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Population Biology of *Avena*

II. Isoenzyme Polymorphisms in Populations of the Mediterranean Region and Central California*

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Summary. Surveys for polymorphisms in natural populations of *A. barbata* sampled in California grasslands had provided evidence for widespread monomorphism and rather localized polymorphic areas in the north coastal and San Francisco regions, based on a set of morphological and isoenzymatic marker loci. Since this species, like many other annuals, was introduced from the Mediterranean region during the Spanish mission period, a comparative study of the Canadian-Welsh collections of *Avena* species from the Mediterranean region was undertaken using various plant characters and starch gel electrophoresis to analyze variants for esterase, phosphatase and peroxidase systems. A total of 96 samples including 73 of *A. barbata* and 23 of *A. hirtula* were studied and the results were scored to compute the polymorphism indices. In both species, only 10 to 15 percent sites showed any significant degree of polymorphism of which a majority seemed to originate from localized regions in Italy and Turkey; a part of this observed lack of within-sample variation might be the result of small sample size. In general, the patterns of variation in *A. barbata* from the California surveys and the present analyses seemed to be very similar and raised some interesting questions on (a) the colonizing history of introduced materials (b) the factors underlying such marked patterns of geographical variation, and (c) the current evolutionary changes occurring in these two broad, disjunct areas of species distribution.

Introduction

Following the pioneering work of A. De Candolle and N. I. Vavilov, numerous recent accounts have been published on the origin and evolution of Old World cereals, their colonizing history in the New World and the patterns of cytogenetic differentiation. Relatively speaking, the genus *Avena* has been given far less attention than *Triticum*, *Hordeum* and *Oryza*. Recent Canadian-Welsh collections of various *Avena* species in the Mediterranean region (Rajhathy, et al., 1966) and cytogenetic analyses by Rajhathy, Zohary and others have stimulated a great deal of research in recent years. Two species, *Avena fatua* (6x, common wild oat) and *A. barbata* (4x, slender oat) occur in the California grasslands as well as in the agrestal or ruderal habitats, accounting for nearly 1 and 2 percent total cover, respectively (Talbot, et al., 1939). Our interest in these species is due to several reasons. As summarized by Burcham (1957), *Avena* species were phenomenally successful colonizers in the valleys and bordering foothills soon after their introduction (1769–1823, Mission period) from the Mediterranean region into California; later, they were reduced in competition with the other introduced annuals to somewhat lower but presently stable proportions. Both species are predominant inbreeders (percent outcrossing rate 5% and below) with high phenotypic plasticity, wide range of adaptability and with interesting patterns of genetic variation in California (Jain, 1969). Moreover, Naveh's (1967) comparison of the Californian and Mediterranean ecosystem reveals some very striking parallels in the species composition and the role of climatic variables as well as man's influence in these successional changes. In studying the population biology and evolution of colonizing species, the genetic homology of populations from these two regions would be interesting in order (a) to retrace the history and evolutionary dynamics of introduced genetic variation and (b) to understand the role of ecogenetic systems in colonizing species.

We have previously reported in a series of papers on the comparative ecogenetics of *Avena fatua* and *A. barbata* (Jain and Marshall, 1967, et seq.; Jain, 1969; Marshall and Jain, 1967). To date over 200 populations of *Avena* have been sampled which show consistently that *A. barbata* is polymorphic at several sites in the San Francisco Bay and adjacent coast ranges, and monomorphic in the interior valley region, whereas in contrast, *A. fatua* has widespread polymorphism over the entire area studied. In the Mediterranean region, as surveyed by Rajhathy, et al. (1966), *Avena barbata* and *A. sterilis* seem to provide ecological analogues of *A. barbata* and *A. fatua* of Central California, respectively. They also reported the distribution of diploid species, *A. hirtula* which very closely resembles *A. barbata* and in fact, requires chromosome counts for definite identification. In this paper, we shall report on an analysis of genetic variability in samples of *A. barbata* and *A. hirtula* (received through the courtesy of Dr. T. Rajhathy) and discuss the pattern comparatively.

