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Associations Between Isozyme Phenotypes and Environment in the Slender Wild Oat (*Avena barbata*) in Israel*

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Summary. Collections from 31 populations of *A. barbata* from diverse habitats in Israel were assayed electrophoretically for seven enzyme systems. Phenotype frequencies were scored in nine enzyme zones, probably representing 27 loci, to determine isozyme variability within and among populations. Many different isozyme phenotypes were found in all of the populations; also the array of isozyme phenotypes found in each population differed distinctly from that found in each other population. Overlays of phenotypic frequencies on map locations showed that isozyme variability is distributed in mosaic patterns not related to geographical distance. Principal-component and multiple-regression analyses revealed that temperature- and moisture-related variables are significantly correlated with particular isozyme phenotypes. Further, the mosaic patterns of isozyme variation were found to correspond closely to mosaic patterns of the habitat. This structuring of the genetic variability into multilocus combinations was attributed to the combined effects of directional and diversifying selection. Comparisons of patterns and extent of genetic variation in Israel and California led to the conclusion that the evolution of 'ecotypes,' each adapted to a specific habitat and marked by a particular set of enzyme alleles, has proceeded further in Israel, where *A. barbata* is endemic, than in California, where it is a recent introduction.

Key words: *Avena barbata* – Isozyme phenotypes – Environment – Evolution of ecotypes.

Introduction

The slender wild oat, *Avena barbata* Brot. (2N = 4X = 28), which was introduced to the New World during the period of Spanish exploration and colonization, has become a prominent component of grassland and oak savannah communities in California (Robbins 1940). Botanical history shows that this species was brought to California from the Western Mediterranean Basin in a large number independent introductions extending over a period of more than a century; it is therefore not surprising that the California gene pool has been found to be very similar to its ancestral Western Mediterranean gene pool in allozyme composition (Clegg and Allard 1972). However, genotypes and patterns of differentiation have developed in California that have not been found in the Mediterranean area (Clegg and Allard 1972; Hamrick and Allard 1972; Allard et al. 1972). Populations in the semi-arid warm-summer region of California are fixed for a specific combination of alleles governing electrophoretically detectable variants and morphological polymorphisms, whereas populations in the most mesic parts of the cool-summer region are usually polymorphic but often contain high frequencies, or are even fixed, for a genotype with a balanced opposite set of alleles. Populations occupying intermediate habitats in the environmentally diverse cool-summer region are highly polymorphic and in these polymorphic populations the frequencies of the 'xeric' and 'mesic' complexes of alleles are correlated with environment, the xeric complex becoming more frequent as the habitat becomes increasingly arid, and vice versa. It seems most unlikely that these patterns can be due to founder effects, or to genetic drift, because the California gene pool received a near random sample of genetic variability from the Western Mediterranean, because of frequent and massive migrations over both short and long distances in California as a result of agricultural activities, and because the numbers of individuals within populations are very large. Also the finding that striking local genetic differentiation correlated with microgeographical variations in aridity involving areas one square meter or smaller occurs within polymorphic populations (Allard et al. 1972; Miller 1977;

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Allard et al. 1978; Hamrick unpubl. data) is inconsistent with founder effects or drift.

In addition to the loci governing enzyme and morphological polymorphisms, several quantitative characters have been studied both in nature and in common-garden experiments (Hamrick and Allard 1972 1975). These studies show that homozygous xeric and homozygous mesic enzyme genotypes taken from the same heterozygous polymorphic population differ in stature, time to maturity, tillering capacity, outcrossing rate, and other quantitative characters and hence that loci governing these quantitative characters are correlated components of the genotypes marked by the enzyme loci. The distribution of *A. barbata* in typical grassland and oak savanna habitats in California can therefore be accounted for in large part on the basis of two 'ecotypes' that can be identified by allozymic Mendelian formulas, although additional allozyme complexes have been found in locations that are environmentally different from the grassland and oak savanna habitats in which the species typically occurs in California (Allard et al. 1972; Miller 1977).

The California results raise three obvious questions: (1) what are the patterns of variability for electrophoretically detectable variants in other localities where *A. barbata* grows; (2) do similar associations between electrophoretically detectable variants and environment occur in other localities; and (3) do the joint distribution patterns of isozyme polymorphisms and factors of the environment provide clues to the processes by which this species has met problems of adaptation to a heterogeneous environment? This paper reports the results of an electrophoretic analysis of populations collected over the spectrum of environments in Israel, where *A. barbata* is endemic. The results show that there is extensive isozyme variation both within and among populations in Israel and that temperature- and moisture-related variables are correlated with particular isozyme phenotypes. The results also show that the isozyme phenotypes are distributed in mosaic patterns that correspond to and are predictable on the basis of environmental mosaicism. Comparisons with the California results led to the conclusion that the evolution of 'ecotypes' marked by specific sets of enzyme alleles has proceeded further in Israel than in the colonial populations of California.

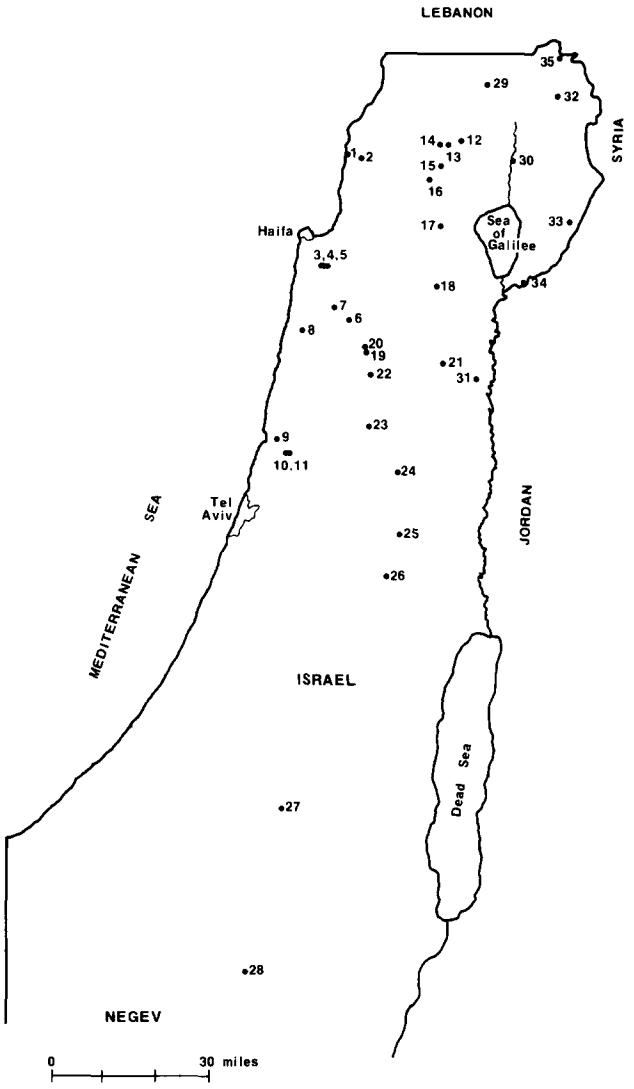

Fig. 1. Geographical locations of the 35 *Avena barbata* populations (including 2N = 14 and 2N = 28 collections) in Israel

Materials and Methods

The materials of this study were derived from 35 populations of *A. barbata* representing the distributional range of the species in Israel (Fig. 1 and Table 2). Sites were chosen for collection that appeared to have been protected from grazing or other activities for long periods of time. In each population panicles were taken from 55 randomly chosen adult plants. Enzyme extracts were obtained from leaf tissue of 21-30 day-old (two-leaf stage) greenhouse-grown seedlings. The leaf material from each plant was crushed using a plexiglass rod and the crude squeezate was absorbed in a filter paper wick (6 X 6 mm, Beckman 319329). The wicks were then inserted into a cut 4.0 cm from the cathodal end of horizontal starch gels. Each gel accommodated 19 samples (the center sample was a standard). The gels were prepared using either a tris-citric acid buffer system (Shaw and Koen 1968), or a histidine tris-citrate system (details given in Kahler et al., in preparation). The enzyme systems examined using the tris-citric acid gel buffer system were esterase (EST), acid phosphatase (AP), peroxidase (PX), and glutamate oxalate transaminase (GOT). The histidine buffer system was used for 6-phosphogluconate dehydrogenase (6-PGDH), malate dehydrogenase (MDH), and phosphoglucoisomerase (PGI). Staining procedures closely followed those described by Shaw and Koen (1968), Shaw and Prasad (1970) and Brewer (1970). Zones of activity were defined for each of these seven enzyme systems and all isozymes that appeared in a zone were considered in identifying isozyme phenotypes. For three enzymes, 6-PGDH, MDH and GOT, all isozymes that appeared on

