ISOZYME POLYMORPHISMS IN NATURAL POPULATIONS OF *AVENA FATUA* AND *A. BARBATA*

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1. Introduction

A number of recent studies (Imam and Allard, 1965; Jain and Marshall, 1967; Marshall and Jain, 1969; review in Jain, 1969) of *Avena fatua* L. (common wild oat) and *A. barbata* (slender oat) have established that natural populations of these heavily inbreeding species (95-98 per cent. selfing) are often polymorphic for morphological characters and that they also contain substantial stores of genetic variability for metrical characters. Further, close correlations are found between patterns of polymorphism and patterns of environmental variation, indicating that much of the genetic variability existing in these species is adaptive and fits them for both the opportunistic settlement and enduring occupation of diverse niches. While populations of both *A. fatua* and *A. barbata* contain substantial genetic variability, there is a major difference between the species in this respect; populations of *A. fatua* are more often highly polymorphic than those of *A. barbata* and they also contain greater genetic variability for measurement characters. Nevertheless populations of *A. barbata* show greater phenotypic variability *in nature* than populations of *A. fatua*. On the basis of this observation, it was postulated that the two species differ in adaptive strategy, namely, that *A. barbata* relies less on genetic diversity and more on developmental flexibility than *A. fatua* in adapting to temporally and spatially heterogeneous environments. Studies under controlled environmental conditions (Marshall and Jain, 1968) provide additional support for the thesis that *A. barbata* is developmentally more flexible than *A. fatua*.

The above hypothesis can, however, be criticised on two main grounds. First, it can be argued that the four morphological traits studied in the above investigations mark only a small fraction of the total genetic complement of these species and this may be insufficient to permit a valid comparison of relative genetic variation in *A. fatua* and *A. barbata*. Second, following Lewontin (1967), it can be argued that morphological polymorphisms, controlled by simple genetic systems, represent a very special sample of genic variation and that the study of iso-allelic variants using electrophoretic techniques may provide a better measure of total genic variation. Since electrophoretic techniques offer a number of other advantages, particularly with respect to the number of *loci* which can be scored simultaneously per individual, a systematic study of allelic variation in natural populations of *A. fatua* and *A. barbata* was initiated using discontinuous starch gel electrophoresis. As a first step in this study answers were sought to the following questions: (1) To what extent are populations of these species polymorphic for electrophoretic variants? and (2) what is the relationship, if any, between

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the geographical patterns of polymorphism for the morphological and biochemical markers? This paper reports on these questions and describes the techniques followed in seeking answers.

2. Materials and methods

The present study was based on eight Central California populations of each species for which information on morphological polymorphisms was available from previous studies. Table 1 describes the sites occupied by these populations and also gives a polymorphic index (PI) which provides a relative measure of the degree of polymorphism for the four morphological marker *loci* in each population. It will be noted that these sites represent a variety of climatic regions and that populations occupying different sites often differ markedly in degree of polymorphism.

Electrophoretic studies were based on greenhouse plants grown from random samples of seeds taken from each of the eight populations. Samples for electrophoretic analysis were obtained from the two uppermost leaves of plants in the jointing stage as this material gave the largest number of identifiable bands for the enzymes assayed. The leaf samples were crushed in a small petri dish with a plexiglass rod and the crude squeezate was adsorbed in with Beckman No. 319329 paper strips, which were then inserted in a cut 4·0 cm. from the cathodal end of the gel. Each gel accommodated 18 samples and 5 or 6 gels (90-108 individuals) were run per population.

The gels were prepared in a manner similar to that described by Kristjansson (1963) and Brown and Allard (1969). The gel buffer was 0·003 M citric acid and 0·0152 M tris (hydroxymethyl) aminomethane (*p*H 8·0). Sponge cloth connected the gel to the electrode chambers containing 0·3 M boric acid and 0·1 M sodium hydroxide (*p*H 8·7). The gels were subjected to 300 V for 15 minutes, after which the sample strips were removed, and electrophoresis continued until the borate front had migrated 8 cm. from the insert cut. Each gel was cut horizontally into three slices. The top cathodal slice was assayed for phosphatase (P), the middle slice for esterase (E) and the bottom slice for leucine amino-peptidase (L.A.P.). The staining procedures used were similar to those described by Shaw and Koen (1968) for esterase and leucine aminopeptidase, and Rendel and Stormont (1964) for phosphatase. In the case of the esterase stain an equiproportional mixture of α- and βnapthyl acetate was used as the substrate to aid in the differentiation and identification of the bands. The gels were developed until the sites of activity reached optimum visibility, after which they were fixed in 50 per cent. methanol.