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Materials and Methods

A total of 73 samples of *A. barbata* and 23 samples of *A. hirtula* were used for this study from a larger collection which Rajhathy, Zillinsky and Hayes (1966) had made in the form of seed bulks from the nine countries of the Mediterranean region. Identification of *barbata* versus *hirtula* was done by Dr. Rajhathy and his associates on the basis of chromosome counts. These 96 samples were grown in a twice replicated randomized experiment in the field at Davis to score variation at marker loci for lemma color (B, b), lemma pubescence (H, h), leaf sheath hairiness (Ls, ls) and node hairiness (Np, np) and for several quantitative characters.

Seed harvested in bulk from these field rows or, in some cases, original bulks were used for scoring electrophoretic variation by the starch gel method as described by Kristjansson (1963), Shaw and Koen (1968), Stormont (unpubl.) and specifically adapted for *Avena* by Dr. D. R. Marshall (personal comm.). Briefly, the method is based on the relative mobilities of protein molecules in an electrical field and specific staining to get the socalled zymogram display of some known isoenzyme systems. Genetic analysis of any variation, of course, requires usual Mendelian ratio studies on parents, hybrids and the segregating generations. Samples for this analysis used two uppermost leaves of the 10–12-days old seedlings; three horizontal slices of each gel were used for scoring the esterase, phosphatase and anodal peroxidase systems (further methodology described by Shaw and Koen, 1968; Rendel and Stormont, 1964).

Data analysis essentially involves certain comparisons of the degree of polymorphism between the two sets of markers, or between the California and Mediterranean collections. A statistic, defined as polymorphism index (PI), calculated as $\sum p_i q_i/n$, or following Brown (1969), for multiallelic loci as $\frac{1}{n}\sum_{i=1}^{n} m_i \sum_{j=1}^{m_i} p_{ij}(1-p_{ij})$, where n = number of loci, m_i = number of alleles at the i^{th} locus, can be computed based on the estimates of allelic frequencies at individual loci, or where genetics has not been worked out, based on the phenotypic proportions for the presence or absence of individual bands.

Results and Discussion

Taking all gels into account, a total of 28 bands (16 for esterase, 9 for phosphatase and 3 for anodal peroxidase) could be scored as shown diagrammatically in Fig. 1. The relative positions as shown in terms of a distance scale from the origin (sample location) are based on a careful evaluation of parallel distributions among the gels and averages based on only the unambiguous reading of gels. The genetics of five sets of bands, governed by five loci, was discussed by Marshall and Allard (1969) as follows: Esterases: (.41, .49) E_4, (.90, .92) E_9, and (.95, .96) E_{10}; Phosphatase: (.75, .83) P_5; Anodal peroxidase: (.71, .85, .88) APX_5. Singh (unpub. data) has further analyzed some segregating materials for the inheritance pattern of these and other bands shown in Fig. 1. For the present, we shall score polymorphisms in terms of the presence or absence of each band; this introduces an overestimation bias that might very well be counterbalanced by the various sources of underestimation as discussed by Lewontin and Hubby (1966), and others.

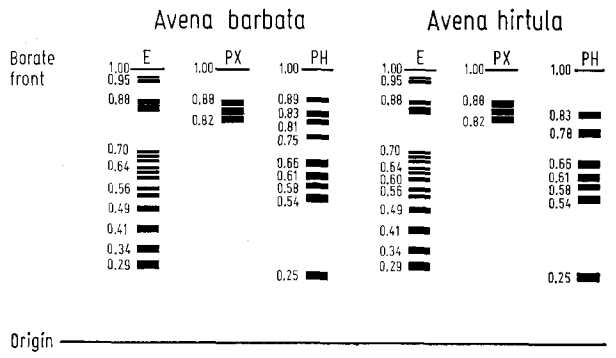

Fig. 1. Diagrams showing the relative position of bands (E = esterase, PX = peroxidase, PH = phosphatase systems) scored as the proportion of distance between origin and borate front. Note a strong similarity between the bands in *A. barbata* and *A. hirtula*