Table 1. Isozymes observed in 35 populations of *A. barbata* in Israel. Schematic representations of the isozyme bands are given in Figures 2-4. Phenotypes marked with an asterisk were observed only in diploids

Phenotype numbers	Enzyme zone								
	6-Pgdh	Mdh	Pgi-I	Est-I	Got	aPx-V	cPx-I	AP-IV	AP-V
1	1a, 3a, 5b, 10a	2a, 7a, 11a, 13a	3a, 4b, 5b, 6a	9a	2a, 3a, 5b, 6a, 7a, 10a, 13a	4a, 6a	1a, 4c, 5a	1a, 2c, 5b, 6a	1a
2	1a, 3a, 5b, 12a	2b, 5a, 7b, 9b, 11b, 13a	3b, 4b, 5a, 6a	9a, 11a	2a, 3a, 5b, 6a, 7a, 9a, 12a	2a, 5a	1a, 4a, 5c	2b, 5a, 6b	3a
3	1a, 3a, 5b, 10d, 11a, 12c	8a, 10a, 12a, 13a	1a, 2a	11a	2a, 3a, 5b, 6a, 7a, 8a, 11a	2a, 5b, 6b	1a, 5b	3a, 6b	2a
4	1a, 3a, 5b, 6c, 7a, 10b	3a, 6a, 7c, 9c, 11c, 13a	4a	12a, 15a, 16b	2b, 3b, 4a, 10a, 13a	1a, 3a, 6b	1a, 4b	2b, 4a, 6b	4a
5	1a, 3a, 5b, 6b, 7a, 10c	7a, 9a, 11a, 13a	3b, 4b	6b, 9a	2b, 3b, 4a, 9a, 12a	6c, 7a	1a, 4b, 5b	1a, 2a*	Null
6	1a, 3a, 5a, 6d, 7a, 10b	1a, 2a, 4a, 7d, 9a, 11a, 13a	5a, 6a, 7a, 8a	4c, 9a	5a, 10a, 13a*	1a, 3a, 5c, 6b	2a, 3a		
7	1a, 3a, 5b, 6d, 7a, 9b, 10b, 12b			1a, 2c, 9a	5a, 9a, 12a*				
8	1a, 3a, 5b, 9a, 10d			9a, 15a	1a, 10a, 13a*				
9	1a, 3a, 5c, 7b, 9a, 10b			4a, 15a					
10	2a, 10b			2a					
11	2a, 12a			2a, 9a					
12	2a, 10d, 11a, 12c			2a, 15a					
13	2a, 6a			4a, 5a					
14	2a, 6c, 7a, 10b			3a, 15a					
15	2a, 6a, 7a, 10b			1a, 9b, 15c					
16	2a, 9a, 10e			4b, 9b					
17	4a, 10b			4c, 9b, 15a					
18	3b, 8a, 10e*			1a, 15a, 17b					
19				9b, 11a, 15a					
20				1a, 9b, 11a					
21				4a, 8a					
22				7a, 9c, 15c, 16a					
23				6a, 9c, 15a					
24				9c, 15c, 17a					
25				4d, 15c, 17a					
26				4c, 8b, 9c, 15a					
27				4c, 8b, 9b					
28				8a, 14a					
29				2b, 4a*					
30				1a, 9c					
31				8a, 13a*					
32				1a, 10a					
33				18a					

the gels were utilized in identifying phenotypes. However, for EST and PGI only isozymes that appeared in a single zone of activity were used while for both PX and AP classification of phenotypes was based on isozymes in two distinct zones. The zones chosen for analysis were ones that could be scored unambiguously in all populations. Thus, isozyme phenotypes were defined in terms of bands appearing in a total of nine different zones. These zones and the phenotypes for each zone are described in detail in the results section. *A. barbata* from Israel usually produces more isozymes per zone than materials from California and we have not been able to interpret the more complicated banding patterns of the Israeli populations fully on the basis of formal genetic studies that have been done on the California materials (Marshall and Allard 1969; Clegg and Allard 1972; and unpublished). Consequently, population analyses in this paper are based on phenotypic rather than genotypic frequencies.

For reasons of statistical efficiency a variant of Brown's (1975) Experimental Design II was adopted for estimating the extent of isozyme phenotype variability within and between populations. Two seedlings were grown from each adult plant collected in nature, one of which was assayed for enzymes requiring the tris-citric acid buffer system and the other for enzymes requiring the histidine system. Populations found to be phenotypically monomorphic for any isozyme system, as determined by the single-seedling assays, were not assayed further for that system. However, for those populations that were found to be phenotypically polymorphic for any enzyme system, additional progeny were grown (all available seeds up to nine progeny per adult plant) and assayed for the polymorphic enzyme systems. In these additional plantings two wicks were prepared from each seedling and all seedlings were assayed for the enzymes requiring the two buffer systems. The number of adults represented in progeny tests varied from 29 to 54 per population.

Chromosome Assays

Because the diploid 'species' or 'races', *A. wiestii* and *A. hirtula* (2N = 14), sometimes occur in mixed stands with the *A. barbata*, and these putative diploid ancestors of *A. barbata* are morphologically similar to the tetraploid (Ladizinski and Zohary 1968; Rajhathy and Thomas 1974), chromosome numbers were determined for all families in all of the 35 populations studied. Chromosome counts were made (by M.K) from root-tip preparations in 1,559 families. Diploid individuals were found in five populations in the following proportions: Population 11 (100%), Population 19 (11%), Population 27 (100%), Population 28 (52%) and Population 34 (79%). The 30 remaining populations included only tetraploid individuals. Electrophoretic data from Populations 11, 27, 28, and 34 were not included in population analyses but those from Population 19 were included, after eliminating data from four diploid families.

Estimation of Phenotypic Polymorphism

Phenotypic polymorphism for each enzyme zone and population (P_j) was estimated by

$$P_j = \sum_{i=1}^{n} p_i(1-p_i) = 1 - \sum_{i=1}^{n} p_i^2,$$

where p_i is the frequency of the ith phenotype and n is the number of phenotypes observed per enzyme zone and population. The weighted average amount of phenotypic polymorphism $\bar{P}$ over all observed enzyme zones is given by

$$\bar{P} = \frac{\sum_{j=1}^{k} (1/N_j)P_j}{\sum_{j=1}^{k} (1/N_j)},$$

where N_j is the total number of phenotypes observed (collection wide) per jth zone for k zones.

Multiple Regression, Principal Components and Correlation Analyses

Multiple-regression, principal-components and simple correlation analyses were used to test whether environmental factors are associated with variation in phenotypic frequencies. Initially, multiple regression on all environmental variables was used to predict arcsin transformed phenotypic frequencies in the 31 tetraploid populations. This analysis, which was done for each phenotype within each enzyme zone, revealed that a significant F value for one phenotype in an enzyme zone was often accompanied by significant F values for one or more other phenotypes in the zone. Further, these phenotypes tended to be highly negatively correlated with one another. For this reason, as well as to reduce the complexity of subsequent analyses, the phenotypic variables (denoted Y_i, i = 1, ...) were transformed to five independent variables by means of principal-components analysis. Each transformed variable (denoted by Z_j, j = 1, ..., 5) is a linear function of the Y_i, i = 1, ... and it should be highly correlated with one or more of the Y_i. Five principal components were sufficient to account for more than 99 percent of the total variation in isozyme phenotype frequencies, with the exception of Est-I and 6-Pgdh phenotypic frequencies, among the 31 populations studied.

Subsequently, multiple regression on latitude, longitude and nine environmental variables was used to predict principal components scores (values of Z_j for each of the 31 populations). F statistics with 11 and 19 degrees of freedom were calculated to determine whether multiple correlation coefficients (R) were significant. Multiple regression was also used to predict phenotypic principal component scores as functions of: (1) temperature-related variables (average coldest monthly temperature, average hottest monthly temperature, mean annual temperature, and altitude) and; (2) moisture-regime variables (water deficit, average evaporation, annual rainfall, and humidity index). F tests with 4 and 26 degrees of freedom were performed to test for the significance of multiple correlation coefficients. In most cases, prediction equations involving four or fewer of the nine environmental variables accounted for very high proportions of the coefficients of multiple determination (R^2) calculated previously.