3. Results

Each gel stained for a particular class of enzymes showed a number of bands or groups of bands. Formal genetic studies have established the inheritance of certain of these bands (Marshall and Allard, 1969) and the remainder are also assumed to represent the products of different genetic units. In order to obtain a quantitative measure of the level of genic variation in the two species, each individual was scored for the presence or absence of each band. If a particular band was present in all individuals of the species, the corresponding *locus* was considered to be monomorphic. However, if a band was present in some individuals and absent in others due

TABLE 1

Description of sites from which seed samples were obtained

Site	Description	Polymorphic Index (PI)*	
		A. fatua	*A. barbata*
D	Yolo County. 3·9 miles west of Winters on Highway 128. Pure *A. fatua* in moderately dense stand occupying one acre plot on roadside with gentle east facing slope	0·13	—
G	Yolo County. 5·8 miles west of Winters on Highway 128. Less than 3 per cent. *A. fatua*. Sparse stand at base of steep rocky south facing slope in grazed area	—	0
H	Yolo County. 7 miles west of Winters on Highway 128. *A. fatua* and *A. barbata* in about 7 : 3 ratio in dense stand. Roadside population on steep south facing slope	0·04	0
J	Napa County. 9·7 miles west of Winters on Highway 128. Less than 1 per cent. *A. fatua*. Roadside population of moderate density on gentle slope with easterly aspect	—	0
L	Napa County. 20·5 miles west of Winters on Highway 128. Pure *A. barbata* population on steep sloping roadside area carrying sparse stand	—	0
0	Napa County. 35 miles west of Winters on Highway 128. Pure *A. fatua* population. Extremely dense stand in moist pocket on the banks of Hennessy reservoir	0·07	—
C1	Napa County. 5 miles south Calistoga on Silverado trail. Heterogeneous mixed population on gentle slope. Sample 0 was taken at the base of the slope in an abandoned orchard, sample 2 was taken mid-way up the slope. The ratio of *A. fatua* to *A. barbata* was 3 : 2 and 1 : 9 respectively	0·09	0·06
CTG-H	Napa County. 2 miles north of Calistoga on Highway 128. *A. fatua* to *A. barbata* in nearly 1 : 1 ratio. Steep dry slope carrying sparse stand in grazed area	0·01	0·05
NVT-E	Marin County. 5·5 miles north of Navato on U.S. 101. Mixed population *A. fatua* to *A. barbata* in about 3 : 7 ratio on steep south-east facing roadside cut	0·16	0·07
M.V.	Marin County. 4 miles south San Rafael on U.S. 101. Pure population of *A. barbata*. Vigorous roadside stand	—	0·12
M.C.	Marin County. 8 miles south San Rafael on U.S. 101. Mixed population of *A. fatua* and *A. barbata* in about 1 : 1 ratio	0·17	—
ABN-A	Placer County. 2 miles north of Auburn on Highway 49. Vacant lot carrying a sparse mixed population with the two species in nearly 1 : 1 ratio	0·11	—

* $PI = \sum_{i=1}^{N} p_i q_i / N$ where p_i is the frequency of the *ith* morph, $q_i = 1 - p_i$ and N is the number of traits considered. Data from Marshall and Jain (1969)

to differences in electrophoretic mobility or to active-inactive (presence-null) differences, the species was considered to be polymorphic for different forms of the enzyme. The bands for each enzyme system which were scored in this manner are shown diagrammatically in fig. 1. It should be emphasised that fig. 1 does not include all bands which appear on staining for phosphatase and esterase but only those bands which could be read unambiguously in all samples. Bands which stained faintly, or which appeared inconsistently in two or more samples from the same individual, were not used in the present study.

