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Review

Isozymes, Plant Population Genetic Structure and Genetic Conservation

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Summary. The exploration, conservation and use of the genetic resources of plants is a contemporary issue which requires a multidisciplinary approach. Here the role of population genetic data, particularly those derived from electrophoretic analysis of protein variation, is reviewed. Measures of the geographic structure of genetic variation are used to check on sampling theory. Current estimates justify the contention that alleles which have a highly localised distribution, yet are in high frequency in some neighbourhoods, represent a substantial fraction of the variation. This class, which is the most important class in the framing of sampling strategies, accounts for about 20-30% of variants found in 12 plant species. The importance of documenting possible coadapted complexes and gene-environment relationships is discussed. Furthermore, the genetic structure of natural populations of crop relatives might suggest the best structure to use in the breeding of crops for reduced vulnerability to pest and disease attack, or for adaptation to inferior environments. The studies reported to date show that whilst monomorphic natural populations do occur, particularly in inbreeding colonisers, or at the extreme margins of the distribution, polymorphism seems to be the more common mode. It is stressed here that the genetic resources of the wild relatives of crop plants should be systematically evaluated. These sources will supplement, and might even rival, the primitive land races in their effectiveness in breeding programmes. We may look forward to a wider application of gel electrophoresis in the evaluation of plant genetic resources because this technique is currently the best available for detecting genetic differences close to the DNA level on samples of reasonable size.

Key words: Allozymes – Genetic diversity – Sampling strategies – Coadapted complexes – Genetic resource evaluation

1.1 Introduction

The last decade has witnessed the emergence of a widespread concern to solve the major problem of the erosion of genetic resources caused by the global extension of modern cultivars of crop plants. Our current appreciation of the problem, and the international action taken thus far, have been clearly detailed at two international conferences held in Rome (Frankel and Bennett 1970; Frankel and Hawkes 1975) and summarized by Harlan (1975). The only realistic solution to the continuing loss of crop germplasm is genetic conservation; that is the collection and systematic preservation in gene resource centres of as wide a representation as practicable of the spectrum of genetic variation within those plant species upon which man depends, including samples of their endangered wild progenitors and relatives Conservation measures can include the storage of seed samples, or tissue cultures; the cultivation of plants in gardens and the protection of in situ populations in natural habitats.

It is important to distinguish the land race materials, or primitive cultivars of crop plants, from their wild relatives (see Frankel 1977). The land races are geographically or ecologically distinctive populations which are conspicuously diverse in their genetic composition both between populations and within them. They differ from their wild relatives because they have evolved under cultivation upon which most of them have come to rely for their survival. They differ from the varieties developed by modern scientific plant breeding in that they have not been deliberately intensively selected to a predetermined level of genetic heterogeneity (e.g. a pure line or hybrid variety, a mixture or a synthetic). Many of the land races have already vanished, and clearly the task is to recover samples from those which remain before they too disappear.

By contrast, many species of wild relatives are today abundant and generally there is much more material available than can possibly be, or should be, conserved. The

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existence of these populations is not dependent on cultivation, and not directly threatened by the extension of modern varieties. However, there is a threat from the reduction in available habitats (Whyte 1963) for species which are relatively rare. The task then for wild relatives is the collection of representative samples, which should be made readily available for critical evaluation and use by plant breeders (Harlan 1976).

This review specifically examines the role of information on population genetic structure – particularly that obtained from isozyme studies – in expediting the sampling, evaluation and use of crop genetic resources. First, it is necessary to define the term population genetic structure, and second, to consider the problem of measuring genetic diversity itself, comparing the isozyme technique with more traditional methods. Next the question of optimal sampling strategies is examined. Procedures often have to be adopted when there is little or no knowledge of the population genetic structure of the target species available. Are the assumptions underlying these procedures sound, in the light of current data?

More general questions which can also be approached using isozyme data are then reviewed. First, can mutagenesis replace conserved variability as the source of genetic variation? Second, should the choice of populations to be sampled and used, be guided by environmental criteria? Third, what genetic structure is likely to be optimal for the continuing productivity of crop varieties? Fourth, how might the relative potential utility of genetic resources be assessed?