Simple correlation coefficients between principal components and environmental variables were also calculated. The procedures used are identical to those described by Walker and Wehrhahn (1971) and by Jeglum et al. (1971) to analyze plant synecological data. Additional information on the use of principal components analyses is presented by Blackith and Reyment (1971) and by Morrison (1967). Examples of applications of principal components in population genetics are found in papers by Johnson et al. (1969), Taylor and Mitton (1974), Gilpin and Ayala (1975) and Hedrick et al. (1976).

Reults

Phenotypes and Phenotypic Frequencies

The criteria used in determining isozyme phenotypes were migration distance (cm) of bands from the origin towards the anode or cathode, width of the bands (broad, intermediate, narrow, judged visually), and staining intensity (light, intermediate, dark, also judged visually). Figures 2-4 give, in schematic fashion, all bands discovered in each zone and identify each band with a number. Table 1 gives the isozyme composition of the phenotypes of each zone. This information, combined with that given in Figures 2-4, allows contruction of the banding patterns of all phenotypes observed in the study. Eighty-six phenotypes were observed in total in the nine zones (Table 1): AP-IV and AP-V had five each, Mdh, Pgi-I, (anodal) aPx-V, and (cathodal) cPx-I, six each, Got, 10; 6-Pgdh, 18; and Est-I, 33. We estimate that the following numbers of loci, respectively, govern the phenotypes for the nine enzyme zones: 6-Pgdh, 4; Mdh, 6; Pgi-I, 2; Est-I, 2; Got, 4; aPx-V, 2; cPx-I, 3; AP-IV, 2; and AP-V, 2, or 27 loci in total. *A. barbata* is a diploidized tetraploid containing genomes A and B (Rajathy 1968). Consequently it seems likely that among these 27 loci many are duplicate loci that were present in both the A and B genomes of the diploid ancestors.

Among the 86 phenotypes observed, 7 were found in diploids but not in tetraploids and, not unexpectedly, considering the much greater number of tetraploids in our sample, a large number of phenotypes (48 in all) found in the tetraploids were not found in the diploids. Studies designed to give quantitative measures of the degree of isozyme relationship between *A. barbata* and its putative diploid ancestors, presently underway, will be reported in a later paper.

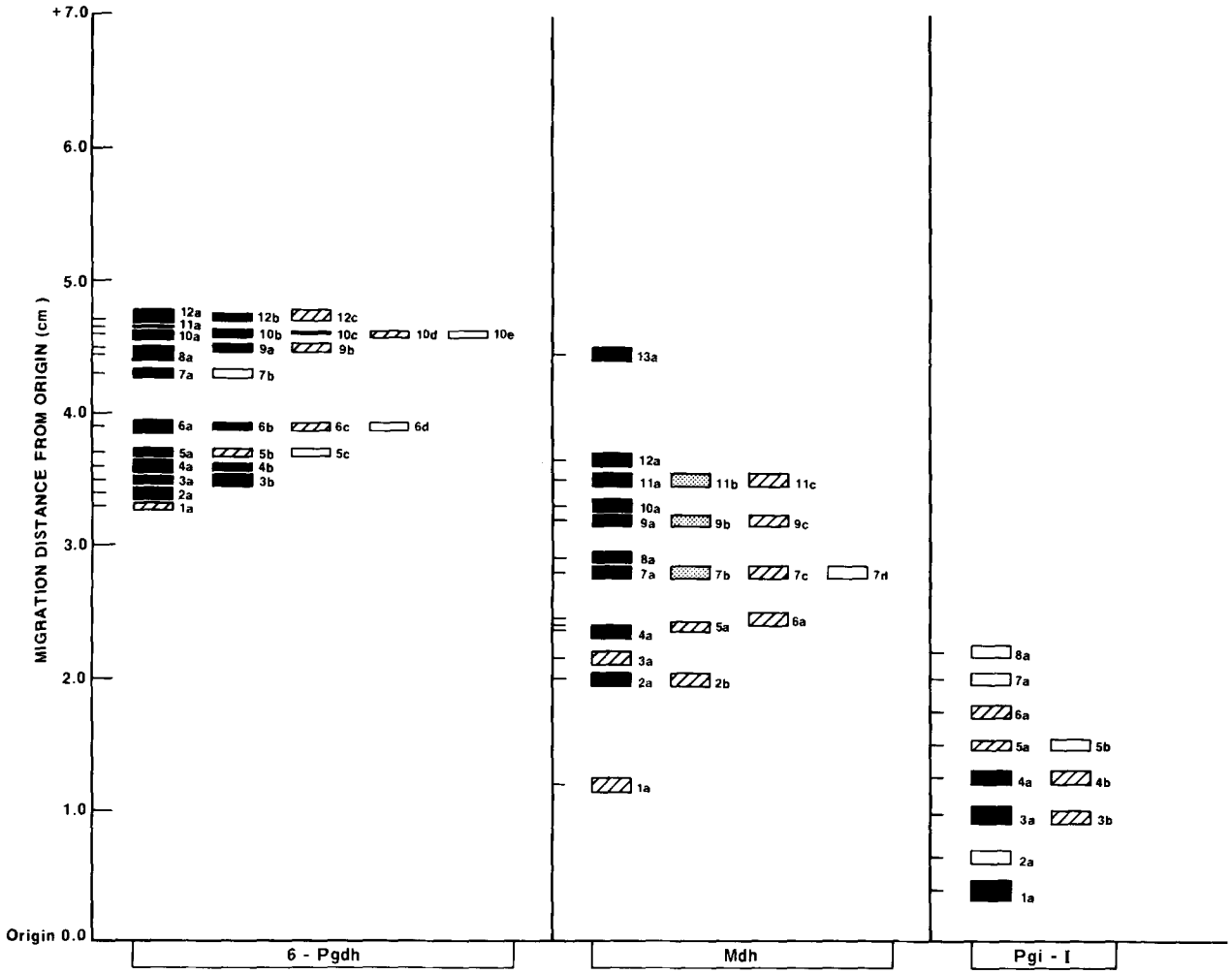

Fig. 2. Schematic diagram of isozyme band types observed for enzyme zones 6-Pgdh, Mdh, and Pgi-I in 35 populations studied. See Table 1 for isozyme phenotypes

Table 2 gives, in summary form, the number of isozyme phenotypes observed for each isozyme zone in each of the 31 tetraploid populations. The data in this table show that Est-I had the largest number of phenotypes in nearly all populations, that 6-Pgdh was intermediate, and that the seven remaining systems were much less variable over all populations, and often monomorphic within individual populations.

As noted in the Methods and Materials section, progeny arrays derived from each adult individual were assayed electrophoretically in polymorphic populations. In all of these assays only one segregating progeny array was found (this progeny array was derived from an individual in population 1 with aPx-V Phenotype 6). This low level of intrafamily phenotypic variation indicates that individuals in these populations of *A. barbata* in Israel are highly homozygous for loci governing the enzyme phenotypes studied. Also no individuals resulting from outcrosses were found in any of the progeny arrays; this indicates that outcrossing rates are much lower in Israel than in California, where it varies from about 0.1% to more than 7%. The uniformity within progeny arrays allows precise ascertainment of the phenotypes of the maternal parents and the data from the progeny arrays were used to infer the phenotypes of the parental plants that had been collected in nature. The phenotypic frequency data for the nine enzyme zones in the 31 populations are voluminous and they will consequently not be reported in total. Instead data for only a single enzyme zone, 6-Pgdh, are reported (Table 3) as an illustration of the pattern of the distribution of phenotypes within and among the 31 populations. (Data for the eight other enzyme zones are available on request to A.L.K. or R.W.A.)