In *A. barbata* 11 out of the 23 bands scored were present in all of the 90 or more individuals examined in each of the eight populations. However, for the remaining 12 bands, at least one variant individual was found in one or more of the populations. In sharp contrast, only six bands were constant in all individuals of the eight populations of *A. fatua*, whereas the remaining 18 bands were variable. This difference between the species was significant ($P < 0{\cdot}05$) indicating that the proportion of polymorphic bands is higher in *A. fatua* than *A. barbata*. It is not possible to obtain a precise estimate of the proportion of unfixed (polymorphic) and fixed (monomorphic) *loci* in these species at the present time, since, as mentioned above, the formal genetics of only certain of the bands has been worked out. However, we can obtain a provisional conservative estimate from the minimum numbers of polymorphic *loci* and the numbers of monomorphic *loci*. The evidence available from segregating families (Marshall and Allard, 1969; and unpublished) indicates that at least seven *loci* are polymorphic in one or more of the populations of *A. fatua* surveyed while in *A. barbata* at least five *loci* are polymorphic. Further, on the assumption that each of the invariant bands represents a single *locus*, the numbers of monomorphic *loci* in *A. fatua* and *A. barbata* are six and eleven, respectively. These figures yield estimates of 54 and 31 per cent. of polymorphic *loci* in *A. fatua* and *A. barbata*, respectively.

Within both species there were marked differences among the populations surveyed with respect to the number of polymorphic bands, and the relative degree of polymorphism for each band. Further, the relative frequencies of the variant bands in both species showed a more or less continuous distribution extending from extreme rarity in a single population to a high degree of polymorphism in a number of populations. The frequencies of a sample of five bands from each species are given in table 2 to illustrate this point.

To facilitate comparisons of the degree of polymorphism, we have calculated a polymorphic index,

$$PI = \sum_{i=1}^{N} p_i\,(1-p_i)/N,$$

in which p_i is the frequency of the *ith* band and N is the number of bands, for each enzyme system and population. This index, which can vary from 0 to 0·25, takes into account both differences in the proportion of polymorphic to monomorphic bands and the degree of polymorphism for each of the variable bands. Values for the index, which is essentially a weighted measure of allelic diversity, are given in table 3. In both species L.A.P. was the least variable of the three enzyme systems. No variant L.A.P. bands were recorded in *A. fatua* while in *A. barbata* a single variant band occurred in low