Note that our tally of all scorable bands shown in Fig. 1 included all except the APX_5 band at the .71 position reported by Marshall and Allard. Instead, a new band at the .82 position was scored in these materials. Preliminary analysis in bulk samples leaves the possibility open that two loci or three different alleles may be involved in the three APX_5 bands. It is interesting to note the band homology of two species, *A. hirtula* and *A. barbata*; *A. hirtula* has the same bands as *A. barbata*, except two of the phosphatase bands (.75 and .89 position). Their close homology supports the findings and interpretation of karyotype features, morphological and ecological similarities, etc., as discussed by Rajhathy and Thomas (1967), and Ladizinsky and Zohary (1968). The „autotetraploid" origin of *A. barbata* from two partially differentiated A_s genome parents, and chromosome pairing controlled by certain pairing gene(s) seems valid. Critical tests of protein similarities will, however, require use of hybrids and progenies, as discussed by Hubby and Throckmorton (1968). The similarity of *hirtula-barbata* bands is in sharp contrast to the *fatua-barbata* differences reported by Marshall and Allard (in press) in which case only 3 potentially homologuous bands were found. Further work on protein similarity will be helpful for a full evaluation of the cytogenetic evidence on their genome relationships.

Our survey of 96 samples (from 96 different collection sites), with a total of nearly 1200 plants, for variability in the occurrence and relative frequency of individual bands showed that only 22 out of 73 *barbata* samples and 6 out of 23 *hirtula* samples had any variation among the individual plants (or plant progenies), i. e. only 26–30 percent of samples were polymorphic for *one or more* bands; the others were monomorphic. It is important to note that although samples are small, the probability of a monomorphic sample for n loci is k^n, if k is the probability of being monomorphic at any one locus. Among different sites, polymorphic or monomorphic, there are marked differences in the presence versus absence of certain bands

Table 1. *Mean polymorphism indices for individual enzyme systems*

Region	*barbata*				*hirtula*				No. of samples showing variation/total
	E	*Ph*	*APX*	No. of samples	*E*	*Ph*	*APX*	No. of samples	
Spain				0	0	0	0	1	0/1
Gibraltar	0	0	0	1				0	0/1
Morocco	0	0	0	1	.020	0	.040	4	1/5
Algeria	.011	.005	.011	11				0	4/11
Tunisia	0	0	0	1				0	1/1
Sardinia	.012	.003	0	6	0	0	0	1	2/7
Corsica	.005	0	0	7				0	1/7
Italy	.018	.023	.011	11				0	5/11
Sicily	.034	.014	0	4	0	0	0	2	1/6
Libya									
a) Cyrenaica	.014	.010	0	6	0	0	0	1	2/7
b) Tripolitanica	.010	0	0	3	0	0	0	1	1/4
Greece									
a) Attica	.004	0	0	5				0	1/5
b) Maced-Thrace	0	0	0	5				0	0/5
c) Crete	0	0	0	8	027	.065	.068	3	2/11
Turkey	.011	.018	.021	4	.004	.030	.041	10	5/14

from the entire bulk analyzed. It should be pointed out that small sample size due to collection procedures imposes uncertainty about both the intersite and the intrasite variability estimates. Yet, taking all samples into account, a general overall estimate is still valid. Table 1 gives estimates of the mean polymorphism index for each region and system. In general, polymorphism is low in the entire region, with some localized areas in Italy, Sicily, Crete and Turkey showing variation. Fig. 2 shows the distribution of polymorphic sites within different regions for the three enzyme systems. Overall there might be a longitudinal gradient, with some important differences in the eastern polymorphic region and the largely monomorphic areas in the western regions.

The pattern of variation in *barbata-hirtula* resembles that of *barbata* in California. Mild climate with relatively cooler summers of Bay region seems to favor polymorphism; moreover, there are localized areas of polymorphic and monomorphic populations within this region such that some local rather than regional factors alone seem to be involved in the maintenance of polymorphisms within individual populations. Monomorphic populations within an area might likewise show different allelic composition in that different alleles might be fixed in them. That allelic composition of *barbata-hirtula* samples was different can be illustrated by the data on APX allelic frequencies in the Mediterranean region:

Species	Percent Populations with Allele		
	.82	.85	.88
barbata	16.4	43.8	95.9
hirtula	8.7	65.2	43.5

With wider and detailed genetic analyses, it should be possible to establish further evidence for the degree of populational differentiation.