The data on phenotypic frequency distributions for adult plants collected in nature show two main features of isozyme variability in wild oats in Israel. First, each popu-

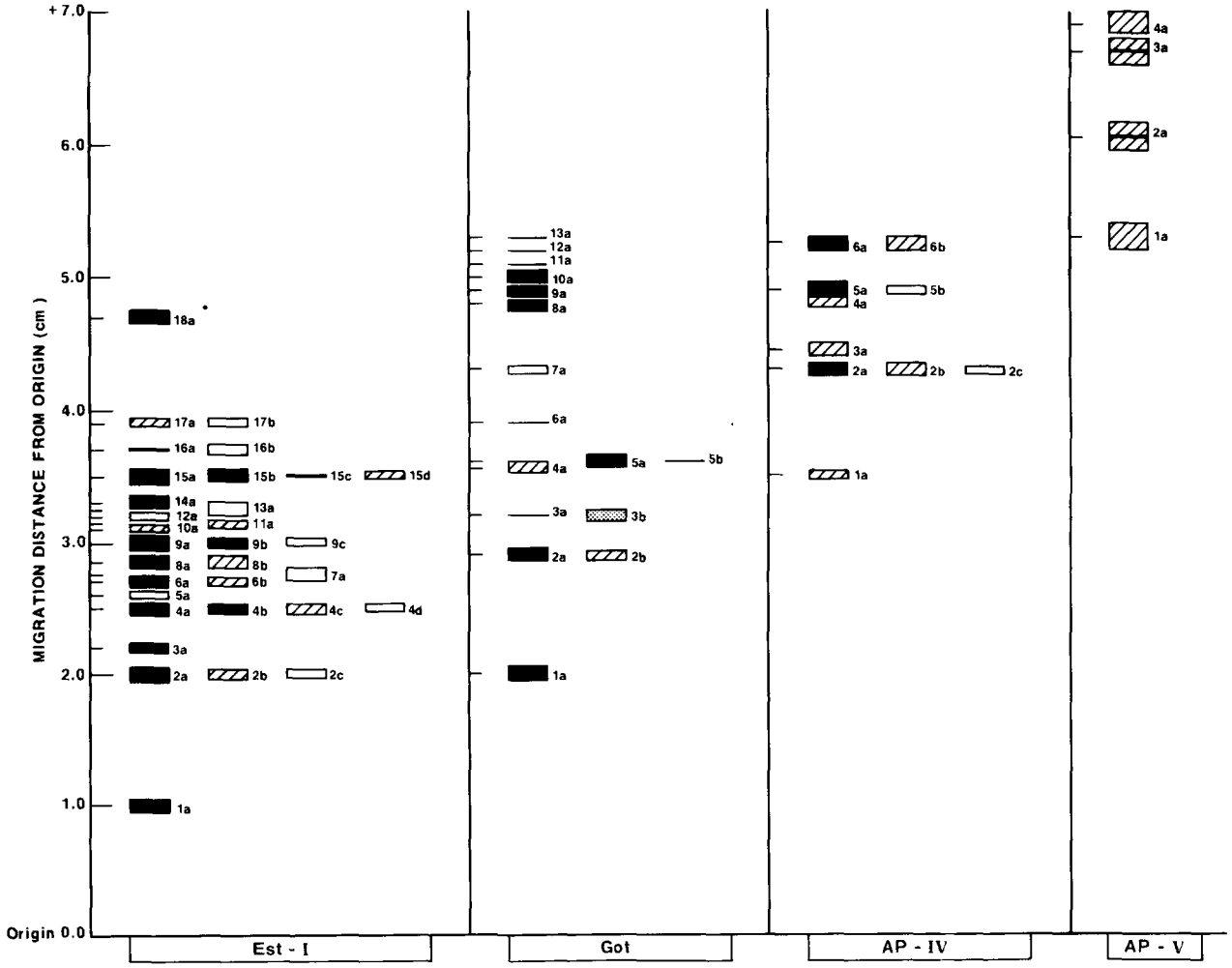

Fig. 3. Schematic diagram of isozyme band types observed for enzyme zones Est-I, Got, AP-IV, and AP-V in 35 populations studied. See Table 1 for isozyme phenotypes

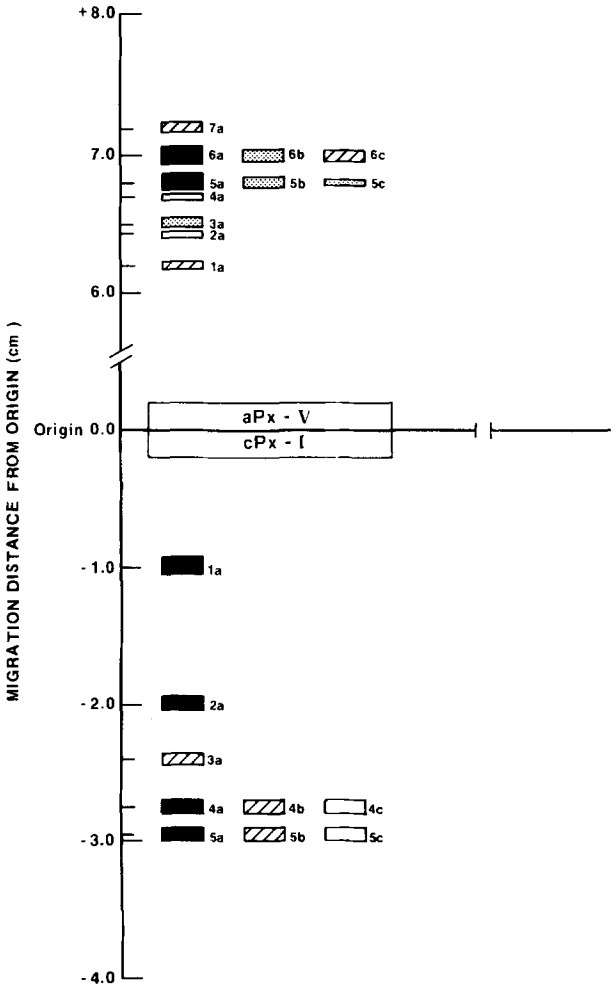

Fig. 4. Schematic diagram of isozyme band types observed for enzyme zones aPx-V and cPx-I in 35 populations studied. See Table 1 for isozyme phenotypes

lation has its own distinctive array of isozyme phenotypes for each enzyme zone, and when all nine zones are considered simultaneously, the population genotype of each population is found to be sharply different from that of each other population. Second, the data show that all of the 31 populations are polymorphic due to the coexistence in each population of several to many fixed phenotypes. Table 4 gives measures of the extent of the polymorphism for the different enzyme zones and for the different populations. Among the nine enzyme zones only two (Pgi-I and Got) were polymorphic in fewer than 50 percent of the populations. Est-I was polymorphic in all 31 populations. Est-I, with a mean P_j value of 0.63, was the most polymorphic enzyme zone whereas Pgi-I and Got with mean P_j values of 0.11 and 0.12, respectively, were the least polymorphic zones.

The extent of polymorphism also differed from population to population (Table 4). Four of the populations (14, 15, 32, 35) were polymorphic for all nine enzyme zones, eight were polymorphic for eight zones, seven for seven zones, five for six zones, six for five zones, and one population (No. 8) was polymorphic for only three zones. Averaged over all enzyme zones Population 32, with a P_j value of 0.43, was the most polymorphic and Population 8, with a P_j value of 0.06 was the least polymorphic. Polymorphic Indices tended to be lower for coastal plain populations, such as Populations 1 through 10, than for populations from the higher elevations, such as Populations 12-16, 24, 25, 29, 32, 33, and 35 (elevations given in Table 5).

Phenotypic-Environmental Associations

Multiple regression of phenotypic frequencies on environmental variables, as well as on latitude and longitude, was used to predict arcsin transformed phenotypic frequencies for all enzyme phenotypes. It should be noted that the meterological data, given in Table 5, were not taken at the collection sites themselves. They are values for the nearest weather station and hence they may depart in some degree from the true values for the collection sites. An F test with 11 and 19 degrees of freedom was used to determine the significance of associations between phenotypic frequencies and environmental variables. Coefficients of multiple determination and associated F values are presented in Table 6 for the phenotypes that were significantly correlated with environmental variables in at least one enzyme zone.

The proportion of the total phenotypic variance accounted for by each of the five principal components for each enzyme zone is presented in Table 7. The five principal components accounted for more than 99% of the total variance in arcsin transformed phenotypic frequencies for all enzyme systems, other than 6-Pgdh (88.9%) and Est-I (64.2%).

Multiple regression on all environmental variables was used to predict principal components scores. Coefficients of multiple determination and F values resulting from this analysis and the corresponding statistics for regression on the four temperature and the four moisture related variables are presented in Table 8.

The F test used in conjunction with multiple regression analysis may not be robust enough to permit reliable inferences if the data to which it is applied deviate too far from multivariate normal. For this reason principal component scores for all 31 populations were checked visually for the occurrence of aberrant distributions (for the occurrence of a few populations with very high scores when most populations had low scores). If a principal component passed this test, and if it was also significantly correlated with some factor of the environment, a detailed analysis of the underlying associations was undertaken.

The main results from these analyses are presented in Table 9. Using the Got isozyme phenotypes as an example, we see that principal component 1 is highly correlated with Phenotype 1 (r = −0.997) and with Phenotype 2 (r = 0.995), and that the coefficient of multiple determination with the environmental variables is R^2 = 0.66**. The simple correlation coefficients between the principal component and annual rainfall, average coldest monthly temperature, mean annual temperature, altitude and humidity index are 0.54**, −0.63**, −0.69**, 0.71**, and 0.61**, respectively. This indicates that a high frequency of Phenotype 2 (and a low frequency of Phenotype 1) is associated with low temperature, high altitude conditions.

