA forms notwithstanding, many features, especially the exon-intron structure of rat aldolase A and B gene, are essentially similar. This indicates that both genes have evolved from a common ancestral gene.

To the variation found hitherto in aldolases based on gene duplications, tissue and ontogenetic specificities, and the multiple promoters to the same gene producing multiple mRNA forms, we now add aldolase polymorphisms (RFLPs) in both aldolase A and B of the mole-rat, *Spalax ehrenbergi.* This discovery is particularly interesting in view of (i) the monomorphism reported earlier for the three isozymes (aldolase A, B, C) in mole rats of the *Spalax ehrenbergi* superspecies (Nevo and Shaw, 1972), (ii) the environmental correlates of the RFLPs, and (iii) polymorphism in the Sede Boqer desert isolate. We will now discuss these three aspects in turn.

Monomorphism of isozyme proteins and polymorphism of DNA

Out of 30 isozymic genes analysed in the *S. ehrenbergi* superspecies, 14 gene loci proved polymor-

Table 4 Discriminant analysis of aldolase RFLPs of four chromosomal species of *Spalax ehrenbergi* in Israel

A. Variables chosen:

		Summary table		
Step	Entered/removed	Vars in	Wilks' lambda	Significance ($P<$)
1	AE2·1	1	0·46211	0·0001
2	BE8·0	2	0·38269	0·0001
3	AE20·0	3	0·29808	0·0001
4	BH6·2	4	0·24818	0·0001
5	BH2·4	5	0·21084	0·0001
6	BE3·3	6	0·17287	0·0001
7	AE2·3	7	0·15169	0·0001
8	BH5·0	8	0·13549	0·0001
9	BE4·9	9	0·12336	0·0001

B. Significance of discriminant functions

Canonical discriminant function	Eigenvalue	Canonical correlation	After function	Wilks' lambda	Chi-squared	df	Significance
1	1·9212	0·811	0	0·1234	74·290	27	0·0001
2	1·0062	0·708	1	0·3604	36·234	16	0·0027
3	0·3832	0·526	2	0·7229	11·517	7	0·1176

C. Pairwise comparison

Species:		1. $2n = 52$	2. $2n = 54$	3. $2n = 58$
2. $2n = 54$	$F =$	1·3696		
	$P =$	0·2439		
3. $2n = 58$	$F =$	5·5785	5·1379	
	$P =$	0·0001	0·0003	
4. $2n = 60$	$F =$	3·0010	3·3148	4·5821
	$P =$	0·0108	0·0061	0·0007

Each F statistic has 10 and 30 degrees of freedom.

D. Classification results

Actual species	No. of cases	Predicted species membership			
		1	2	3	4
1. $2n = 52$	11	5	5	0	1
		45·5%	45·5%	0·0%	9·1%
2. $2n = 54$	11	1	9	0	1
		9·1%	81·8%	0·0%	9·1%
3. $2n = 58$	10	0	2	8	0
		0·0%	20·0%	80·0%	0·0%
4. $2n = 60$	11	0	0	1	10
		0·0%	0·0%	9·1%	90·9%

74·4 per cent of the cases were correctly classified.

phic (with low level of polymorphism), whereas 16, including the three aldolase genes, proved monomorphic. The discrepancy between the isozyme monomorphism and extensive DNA polymorphism of aldolase A and B reported here is explicable on several grounds. First, routine gel electrophoresis reveals only some, not all, of the variation involved. Second, the redundancy of the code permits changes in codons without a change in amino acid sequence. Third, mutations in introns are not expressed in the translated message. Fourth, mutations at the flanking regions of the gene are not expressed in gene product, but they may affect regulation.

Table 5 Spearman (r_s) rank correlations between aldolase RFLPs and ecological and climatic factors, in 12 populations of mole rats, *Spalax ehrenbergi*, in Israel. The Sede Boqer desert isolate was excluded due to its isolation. Variables are defined in table 1 legend. Only significant correlations were included in the table ($r_s > 0\cdot30$)

Variables	KB	Aldolase A RFLPs by *EcoRI*					Aldolase B RFLPs by *EcoRI*			*HindIII*	
		20·0	6·0	4·2	2·3	2·1	8·0	4·9	3·3	5·0	2·4
Biotic	Plant cover (Cov)	−0·453**	—	—	0·346*	—	—	—	—	—	—
	Density (Dev)	−0·349*	—	—	0·345*	—	0·306*	—	—	—	—
Geographic	Longitude (Lon)	−0·378*	—	—	0·345*	—	—	—	—	—	—
	Latitude (Lat)	−0·368*	0·402*	0·315*	0·345*	—	0·429**	0·381*	—	—	—
	Altitude (Alt)	—	—	—	—	0·453**	0·515***	—	−0·353*	—	0·332*
Temperature	January (Tj)	—	—	—	−0·346*	−0·491***	−0·443**	−0·415**	0·322*	—	—
	Daily difference (Tdd)	—	—	—	−0·312*	−0·481***	−0·422***	−0·407**	—	—	—
	No. of Sharav days (Sh)	—	—	—	—	0·434**	—	0·346*	−0·335*	—	—
Water availability	Rainfall (Rn)	—	0·306*	—	0·345*	0·359*	0·427**	0·404**	−0·370*	—	—
	Rainy days (Rd)	−0·325*	—	0·316*	0·346*	—	0·397***	0·324*	—	—	—
	Midday humidity (Hu14)	—	—	—	—	—	0·350*	0·308*	—	—	—
	Thornwaite's Index (Th)	—	—	—	0·378*	—	—	0·345*	—	—	—

Levels of significance: * $P<0\cdot05$; ** $P<0\cdot01$; *** $P<0\cdot001$.