Data on morphological markers (lemma color, leafsheath hairiness) are summarized in Table 2. Since

Table 2. *Phenotypic variation in Mediterranean region sites*

Class	No. of sites scored	
	barbata	*hirtula*
BB	42	8
B + b	7	2
bb	10	12
Ls Ls	38	15
Ls + Ls	9	1
ls ls	12	6

bulks and not individual plant progenies were scored for each site, we cannot distinguish at this stage for a site between its being polymorphic with a mixture of homozygotes or with some heterozygotes. However, note that only a small proportion of samples had both alleles present for loci *B/b* or *Ls/ls*; all samples were monomorphic with allele *H* (hairy lemma). Other variations were observed for growth habit, lemma hair color, leaf width in the young seedling stage, and the occurrence of albino seedling character in a few cases. In general, most samples are monomorphic and show no consistent geographical cline over the entire region. The main point of interest in these data is the overall concordance between the variation for electrophoretic bands and the morphological markers, although sample size and number of samples from each subregion are too small for further quantitative analyses. For California, Marshall and

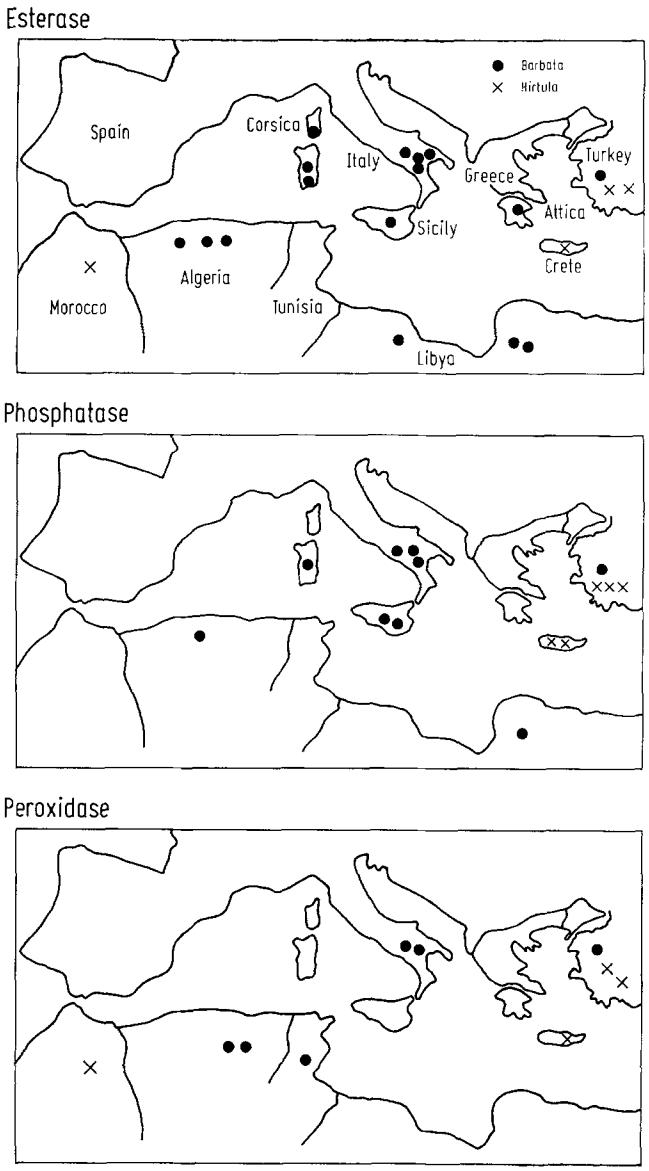

Fig. 2. Map of Mediterranean region showing the location of polymorphic samples for the two species and individual enzyme systems

Allard had reported a high correlation coefficient based on eight samples scored for polymorphisms at the morphological and isoenzymatic marker loci. With the results of our additional 11 samples given in Table 3, we can show the degree of concordance in a correlogram (Fig. 3). The value of correlation coefficient, $r = 0.74$ is statistically significant ($P < .05$). Similar analyses of Mediterranean samples of larger size would be very worthwhile. With the two systems of variants taken together, the estimates of variability between species, between regions or among localities within regions, all become more reliable and meaningful measures of the total genetic diversity patterns.