To determine whether geographic proximity could account for the observed associations, an overlay of Phenotype 2 frequencies was made on the map locations of these populations. It was found that populations with relatively high frequencies of this phenotype were widely dispersed geographically. Further, when phenotypic frequencies were similar in two nearby populations, these populations were usually separated, geographically, by a population with a quite different phenotypic frequency. We also calculated fourth order polynomial regressions and used them to predict differences in phenotypic frequencies as a function of geographic distances. No significant associations were found for the phenotypes included in Table 9. As a further test of the question whether geographic proximity influences genetic similarities among populations we calculated fourth order polynomial regressions of Hedrick's (1971) genetic similarity index (modified for use with phenotypic frequencies) on geographic distances. The regressions were not significant for any enzyme system. Thus, the hypothesis that sites which are located near each other have similar environments and hence similar phenotypic frequencies does not appear to

Table 2. Observed number of isozyme phenotypes in 31 tetraploid populations

Pop. no.	Collection location	Enzyme zone								
		6Pgdh	Mdh	Pgi-I	Est-I	Got	aPx-V	cPx-I	AP-IV	AP-V
1	Sa'ar	3	2	1	10	2	4	3	2	3
2	Kabri	3	1	1	8	1	3	4	1	3
3	Mt. Carmel	3	2	1	2	1	2	2	2	3
4	Mt. Carmel	1	2	1	8	1	2	1	2	4
5	Mt. Carmel	2	2	1	4	1	3	2	1	3
6	Juara	1	2	2	6	1	2	1	1	2
7	Eliyagim	5	1	2	4	1	2	2	1	4
8	Zikhron-Yaakov	1	1	1	3	1	1	2	1	2
9	Even-Yehuda	6	1	3	7	1	2	3	3	3
10	Tel-Mond	2	1	1	6	1	3	2	2	1
12	Yiron	3	1	1	7	2	2	4	2	3
13	Bar'am	7	3	2	10	2	1	3	2	3
14	Dovev	7	2	2	9	3	2	4	3	3
15	Mt. Meron	2	2	2	7	2	2	3	3	2
16	Beit Jann	6	2	2	7	1	3	4	3	2
17	Eilabun	1	3	–	4	1	2	2	2	3
18	Mt. Tabor	4	2	1	8	1	3	4	3	4
19	Anin	4	2	2	4	1	1	3	2	4
20	Umm El Fahm	3	4	2	8	1	1	5	1	3
21	Máale Gilboa	3	3	–	7	1	2	2	2	2
22	Yabad	2	2	2	7	1	2	2	1	2
23	Bazzariya	6	2	1	4	1	2	2	3	1
24	Mt. Gerizim	4	1	1	7	3	3	2	4	4
25	Shillo	6	2	1	9	2	4	3	3	2
26	Beit-El	8	2	2	6	3	2	3	2	1
29	Margaliyyot	3	1	1	10	1	2	5	2	2
30	Gesher Benot Yaaqov	6	1	1	7	1	1	4	3	4
31	Sede Eliyyahu	2	4	3	5	1	3	4	2	3
32	El-Rom	5	2	2	4	3	3	3	2	2
33	Ramat-Magshimin	1	2	1	4	1	2	3	1	3
35	Hermon	5	2	2	7	2	2	2	4	4
Total[a]		17	6	6	31	5	6	6	4	5

[a] Total number of phenotypes observed for each enzyme zone over all populations studied

be a valid explanation for the associations found in this study.

Three other points are worthy of note. First, not all enzyme zones (Pgi-I, Est-I, and cPx-I) have phenotypes that are associated with the particular environmental variables included in this study. However, it is possible that phenotypes in these zones are associated with physical factors of the environment for which data were not available, or for any of a vast array of biotic and edaphic factors that have not as yet been defined. Second, the associations that were observed (those with 6-Pgdh, Got, aPx-V, AP-IV and AP-V) involved frequent phenotypes. Third, in six out of seven instances, temperature was moderately to highly correlated with phenotype frequency variations and in four cases, altitude was also highly correlated with variation in phenotype frequencies. This suggests that environmental conditions affect phenotypic frequencies in these enzyme zones. We also note that certain phenotypes of 6-Pgdh (Phenotypes 6, 12, and 14), Got (Phenotypes 1 and 2) and AP-V (Phenotype 2) show close associations with average coldest monthly temperature, mean annual temperature and altitude. This suggests that these isozyme phenotypes mark chromosome blocks that contribute to adaptation, i.e. that the isozyme phenotypes mark coadapted allelic complexes. The fact that temperature is the most important single correlate with isozyme-phenotype frequencies in this study is perhaps not surprising because earlier studies in California suggest that late spring and early summer temperatures are important environmental factors associated with monomorphism and polymorphism for morphological characters in *A. barbata* (Rai 1974). Studies of the landsnail *Theba pisana* have shown that a combination of temperature and humidity indices explain a large proportion of the variation observed in this species in the coastal plain of Israel (Nevo and Bar 1976). Also, there is evidence, particularly for ectothermic fish, that one way in which organisms compensate for, or adapt metabolically to, dif-

Table 3. Observed phenotypic frequencies for 6-Pgdh in 31 populations.

Pop. no.	N	Phenotypes																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	47			0.91		0.06		0.02										
2	47	0.11		0.57	0.32													
3	33	0.76		0.03	0.21													
4	44	1																
5	44	0.98		0.02														
6	49			1														
7	50	0.70	0.06	0.06							0.08	0.10						
8	41			1														
9	46	0.20	0.13	0.17	0.02						0.41		0.06					
10	42		0.98								0.02							
12	42			0.14			0.79								0.07			
13	44	0.02		0.29		0.33	0.07				0.04		0.11		0.13			
14	39		0.10	0.38		0.10	0.36						0.03		0.03			
15	33				0.97								0.03					
16	40			0.31	0.31		0.05		0.15				0.05		0.10			
17	54	1																
18	45	0.76		0.13										0.09	0.02			
19	32	0.41	0.53	0.03							0.03							
20	39	0.54		0.44							0.03							
21	51	0.08		0.67		0.25												
22	36	0.19		0.81														
23	49	0.14	0.16	0.39		0.04					0.10		0.16					
24	36	0.31				0.44					0.17					0.08		
25	51	0.18	0.06		0.29	0.23					0.06					0.18		
26	39				0.20	0.20	0.03	0.03	0.03		0.26					0.23	0.03	
29	42	0.02		0.05		0.93												
30	48	0.27			0.19	0.10			0.02	0.37					0.04			
31	54		0.96								0.04							
32	29			0.10	0.03						0.07		0.62		0.17			
33	29				1													
35	45			0.02	0.73								0.11		0.04			0.09

N = the number of individuals assayed per population

ferent temperatures is to produce isozymes whose enzyme-substrate affinities are optimal over the temperature range encountered (Hochachka and Somero 1973). Selection favoring isozymes with near optimal properties at temperatures prevailing during the growing season at a location thus might be a direct and simple way for sedentary species, such as *A. barbata*, to achieve a high level of microgeographic adaptation.

Discussion

Among the nine enzyme zones scored in this study, all were found to be variable. The two least variable zones, Pgi-I and Got, were polymorphic in 39 and 48 percent of the populations, respectively, while the most variable zone, Est-I, was polymorphic in all populations. However, heterozygotes were rare and the variation was carried almost entirely as differences among homozygotes in each zone. When the nine zones were considered simultaneously the total number of phenotypes was large in all of the 31 tetraploid populations analyzed. Also the array of multiple enzyme phenotypes found in each population was distinctly different from that of each other population; each population had its own unique genotype. Thus with respect to the first introductory question of this investigation, it is apparent that there is extensive isozyme phenotype variability both within and among populations of *A. barbata* in Israel.