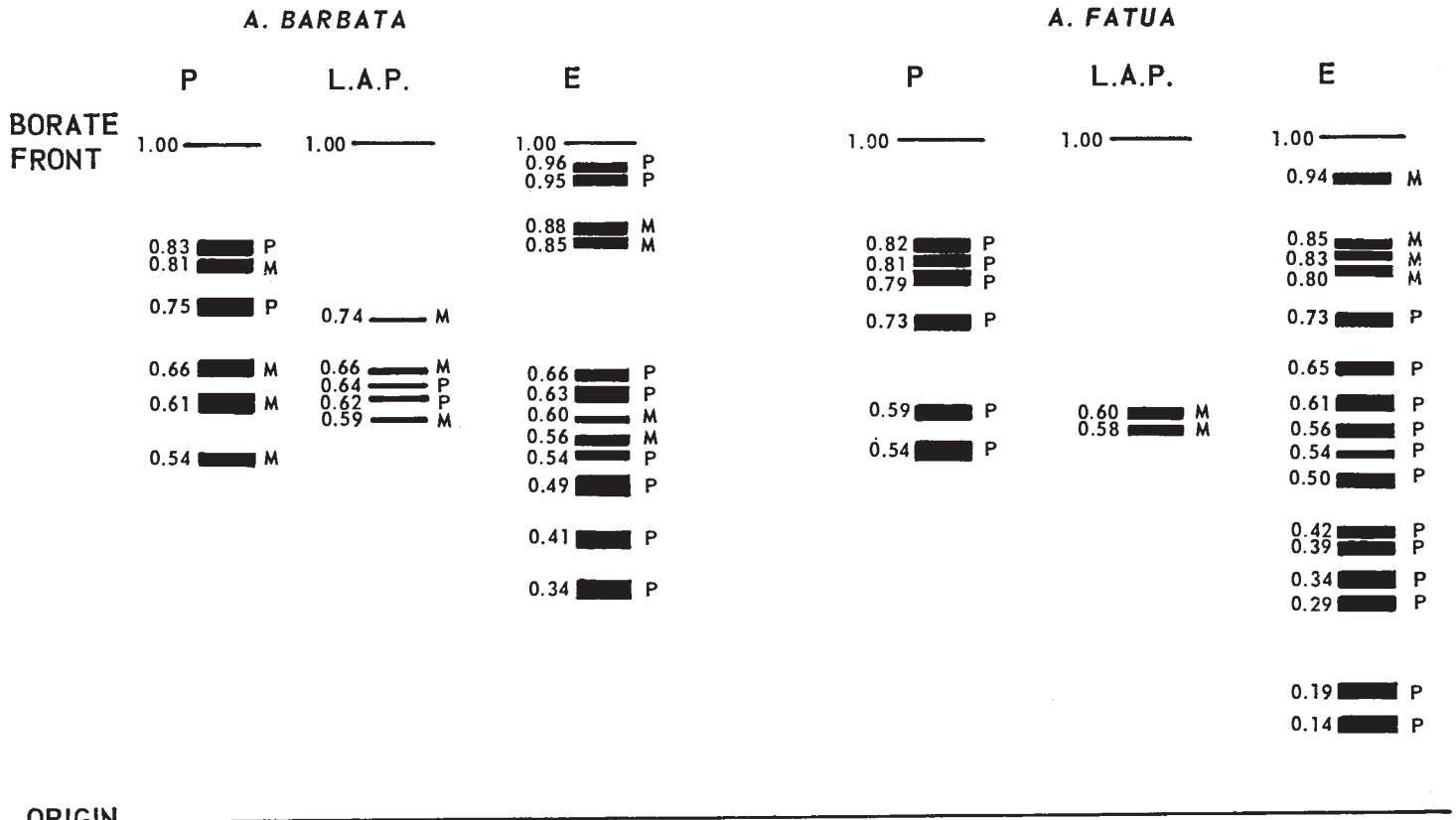

FIG. 1.—Electrophoretic bands for phosphatase, leucine amino pepsidase and esterase in *A. barbata* and *A. fatua*. Additional bands occur but were omitted because they could not be read reliably. The Rf values (relative to the distance migrated by the borate front) are given to the left of each set of bands. The letters given to the right of each band indicate whether it was polymorphic (P) or monomorphic (M) in the populations surveyed.

frequency in three of the eight populations surveyed. The esterase and phosphatase systems were equally variable in *A. fatua* but differed markedly in their relative variabilities in *A. barbata*. Comparing the populations

TABLE 2

Relative frequencies of a sample of five polymorphic bands in natural populations of A. fatua *and* A. barbata *(see text)*

A. fatua

				Site				
Band	D	H	O	NVT-E	C10	CTG-H	ABN-A	M.C.
$E^{0.14}$	0·10	0·04	0·05	0·54	0	0·98	0	0·09
$E^{0.29}$	0·27	0·03	0·25	0·15	0·31	0·02	0	0·02
$E^{0.34}$	0·73	0·97	0·74	0·98	0·97	0·98	1·00	0·76
$P^{0.54}$	0·69	1·00	0·41	0·26	1·00	1·00	0·22	1·00
$P^{0.73}$	0	0	0	0·13	0	0	0	0

A. barbata

				Site				
Band	G	H	J	L	NVT-E	C12	CTG-H	M.V.
$P^{0.83}$	1·00	1·00	1·00	1·00	0·00	0·33	0·60	0·98
$E^{0.34}$	0	0	0	0	0·01	0	0	0·26
$E^{0.41}$	0·99	1·00	1·00	1·00	0·88	0·33	0·71	0·43
$E^{0.54}$	0	0	0	0	0·11	0·58	0·39	0·49
$E^{0.96}$	1·00	1·00	1·00	1·00	0	0·52	0·72	0·92

within each species, it will be noted that none of the *A. fatua* populations was completely monomorphic. However, in several instances, *e.g.* populations H, CTG-H and ABN-A, the degree of polymorphism was relatively low.

TABLE 3

Polymorphic indices for each enzyme system and population

A. fatua

				Population					
Enzyme system	D	H	O	NVT-E	C10	CTG-H	ABN-A	M.C.	Weighted mean
Phosphatase	0·05	0·02	0·08	0·17	0·04	0·02	0·07	0·02	0·06
L.A.P.	0	0	0	0	0	0	0	0	0
Esterase	0·11	0·01	0·08	0·10	0·06	<0·01	0·02	0·12	0·06
Weighted mean	0·09	0·01	0·08	0·12	0·05	0·01	0·03	0·09	0·06

A. barbata

				Population					
Enzyme system	G	H	J	L	NVT-E	Cl_2	GTG-H	M.V.	Weighted mean
Phosphatase	0	0	0	0	0	0·08	0·07	0·03	0·02
L.A.P.	0	0	0	0	0	0·02	0·01	0·02	<0·01
Esterase	<0·01	0	0	0	0·04	0·08	0·07	0·10	0·04
Weighted mean	<0·01	0	0	0	0·02	0·07	0·06	0·07	0·03

In contrast the four *A. barbata* populations (G, H. J and L) for the Central Valley and the bordering foothills were largely or completely monomorphic. Further, the remaining populations showed only low to intermediate levels of polymorphism. Averaged over enzyme systems and populations, the mean PI value of *A. fatua* was twice that of *A. barbata*.