Table 3. *Estimates of mean PI for some Californian populations of A. barbata*

Site	Morphological markers		Electrophoretic markers	
	No. of plants	$\overline{PI}$	No. of plants	$\overline{PI}$
Catalina I., Middle Ranch	71	.003	32	.014
Catalina I., Salta Verde	66	0	16	.009
Near Camarillo (Hy. 1)	64	.004	39	.014
Angeles Natl. Forest	69	0	44	.005
Los Padres Natl. Forest	100	0	33	.008
9 m. N. of Santa Maria	21	0	25	.002
5 m. N. of Moro Bay	101	0	16	0
Carmel	100	.144	27	.040
Monterey, 17-Mile-Drive	20	.052	22	.011
Ukiah, Hy. 101	100	.077	44	.085
1 m. N. of Ukiah Hy. 101	103	.062	31	.030

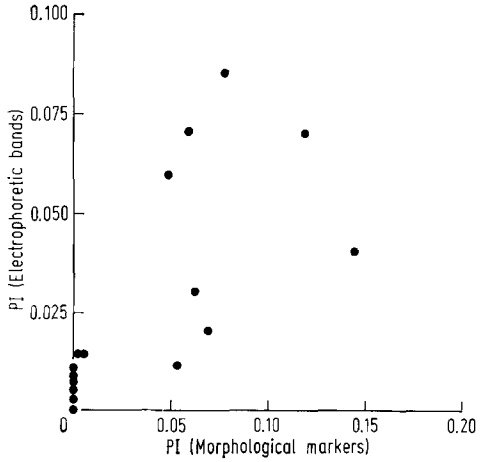

Fig. 3. Correlogram showing the joint distribution of PI values estimated from morphological and isoenzymatic markers in California samples of *A. barbata*

Finally, a summary of quantitative variation for such easily scored characters as the length of primary panicle and number of spikelets per plant, is given in Table 4 to compare the two species and for *A. barbata*, for a comparison of two regions. For both characters, *hirtula* had lower mean and a higher C.V. than *barbata*, indicating a relatively broader range of variation among and within populations. Also, note that *barbata* shows a narrower range of C. V. values and thus, on an average lower amount of variation. However, the amount of quantitative variation as measured here for individual sites was not correlated with the pattern of variation for isoenzymatic markers. Nongenetic sources of variation perhaps masked any consistent pattern for these two sets of phenotypic characters.

General Discussion and Conclusions

There are clearly notable similarities between the pattern of geographic variation reported here for the Mediterranean region and that for Central California

Table 4. *Estimates of variability parameters from data on bulks*

Region and species	No. of bulks	Panicle length $\bar{\bar{X}}$ (cms.)	Range of bulk means	$\overline{C.V.}$	Range of *C. V.,s*	No. of spikelets/plant $\bar{\bar{X}}$	Range of bulk means	$\overline{C.V.}$	Range of *C. V.'s*
A. Mediterranean									
barbata	37	42.2	34.3—50.8	.099	.034—.266	100.1	40.0—136.5	.207	.095—.394
hirtula	17	34.9	27.0—49.6	.120	.038—.253	73.4	21.7—113.4	.235	.100—.437
B. California									
barbata	16	38.2	29.2—49.1	.098	.041—.203	74.7	61.7—109.8	.195	.103—.370

$$C.V. = \frac{\text{standard deviation} \times 100}{\text{mean}}$$

reported earlier (Jain, 1969). *Avena barbata* has a majority of populations monomorphic with certain amounts of localized polymorphisms occurring in the Eastern Mediterranean region and the San Francisco Bay region in Central California. This similarity is reinforced by a significant concordance between the polymorphism indices for morphological markers and the electrophoretic variants. As noted above, the two main deficiencies, small sample size and lack of knowledge about the genetics of a number of bands, allow us to draw at present only broad, tentative conclusions; further studies have been undertaken to remedy these shortcomings in this study. However, it appears that either multiple sources of introduction have brought in the observed allelic diversity into California, or that it has originated more or less independently within these two regions, through mutation or hybridization with the other species, mainly the cultivated oats, *A. sativa*. Since polymorphic sites are patchily distributed *without a significant correlation of gene frequencies among the local neighborhoods*, local variation in gene flow alone is not sufficient to explain the pattern. The presence of clines in *A. barbata* over short distances (Jain and Marshall, unpub. data) and a more or less regional distribution of polymorphic populations suggest that both climatic and local habitat factors of adaptation might be involved. Moreover, the relative abundance of *A. barbata* in a given site and the pattern of its cohabitation with *A. fatua* were found to influence the estimates of polymorphism index in California populations (Jain, 1969). Thus, a detailed study of community diversity, population numbers and physical factors would be required to understand the factors maintaining variation and its role in colonizing ability. Whether these comparative patterns are stable or evolving under an increasing pressure of man's activities, would be important for an understanding of the successional events and the regulation of ecosystem as a whole due to new colonizing events. Comparative studies on other introduced Mediterranean annuals, e. g., softchess, rose clover and burclover, have been undertaken with these same objectives in mind.