When overlays were made of isozyme-phenotype fre-

Table 4. Measures of the amount of phenotypic polymorphism (P_j)[a] over enzyme zones and populations, the average (avg. P_j) and the weighted average ($\bar{P}$)[a] amount of phenotypic polymorphism over populations

Pop. no.	Enzyme zone										
	6Pgdh	Mdh	Pgi-I	Est-I	Got	aPx-V	cPx-I	AP-IV	AP-V	Avg. P_j	$\bar{P}$
1	0.16	0.16	0	0.71	0.24	0.28	0.39	0.04	0.21	0.24	0.19
2	0.56	0	0	0.60	0	0.29	0.65	0	0.60	0.30	0.23
3	0.38	0.33	0	0.44	0	0.29	0.29	0.44	0.46	0.29	0.28
4	0	0.35	0	0.60	0	0.24	0	0.09	0.42	0.19	0.16
5	0.04	0.20	0	0.21	0	0.17	0.04	0	0.65	0.14	0.15
6	0	0.04	0.48	0.70	0	0.46	0	0	0.04	0.19	0.14
7	0.49	0	0.11	0.59	0	0.47	0.42	0	0.29	0.26	0.19
8	0	0	0	0.16	0	0	0.16	0	0.29	0.07	0.06
9	0.74	0	0.29	0.62	0	0.04	0.35	0.41	0.11	0.28	0.21
10	0.05	0	0	0.44	0	0.40	0.19	0.19	0	0.14	0.12
12	0.36	0	0	0.77	0.17	0.28	0.72	0.31	0.40	0.33	0.29
13	0.77	0.09	0.42	0.84	0.14	0	0.56	0.14	0.39	0.37	0.28
14	0.70	0.05	0.05	0.72	0.35	0.50	0.67	0.39	0.55	0.44	0.39
15	0.06	0.06	0.16	0.60	0.48	0.06	0.62	0.64	0.50	0.35	0.37
16	0.75	0.29	0.29	0.75	0	0.10	0.56	0.49	0.05	0.36	0.29
17	0	0.07	—	0.65	0	0.33	0.27	0.27	0.13	0.21	0.18
18	0.40	0.04	0	0.88	0	0.44	0.41	0.30	0.54	0.33	0.27
19	0.55	0.06	0.12	0.39	0.22	0	0.24	0.21	0.64	0.27	0.24
20	0.52	0.27	0.26	0.74	0	0	0.66	0	0.12	0.28	0.20
21	0.48	0.38	—	0.37	0	0.39	0.33	0.22	0.30	0.31	0.27
22	0.31	0.20	0.11	0.79	0	0.39	0.13	0	0.18	0.23	0.15
23	0.76	0.18	0	0.44	0	0.35	0.13	0.50	0	0.26	0.21
24	0.67	0	0	0.77	0.41	0.65	0.37	0.36	0.47	0.41	0.35
25	0.79	0.50	0	0.81	0.24	0.56	0.45	0.15	0.08	0.40	0.30
26	0.79	0.46	0.05	0.65	0.50	0.11	0.18	0.16	0	0.32	0.24
29	0.13	0	0	0.77	0	0.08	0.63	0.15	0.50	0.25	0.20
30	0.74	0	0	0.68	0	0	0.65	0.41	0.55	0.34	0.27
31	0.17	0.51	0.20	0.72	0	0.50	0.58	0.06	0.11	0.32	0.26
32	0.57	0.07	0.43	0.62	0.49	0.65	0.32	0.50	0.41	0.45	0.43
33	0	0.07	0	0.73	0	0.49	0.25	0	0.39	0.21	0.17
35	0.44	0.20	0.09	0.60	0.36	0.04	0.04	0.68	0.56	0.33	0.33
Mean	0.40	0.15	0.11	0.63	0.12	0.28	0.36	0.23	0.32	0.29	0.24
%[b]	84	71	48	100	39	84	94	74	90		

[a] See the text for a description of models used to calculate P_j and $\bar{P}$

[b] The percent (%) of populations phenotypically polymorphic per enzyme zone

quency distributions on map locations, it was found that high frequencies of particular phenotypes were widely dispersed geographically. Also when phenotype frequencies were similar in a pair of populations located near each other, a population with a very different phenotype frequency was often found between the pair. In addition, regressions of genetic similarity indices on geographic distances were not significant for any enzyme zone. It is therefore clear that isozyme-phenotype frequency variations in Israel follow a mosaic pattern not associated with geographical distance. This is in direct contrast to the results obtained with certain environmental variables. Principle component and multiple regression analyses showed that temperature-related variables and, to a lesser degree, moisture-related variables are significantly correlated with particular isozyme phenotypes. For example, the frequencies of Phenotypes 6, 12 and 14 of 6-Pgdh, Phenotypes 1 and 2 of Got, and Phenotype 2 of AP-V, were correlated with coldest monthly temperature, annual mean temperature and altitude. Thus, with respect to the second introductory question of this investigation, it is apparent that associations between isozyme phenotype variation and environment occur in Israel and that they are similar to the associations found earlier in California, i.e. phenotypic variation is distributed in both places in mosaic patterns that correspond to ecologically specifiable mosaicism of the environment. The importance of this result is that, while clinal patterns of genetic variability can arise through drift of neutral alleles (Karlin and Rickter-Dyn 1976), patchwork patterns correlated with environment

Table 5. Environmental data for 31 sites at which collections of *A. barbata* were made

	Water/Rainfall			Temperatures (C°)							
Pop. no.	Water deficit (UL)	Avg. evap. (cm)	Annual (mm)	Avg. coldest months	Avg. hottest months	Mean annual	Humidity index[a]	Soil type	Alt. (m)	Long. ° 'E	Lat. ° 'N
1	8	130	584	12	25	20	−10	Loamy sand	35	3506	3301
2	8	130	536	12	25	20	−10	Terra rossa	100	3509	3301
3	8	140	686	14	23	19	10	Terra rossa	300	3500	3244
4	8	140	686	14	23	19	10	Rendzina	250	3500	3244
5	8	140	686	14	23	19	10	Terra rossa	400	3500	3244
6	8	130	657	12	25	18	−10	Rendzina	200	3505	3237
7	8	150	700	12	25	19	−10	Rendzina	225	3503	3238
8	8	130	560	13	26	20	−10	Terra rossa	140	3455	3234
9	8	130	550	13	26	20	−30	Loamy sand	25	3453	3215
10	8	130	550	13	26	20	−30	Loamy sand	30	3454	3215
12	8	150	702	7	23	16	−10	Basalt	690	3527	3304
13	8	150	646	7	23	16	−10	Rendzina	750	3525	3304
14	8	150	750	7	23	16	30	Terra rossa	750	3524	3303
15	8	150	1010	6	22	14	50	Terra rossa	1150	3524	3300
16	8	150	925	7	23	15	50	Gravels	800	3521	3258
17	9	150	591	11	27	19	−10	Marl	200	3524	3250
18	10	160	580	12	28	18	−30	?	588	3523	3241
19	9	160	580	12	26	19	−10	Terra rossa	400	3510	3230
20	9	160	580	12	26	19	−10	Basalt	450	3509	3231
21	11	160	400	12	28	20	−30	Terra rossa	500	3524	3228
22	9	160	590	12	27	20	−10	Rendzina	350	3510	3226
23	9	150	550	10	25	18	−10	Rendzina	500	3510	3218
24	9	150	700	10	23	17	10	Terra rossa	880	3517	3212
25	9	150	500	8	23	17	10	Terra rossa	700	3517	3202
26	8	150	685	10	23	17	10	Terra rossa	830	3513	3155
29	9	150	758	9	27	18	10	Terra rossa	650	3533	3313
30	9	150	470	11	29	20	−30	Marl	60	3538	3301
31	11	170	280	13	30	22	−50	Marl	−175	3531	3227
32	—	—	830[b]	−2.6[b]	35[b]	15.35[b]		Basalt	1050	3547	3311
33	—	—	450[c]	1.8[c]	32[c]	19.2[c]		Basalt	525	3548	3251
35	—	—	830[b]	−2.6[b]	35[b]	15.3[b]		Marl	1050	3545	3316

[a] Mean values for Thornwaite's (1948) humidity index. The index is defined as follows: 50 = humid (B_2); 30 = humid (B_1); 10 = semihumid-humid; −10 = semihumid-arid; −30 = semiarid; −50 = arid

[b] According to Quneitra

[c] According to Afig

Table 6. Coefficients of multiple determination (R^2) from multiple-regression analysis on latitude, longitude, and nine environmental variables[a]