The PI values for the electrophoretic variants, in conjunction with analogous values for morphological traits (table 1), permit us to examine relationships between the two types of characters (fig. 2). In both species the correlation between the indices was significant ($r = 0{\cdot}84$, $P < 0{\cdot}01$ in *A. barbata*; $r = 0{\cdot}82$, $P < 0{\cdot}01$ in *A. fatua*). Thus, it would appear that populations which are monomorphic for morphological markers also tend to be monomorphic for isozyme variants, and *vice versa*.

4. Discussion

The results reported here complement previous findings from studies based on morphological markers and quantitative traits (Jain and Marshall, 1967; Marshall and Jain, 1969) and indicate that natural populations of *A. fatua* and *A. barbata* contain considerable stores of genetic variability. Further, they provide additional support for the thesis that, on the average, populations of *A. fatua* are genetically more variable than populations of *A. barbata*. However, there are several potential sources of bias in estimates of the relative levels of iso-enzymatic variability in different species derived from data of this sort. These have been discussed in considerable detail by a number of authors including Hubby (1963), Hubby and Throckmorton (1965, 1968), Lewontin and Hubby (1966), Johnson *et al.* (1966) and Gillespie and Kojima (1968) and need not be reiterated here. It is sufficient to note that, at the present time, we cannot say whether we are underestimating or overestimating the total variation in the genome, or to what degree, if any, our estimates are biased. As a consequence, the estimates of the proportion of polymorphic *loci* in *A. fatua* and *A. barbata* given here should be regarded as strictly provisional.

Our findings raise two interrelated questions:

(i) What is the balance of forces responsible for the maintenance of the observed genetic variation? and
(ii) What is the function of this variation:

Two main hypotheses can be proposed to explain the occurrence of significant amounts of iso-enzymatic variation in natural populations of these species. First, since the electrophoretic mutants with the exception of null or silent alleles show little, if any, variation detectable in catalytic activity *in vitro*, it can be argued that they have little, if any, effect on their carriers and that the observed polymorphisms are maintained by a balance between genetic drift and recurrent mutation and/or migration. Under this hypothesis, the polymorphisms observed would have no discernible effect on the fitness of the population and differences among populations could be attributed entirely to chance. Further, no correlation would be expected between the patterns of variation for morphological markers, which presumably represent adaptive polymorphisms, and the isozyme markers. Alternatively we can postulate that the majority of the mutant enzymes have a distinct effect on the fitness of their carriers. In this case, some form of balanced selection (heterozygote advantage, frequency dependent selection, differential selection at two or more life stages, etc.) must be invoked to account for the observed polymorphisms. Under this hypothesis differences between populations can be assumed to have developed in response to, and to be maintained by, differences in the environment. Further, since the genetic