The genome homology of *barbata* and *hirtula*, a pair of morphologically similar coancestral species (like sibling species), deserves further comment. Genetic studies on lemma color and leafsheath hairiness in *barbata* suggest duplicate factors in each case, whereas several isoenzyme variants reported so far in this species seem to be governed by single Mendelian factors. Genome duplication may have been a significant factor in the evolution of enzymatic diversity (Spafford, 1969; Watts and Watts, 1968). The work of Hubby and Throckmorton (1968) on the evidence for protein homologies between the sibling species in *Drosophila* and Ashburner's (1969) study on the puffing regions showing a parallel evidence for homology illustrate some new approaches to systematics and genome analyses. Rajhathy and Thomas (1967) and Ladizinsky and Zohary (1968) have discussed the cytogenetic aspects of genome analysis in *Avena* and hopefully, further electrophoretic studies on protein markers would also bear directly on their evidence.

Lewontin (1967) pointed out that the morphological markers are useful case histories „in which the adequacy of evolutionary theory can be tested", whereas isoallelic variation „uses gene-determined differences in the primary and tertiary structure of polypeptides and so has some claim to being a measure of total variation". Clearly, various genetic tools of analyzing the variation and adaptive processes in totality would provide a wholesome picture, and even when different in the direction of evidence, these different approaches might possibly yield a more balanced view of a species' genetic system.

Zusammenfassung

Untersuchungen der Polymorphismen in natürlichen Populationen von *A. barbata* im kalifornischen Weideland hatten einerseits zum Nachweis eines weit verbreiteten Monomorphismus und andererseits streng lokalisierter polymorpher Bereiche in der nördlichen Küsten- und San Francisco-Region geführt, wobei eine Anzahl morphologischer und isoenzymatischer Markerloci zugrunde gelegt wurde. Da diese Art, wie viele andere Annuelle auch, während der spanischen Missionsperiode aus der Mittelmeerregion eingeführt

wurde, wurde eine vergleichende Untersuchung der Canadian-Welsh-Sammlungen von *Avena*-Arten aus der Mittelmeerregion anhand verschiedener Merkmale der Pflanzen und der Stärkegelelektrophorese-Untersuchung auf Esterase-, Phosphatase- und Peroxydase-Systeme durchgeführt. Es wurde eine Gesamtheit von 96 Stichproben, bestehend aus 73 *A. barbata* und 23 *A. hirtula*, untersucht und die Ergebnisse zur Berechnung von Polymorphismus-Indices verwendet. In beiden Arten zeigten nur 10 bis 15% der Herkünfte einen signifikanten Polymorphismusgrad. Von ihnen scheint die Mehrzahl von lokalisierten Regionen in Italien und Griechenland abzustammen. Ein Teil des beobachteten Fehlens einer Variation innerhalb der Stichproben könnte eine Folge des geringen Stichprobenumfangs sein. Im allgemeinen scheinen die Variationsmuster der kalifornischen Untersuchungen und die der vorliegenden Analysen von *A. barbata* sehr ähnlich zu sein. Das führt zu einigen interessanten Fragen nach a) der Besiedelungsgeschichte des eingeführten Materials, b) den Faktoren, die derart auffallenden Mustern der geographischen Variation unterliegen und c) den laufenden evolutionären Änderungen, die in diesen beiden großen, voneinander getrennten Gebieten der Artverteilung auftreten.

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