Phenotypes	Enzyme zones[b]							
	6-Pgdh	Mdh	Pgi-I	Got	aPx-V	cPx-I	AP-IV	AP-V
1	0.60 (2.61)[c]	0.43 (1.29)	0.21 (0.42)	0.67 (3.52)[d]	0.69 (3.84)[d]	0.52 (1.89)	0.44 (1.34)	0.36 (0.95)
2	0.53 (2.02)	0.40 (1.13)	0.35 (0.83)	0.63 (3.07)[c]	0.27 (0.64)	0.35 (0.93)	0.49 (1.67)	0.58 (2.34)[c]
3	0.20 (0.42)	0.59 (2.47)[c]	0.66 (3.03)[c]	0.27 (0.62)	0.67 (3.49)[d]	0.49 (1.63)	0.42 (1.25)	0.29 (0.72)
4	0.45 (1.45)	0.48 (1.61)	0.56 (1.96)	0.29 (0.72)	0.27 (0.63)	0.35 (0.92)	0.57 (2.28)	0.59 (2.49)[c]
5	0.52 (1.89)	0.34 (0.89)	0.83 (7.62)[d]	0.45 (1.43)	0.50 (1.73)	0.50 (1.73)	--	0.70 (3.95)[d]
12	0.63 (2.97)[c]	--	--	--	--	--	--	--
13	0.64 (3.11)[c]	--	--	--	--	--	--	--
14	0.68 (3.70)[d]	--	--	--	--	--	--	--

[a] Only phenotypes significant in at least one enzyme zone are included
[b] F values [with 11 and 19 degrees of freedom] are in parentheses
[c] Significant at 5% probability level
[d] Significant at the 1% probability level

Table 7. Proportion of total variance in arcsin transformed phenotypic frequencies attributed to principal components

Variable	Principal components					Total
	1	2	3	4	5	
6-Pgdh	29.4	25.4	16.9	11.2	6.1	88.9
Mdh	64.6	22.1	5.9	4.72	2.7	99.9
Pgi-I	76.2	18.0	5.6	2.3	0.1	100.0
Est-I	21.6	13.7	12.7	9.4	6.9	64.2
Got	94.2	4.7	3.7	0.4	--	100.0
aPx-V	75.1	21.7	2.5	0.3	0.2	99.9
cPx-I	56.0	37.0	4.1	1.4	1.0	99.5
AP-IV	71.3	24.2	4.1	--	--	99.7
AP-V	67.0	22.8	8.3	1.5	--	99.6
All Zones	18.7	13.6	9.7	8.8	7.7	--
Amount of polymorphim	31.2	17.3	13.8	12.7	7.6	--

are difficult to explain except on the basis of selection.

With respect to our third introductory question, comparisons and contrasts of the above results with the extent of genetic variability and its spatial and environmental correlates in California provide a basis for four main inferences concerning 'strategies' of adaptation in *A. barbata*. The gene pool of this species is rich in electrophoretically detectable variability in both Israel and California. It is also clear from visual assessment of factors such as soil type, slope, and associated vegetation, as well as from measurement data on climatic factors such as temperature and humidity, that this species occupies a great diversity of habitats in both Israel and California. Thus the idea that genetic variation is related to environmental variation (Dobzhansky 1955; Levins 1968; Nevo 1978; Allard et al. 1978) appears to fit the slender wild oat and it leads to our first inference: the high observed level of genetic variability is a primary feature of the 'strategy' by which this sedentary species meets the challenges of a spatially heterogeneous environment; it is the raw material on which continuing adaptive response to both local and regional environmental variation depends.

Additional major features of isozyme-phenotype variability are the significant correlations of this variability with temperature and moisture as they change progressively from low to higher elevations, and the close correspondence between the patchwork distribution of this variability and the patchiness of the local environment. As noted above, these features of the isozyme variability are difficult to explain other than on the basis of selection and we interpret them as indicating that the observed genetic differentiation in *A. barbata* has a substantial adaptive component. The observed patterns of genetic variation suggest that two forms of natural selection have

Table 8. Coefficients of multiple determination from predictions of principal component scores by multiple regression on environmental variability

	Principal component														
	1			2			3			4			5		
Variable	A[a]	T	M	A	T	M	A	T	M	A	T	M	A	T	M
6-Pgdh	0.54	0.41	0.08	0.33	0.30	0.18	0.47	0.15	0.19	0.49	0.29	0.05	0.64	0.36	0.28
	(2.02)	(4.58)**		(0.86)	(2.83)*		(1.54)			(1.69)			(3.04)*	(3.96)*	
Mdh	0.35	0.14	0.17	0.44	0.25	0.18	0.31	0.11	0.12	0.57	0.19	0.29	0.64	0.12	0.44
	(0.95)			(1.38)			(0.77)			(2.27)			(2.99)*		(5.02)**
Pgi-I	0.26	0.03	0.14	0.42	0.26	0.23	0.67	0.08	0.13	0.66	0.19	0.44	0.60	0.02	0.21
	(0.53)			(1.12)			(3.08)*			(2.93)*		(4.70)**	(2.27)		
Est-I	0.47	0.16	0.20	0.35	0.23	0.07	0.24	0.08	0.09	0.27	0.12	0.12	0.30	0.09	0.14
	(1.56)			(0.92)			(0.56)			(0.65)			(0.75)		
Got	0.66	0.55	0.38	0.41	0.32	0.12	0.23	0.04	0.18	0.26	0.21	0.04	--	--	--
	(3.36)**	(7.85)***	(4.10)*	(1.19)	(3.06)*		(0.50)			(0.60)			--		
aPx-V	0.73	0.24	0.30	0.24	0.16	0.09	0.22	0.12	0.09	0.46	0.05	0.27	0.39	0.14	0.18
	(4.56)**		(2.80)*	(0.54)			(0.49)			(1.49)			(1.11)		
cPx-I	0.56	0.12	0.14	0.34	0.07	0.09	0.21	0.06	0.15	0.43	0.10	0.18	0.32	0.04	0.15
	(2.23)			(0.90)			(0.46)			(1.31)			(0.82)		
AP-IV	0.47	0.39	0.15	0.42	0.12	0.23	0.53	0.24	0.13	--	--	--	--	--	--
	(1.56)	(4.20)*		(1.23)			(1.94)			--			--		
AP-V	0.63	0.44	0.29	0.42	0.11	0.31	0.32	0.18	0.10	0.59	0.09	0.08	--	--	--
	(2.95)*	(5.00)**		(1.24)		(2.96)*	(0.83)			(2.48)*			--		
All zones	0.72			0.39			0.53			0.46			0.61		
	(3.89)**			(0.98)			(1.75)			(1.31)			(2.42)*		
Amt. polym.	0.63			0.41			0.64			0.40			0.47		
	(2.62)*			(1.08)			(2.69)*			(1.02)			(1.35)		

[a] A, T and M are defined as follows: A = multiple regression on all eleven environmental variables; T = multiple regression on temperature variables (average coldest monthly temperature, average hottest monthly temperature, mean annual temperature and altitude); M = multiple regression on moisture variables (water deficit, average evaporation, annual rainfall, and humidity index). F [11 and 19 degrees of freedom] values for A are in parentheses and $*F_{0.05} = 2.34$, $**F_{0.01} = 3.36$ and for T and M, values of F have 4 and 26 degrees of freedom and $*F_{0.05} = 2.75$, $**F_{0.01} = 4.75$, and $***F_{0.001} = 6.40$. Only significant F values are given for T and M

played major roles in structuring the genetic variation: (1) directional selection leading to clines over transects in which there is progressive change in the environment, such as from hot to cold; and (2) diversifying selection, leading to local differentiation. The California results indicate that selection operates on specific combinations of alleles at different loci, leading to the assemblage of coadapted multilocus complexes (sensu Dobzhansky, 1955) that confer adaptive advantage to particular sets of environmental conditions (Allard et al. 1972). These multilocus complexes include not only the enzyme loci but also loci governing morphological polymorphisms, e.g. black vs. white lemma, and loci governing various measurement characters, e.g. plant height and maturity date (Hamrick and Allard 1975). In inbreeding species selective effects are transmitted throughout the entire genome (Cockerham and Rawlings 1967; Allard et al. 1968; Weir and Cockerham 1973; Allard 1975; Clegg et al. 1978), and population data of the type available in *A. barbata* do not allow us to resolve the question of selection at either individual loci, or over the linkage blocks in which the marker loci are located. Thus, while we can conclude that there is substantial selection, the correlational structure of the entire multilocus distribution allows us to see only its effects over the entire genome. The finding that specific combinations of enzyme phenotypes (such as Phenotypes 6, 12 and 14 of 6-Pgdh, 1 and 2 of Got, and 2 of AP-V) occur together suggests that selection operates on multilocus combinations in Israel as well as in California. The above results lead us to our second inference: directional and diversifying selection have played major roles in structuring the total genetic variation into coadapted multi-