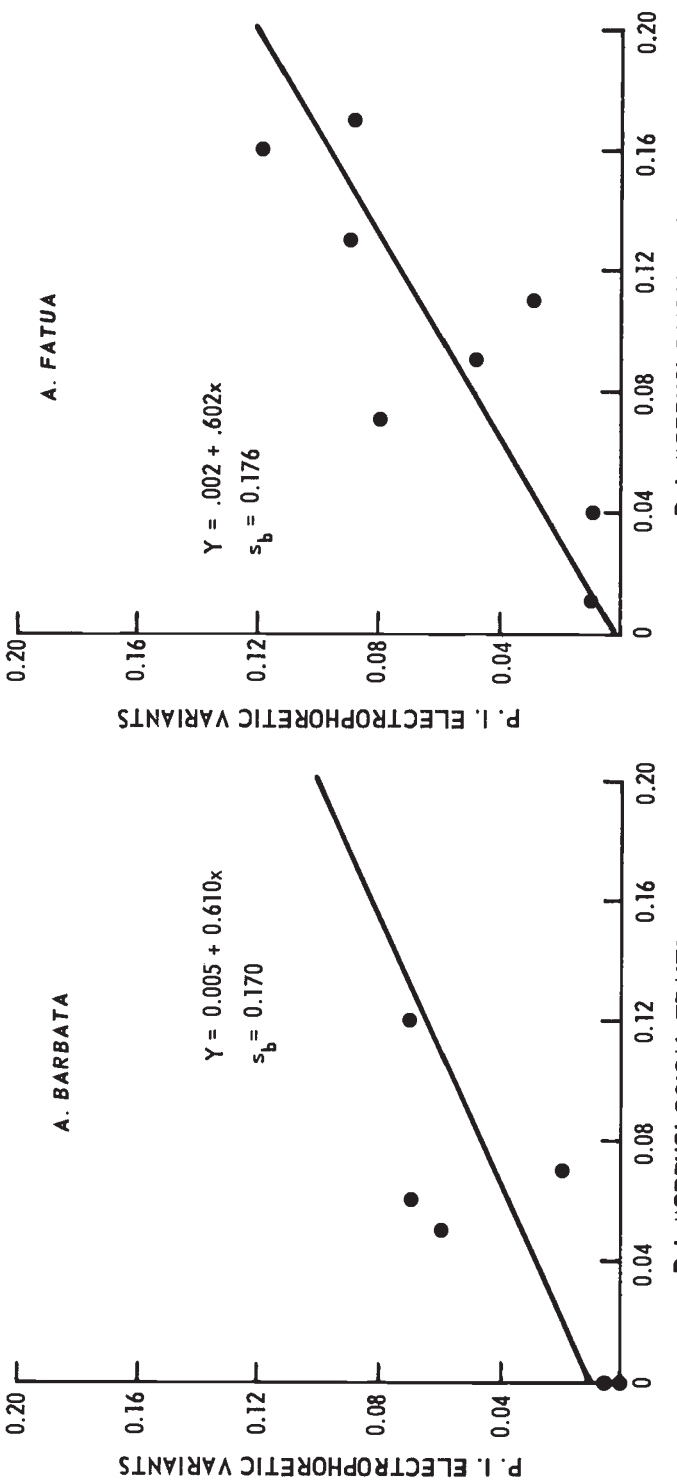

FIG. 2.—Regressions of polymorphic indices for electrophoretic variants with polymorphic indices for morphological characters.

system as a whole is presumably moulded by the environment, strong correlations are expected between morphological and isozyme markers. It should be emphasised that the above hypotheses are by no means mutually exclusive. In any population at least some variation is almost certainly maintained by a balance between selection and mutation and/or drift and mutation. Also not all variation maintained by balanced selection is likely to be adaptive. For example, Crosby (1949) showed theoretically that a gene affecting the breeding system can be strongly selected despite the fact it markedly reduces species fitness and argued that such a system could account for the high frequency of homostyles encountered in a number of natural populations of *Primula vulgaris*. The question is whether the bulk of the variation is maintained by balanced selection and is adaptive.

The present data, in particular the strong correlations between the patterns of variation for the isozyme and morphological markers, support the thesis that the bulk of the isozyme polymorphisms are selectively maintained and are adaptive. The precise nature of the selective mechanisms for the individual systems are not known at the present time. However, preliminary studies with *A. barbata* suggest that heterozygote advantage may be of importance in this regard (Marshall and Allard, in press). Additional studies have been initiated with both *A. fatua* and *A. barbata* in an attempt to elucidate the mechanisms underlying the maintenance of isozyme polymorphisms in these species.

5. Summary

1. Analysis of electrophoretic variations for three enzyme systems, (esterase, phosphatase and leucine amino-peptidase), indicate that *A. fatua* is genetically more variable than *A. barbata*.

2. The greater variability in *A. fatua* is due, in part, to a greater proportion of polymorphic *loci* and, in part to a greater average degree of polymorphism at each *locus*.

3. Provisional estimates indicate that 31 per cent. of *loci* in *A. barbata* and 54 per cent. of *loci* in *A. fatua* are polymorphic for electrophoretic variants in the population surveyed. Further a close correlation was found to exist between the degree of polymorphism for the electrophoretic traits and the degree of polymorphism for morphological variants.

4. These results were compared with previous findings and discussed briefly in terms of the adaptive nature of isozyme variants.

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