Table 6 Coefficients of multiple regressions (R^2) of aldolase DNA fragments frequencies (as the dependent variables) and climatic and ecological variables (as independent variables) in 12 populations of *Spalax ehrenbergi*, (for abbreviations see table 1)

Fragment	Stepwise model		
AE 20·0	Td 0·434*	Td Cov 0·619*	
AE 10·0	Hu14 0·212	Hu14 Dw 0·338	Hu14 Dw Rd 0·450
AE 6·0	Rd 0·236		
AE 4·2	Rd 0·501**	Rd Alt 0·706**	Rd Alt Tm 0·792**
AE 2·3	Cov 0·441*	Cov Rd 0·571*	
AE 2·1	Dw 0·352*	Dw Ta 0·717**	
AE 1·0	Cov 0·441*	Cov Rd 0·571*	
BE 8·0	Tj 0·428*		
BE 4·9	Tdd 0·512**	Tdd Dw 0·655**	Tdd Dw Ev 0·739**
BE 3·3	Tm 0·206†		
BH 6·2	Ta 0·152	Ta Rd 0·586*	Ta Rd Cov 0·749**
BH 5·0	Tm 0·339*	Tm Dw 0·514*	
BH 2·4	Dw 0·207	Dw Td 0·451†	

Level of significance: *** $P<0.001$; ** $P<0.01$; * $P<0.05$; † $P<0.10$.

Environmental correlates of aldolase RFLPs

We hypothesize that the RFLPs found in aldolase A and B, may, at least partly, function in regulation and gene expression. The evidence supporting this hypothesis comes primarily from the environmental correlates (tables 5 and 6), and from the fragment profile diversities (table 2). The climatic correlates of six fragments, primarily with temperature and rainfall variation, and their high level and significant explanation in the multiple regression analysis, are impressive. In particular, it appears that while nine fragments show no, or few significant environmental correlates, hence might be the result of chance alone, the six fragments significantly correlated with climate may suggest deterministic climatic selection on these variants, either directly or indirectly.

The substantial importance of climatic selection, particularly by aridity stress on allozymic variation, has been demonstrated in 38 species of plants, invertebrates and vertebrates across Israel (Nevo, 1983, 1990). In particular 21 of the 38 species which were sampled across the entire mesic-xeric gradient, displayed positive correlation between heterozygosity of individual loci, as well as overall heterozygosity, with climatic heterogeneity and unpredictability (Nevo and Beiles, 1988*a*). Recently, we have also demonstrated climatic selection between ribosomal DNA nontranscribed spacer polymorphism in *S. ehrenbergi* across the same transect of increasing aridity stress analyzed here for aldolase A and B (Nevo and Beiles, 1988*b*).

Remarkably, aldolase fragment profile diversity increases from the northern ($2n = 52, 54$) to southern ($2n = 58, 60$) species. This pattern parallels that of allozymic heterozygosity discussed above. Aldolase DNA diversity, as well as allozymic heterozygosity are positively correlated with rainfall variation and unpredictability; both increase southward from the Mediterranean to the steppic and desert regions. This positive correlation between genetic and environmental variation, *i.e.*, the niche width variation hypothesis (Van Valen, 1965), substantiates the importance of ecological heterogeneity in population genetic differentiation of aldolases.

It is plausible to hypothesize that the level of genetic diversity may primarily relate to the buffering and homeostatic effect of heterozygosity of metabolically vital enzymes related to the energetic balance (Koehn, 1990). Protein heterozygosity is apparently associated with developmental homeostasis and physiological function. The fitness of individuals in ecologically uncertain environments appears at least partially related to the level of heterozygosity. This pattern which corroborates theoretical predictions (Turelli and Ginzburg, 1983) may also be true for DNA polymorphism as demonstrated here for aldolase DNA diversity. Testing of additional DNA polymorphisms is needed before any generality may be drawn.

Polymorphism in the small desert Sede Boqer isolate

The Sede Boqer desert isolate in the Northern Negev may have been disjuncted more than 10,000 years ago from the main range of $2n = 60$, and its current population size is very small, in the order of 100 individuals (Nevo, 1989). Theoretically, this population which underwent a distinct bottleneck-

ing during the late Pleistocene and Holocene times, and is therefore expected to be highly inbred, should have been highly homozygous for most genotypic and phenotypic systems (Nei *et al.*, 1975). By contrast, the results obtained here for aldolases, indicate polymorphism in aldolases with a sample of two individuals only! Additional genetic polymorphisms were found in the Sede Boqer isolate for other allozymes (esterases), mtDNA, and myosin heavy chain, Per-homologous sequence and chromosomal polymorphisms (reviewed in Nevo 1989 and 1990). Recently, we have also found polymorphism in the Sede Boqer isolate in the major histocompatibility complex (MHC) (Ben Shlomo *et al.*, 1988). In addition phenotypic variances have been also found in the Sede Boqer isolate in morphological, physiological and behavioural parameters. These genotypic polymorphisms and phenotypic diversities are probably the result of climatic balancing selection (Nevo, 1988b, 1989). We suggest that direct climatic selection also affects the evolution of aldolase polymorphisms at the DNA level. This hypothesis is testable indirectly by the correlative methodology and directly by biochemical and physiological analyses.

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