Table 9. Principal components, phenotypes discriminated by principal components (single correlation coefficients (r) are in parentheses), coefficients of multiple determination (R^2 for all variables, for temperature variables and for moisture variables), and environmental correlates for six enzyme zones

Enzyme zone	Principal component	Phenotypes discriminated (r)	Coefficient of multiple determination			Environmental correlates (r)	
			All variables	Temperature variables	Moisture variables		
6-Pgdh	1	1(−0.947)	0.54	0.41**	0.08	Avg. coldest mo. temp.	(−0.45)
		3(0.514)				Mean annual temp.	(−0.28)
		4(0.342)					
	5	6(0.792)	0.64*	0.36*	0.28	Avg. coldest mo. temp.	(−0.37)*
		12(0.414)				Mean annual temp.	(−0.49)**
		14(0.736)				Annual rainfall	(0.38)*
						Altitude	(0.33)
Mdh	5	1(−0.303)	0.64*	0.12	0.44**	Water deficit	(0.53)**
		3(0.673)				Avg. evaporation	(0.45)*
						Humidity index	(−0.43)*
Got	1	1(−0.997)	0.66**	0.55***	0.38*	Annual rainfall	(0.54)**
		2(0.995)				Avg. coldest mo. temp.	(−0.63)**
						Mean annual temp.	(−0.69)**
						Altitude	(0.71)**
						Humidity index	(0.61)**
aPx-V	1	1(0.953)	0.73**	0.24	0.30*	Avg. evaporation	(0.38)*
		3(−0.961)				Avg. coldest mo. temp.	(−0.45)*
						Mean annual temp.	(−0.40)*
						Longitude	(0.67)**
AP-IV	1	1(0.893)	0.47	0.39*	0.15	Avg. coldest mo. temp.	(−0.59)**
		2(−0.983)				Avg. hottest mo. temp.	(0.43)*
						Altitude	(0.38)*
						Longitude	(0.48)**
AP-V	1	2(−0.975)	0.63*	0.44**	0.29	Avg. coldest mo. temp.	(−0.40)*
		5(0.936)				Avg. hottest mo. temp.	(0.38)*
						Mean annual temp.	(−0.36)*
						Altitude	(0.45)*
						Annual rainfall	(0.43)*
						Humidity index	(0.42)*

locus complexes of alleles that confer adaptive advantage under specific sets of environmental conditions on both micro- and macrogeographical scales.

The level of isozyme-phenotype variation differs substantially from place to place in Israel. The level of enzyme polymorphism differs even more in California; in fact, many California populations are entirely monomorphic for 35 electrophoretically detectable loci and in the most xeric areas in which the species can survive (250-500 mm of rainfall), including the entire southern half of the state, all populations are monomorphic for a specific 35-locus enzyme genotype (Miller 1977). In considering the extent of genetic variability of *A. barbata* it should be noted that studies of measurement characters carried out before electrophoretic methods were used in population genetics revealed extensive variability for such characters both within and among populations, including populations that were later found to be monomorphic for enzyme variants (Allard 1965; Allard et al. 1968). In these studies seeds collected from random individuals in nature were sown in replicated common garden experiments and the resulting progenies were measured for continuously varying characters, such as flowering time and height. Significant differences were found in progeny means which indicates that the maternal individuals differed from each other genetically and hence that the populations from which they were taken were genetically variable respecting quantitative characters. In addition, responses were obtained when plus and minus selection was practiced within progenies derived from single plants. Response to such selection provides clear evidence that at least some of the loci governing each measurement character are heterozygous in the natural populations. These common garden experiments also showed that the mean value for each population differed significantly from the mean of each other population, and hence that all populations studied, including the electrophoretically identical populations, differed genetically from each other population respecting measurements characters. Thus, if only allozyme data were available *A. barbata* would be judged to be genetically invariant, both within and among populations, over much of its range in California. If, on the other hand, only measurement character data were available, the species would be judged to be genetically variable within populations and also genetically differentiated among populations. It is therefore apparent that no single class of loci, such as those governing enzyme variants, or those governing morphological polymorphisms, or those governing measurement characters gives a complete picture of the extent of genetic variability within populations or of degree of evolutionary divergence among populations in this species (Allard et al. 1978).

The above results lead us to our third inference: the process of evolving locally adapted ecotypes has proceded further in Israel, which is located in the area of origin of *A. barbata*, than in California, where the species is a recent introduction. In California, *A. barbata* appears to be in an exploratory stage featuring, first, the formation of new gene combinations from the large sample of genetic variability introduced to the state from the Western Mediterranean, and second, the testing of these combinations in the wide array of new environments that have become available to the species. The formation of numerous new gene combinations is promoted by the high levels of heterozygosity that have been observed in California populations for loci governing measurement characters, discussed above, as well as for allozyme variants, whereas rapid exploration of the range of habitats is facilitated by the frequent migrations that occur from place to place. In the two centuries or more that *A. barbata* has been in California it has evolved at least seven multilocus complexes marked by electrophoretically detectable loci; these complexes represent 'ecotypes' (Turesson 1922) adapted to regions with strikingly different environments, particularly the 'xeric' and 'mesic' regions of the state. However, individual populations within each 'ecotype' exhibit an intricate and complex pattern of variation for measurement characters. This suggests that diversifying selection is occurring and that it has produced a large number of variations on the major themes, to a series of local adaptations each characterized by its own unique combination of values for various measurement characters, but still undifferentiated respecting isozyme characters. Diversifying selection has evidently progressed further in Israel than in California because visual appraisal indicates interpopulation differentiation for measurement characters is greater in Israel and because all populations from Israel were electrophoretically unique.

The correlation between low levels of heterozygosity and low outcrossing rates ($<<$ one percent) in Israel and high levels of heterozygosity and high outcrossing rates ($>$ seven percent) in some populations in California (Marshall and Allard 1970; Allard et al. 1972; Hamrick and Allard 1972) lead us to our fourth inference: modification of the mating system has been the major strategy by which *A. barbata* adjusts its genetic variability system to allow response to changing evolutionary circumstances (this strategy appears to be a common one in plants, as discussed by Allard et al. 1968, and Allard 1975). Thus, if the premium is on genetic experimentation, as may be the case during the colonization of new habitats, heterozygotes are likely to leave more offspring than homozygotes; and, because individuals produced by outcrossing are more likely to be heterozygous than individuals produced by selfing, selection favoring heterozygotes will increase the frequency of outcrossing alleles at loci that affect the mating system. Conversely, selection is expected to favor individuals carrying alleles for outcrossing rates when the

premium is on preserving existing adaptive genomic configurations of alleles. There have recently been declines in grazing and increases in cropping in Israel which have affected the distribution and abundance of many elements of the agrestral vegetation, including *A. barbata*. Such changes do not appear, at least yet, to have affected the mating system; it was the same in all of the 31 populations studied even though changes in grazing and cropping were unlikely to have affected all of these populations equally. It would be interesting to follow both short and long-term changes in mating system in populations occupying sites in Israel which have clearly been affected by the recent changes in grazing and agricultural patterns.

When considering the extent of genetic variability in *A. barbata* it is also important to recall that this species is a diploidized tetraploid, that regular bivalent formation occurs between the seven pairs of chromosomes of the two ancestral genomes and there are 14 chromosome pairs at meiosis. This makes it possible for individuals that have the same phenotype, and hence apparently the same genotype, to produce different progeny arrays on self fertilization. As an example, adult plants with 6-Pgdh isozyme bands 1a, 3a and 5b have been observed on self fertilization, to produce three different progeny arrays respecting these bands: (1) arrays that segregate in a 1:4:6:4:1 two-locus ratio (the identical loci in the two genomes are both heterozygous for the same two alleles); (2) arrays that segregate in a 1:2:1 single-locus ratio (the locus in one genome is heterozygous and the locus in the second genome is homozygous for a recessive null allele, producing no band); or (3) arrays that include only individuals like the maternal parent (the loci in the two genomes are homozygous for different alleles, with the middle band, 3a, representing a 'hybrid' enzyme of intercistronic origin). The fixed genetic state implied by the third type of array has important implications concerning genetic variability. Populations that are judged to be devoid of genetic heterogeneity on the basis of enzyme monomorphism may, in fact, be little if any less diverse biochemically than populations that are polymorphic for the same enzyme. Thus, for example, Population 6 in Israel is fixed for 6-Pgdh Phenotype 3 but all individuals in this population have the three enzymes which are represented by bands 1a, 3a and 5b. Allard, Miller and Kahler (1978) have discussed the importance of biochemical diversity due to 'fixed heterozygosity' in *A. barbata* in both Israel and California.

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