1.2 Population Genetic Structure

The variation pattern of a species can be measured in terms which collectively define the population genetic structure of the species.
These measures include –
(i) the genetic diversity in the average population,
(ii) the range in levels of diversity in different populations,
(iii) the extent and variation in genetic correlation or genetic distance between different populations.
The components of genetic diversity to be considered in all three cases include the kinds and numbers of alleles present, the heterozygosity, and the correlation of alleles between loci.

The values which these measures assume are the outcome of two groups of evolutionary forces. The first group arises from *population structure*, that is the effective size and demographic composition of populations (their age structure, reproductive dynamics and regulation) and their mating system, their degree of isolation and rate and pattern of migration. These forces can vary in space and time. Jain (1975) defines population structure as 'the totality of ecological and genetic relationships among the member individuals as well as the subdivisions of a biological species'. The second group is the *spatial and temporal distribution of selection intensities.*

Some authors (e.g. Levins 1963) have used the term population structure to denote the realised or observed distribution of genetic variation between populations. This review follows Wright (1969), and Cavalli-Sforza and Bodmer (1971) and reserves it for one of the causes of the observed population genetic structure.

1.3 Measurement of Genetic Diversity

Given a sample of material, how does one assess its level of genetic variation? This is just another form of the question discussed by Lewontin (1974) as 'the struggle to measure variation'. Ideally we would like an inventory of nucleotide sequence differences in the DNA. Then all measures of population genetic structure, which were mentioned above, could be computed. It appears that this ideal might become a reality, at least for certain classes of DNA (e.g. chloroplast and mitochondrial DNA, and highly repeated DNA). However, in general it is obvious that we must be satisfied with much less than the ideal. In the case of crop genetic resources, three kinds of measurements have commonly been employed: (i) economic and other metric traits (e.g. Goodman 1968; Brown, Daniels, Latter and Krishamurthi 1969), (ii) disease resistance genes (e.g. Ashri 1971), (iii) Mendelian morphological markers (e.g. Jain, Qualset, Bhatt and Wu 1975).

It would indeed be most desirable to measure genetic variation for such characters as yield and range of adaptation. However it is impossible to recognise and enumerate in a population the different genotypes at loci which affect these basic characters because such variation is generally manifest as subtle differences. These differences are blurred beyond the range of experimental detection by environmentally induced variation (plasticity). It should be noted that the evaluation of the level of genetic diversity in a sample is a problem different from the evaluation for its agronomic merit.

Disease resistance screening has been widely used in both the land races (e.g. Qualset 1975; Harlan 1977) and the wild relatives (e.g. Harlan 1976; Dinoor 1977) of crop plants. However this is clearly only one fraction, albeit a very important one, of genetic variation. Morphological characters also are of great interest in themselves. An example of the importance of such characters is the disarticulation complex which distinguishes the wild and cultivated forms of a species (Zohary 1969). Land races of wheat and barley are often obviously variable for spike shape, basal node fertility, glume colour and pubescence,

seed colour, awn colour, length and angle (Jain *et al.* 1975). Morphological polymorphisms have been extensively used in studying the genetics of crop and wild species inbreeding populations (Bal, Suneson and Ramage 1959; Jain and Allard 1960; Allard and Hansche 1964; Jain 1976). Despite their obvious advantage of speed in classifying large numbers, these characters again represent only a limited fraction of the genome, and suffer from the technical problems caused by dominance, and the difficulty of distinguishing between multiple alleles or between loci. Polymorphisms for plant metabolites such as the flavonoid pigments (Fröst *et al.* 1975) have similar technical difficulties. Furthermore, the number of genes involved in all these characters is only discernible if in fact variants to them exist.

Lewontin (1974) has clearly expounded the advantages of electrophoretic surveys of proteins as measures of genetic diversity. These are as follows – (1) The effect of an allelic substitution on the phenotype used for its detection (mobility in a gel subject to an electric field) is usually unambiguous and does not depend on the environment (for an exception see Cullis 1977). (2) Allelic substitution at one locus is normally distinguishable from that at another. (Generally, polyploidy can lead to an exception, as for the *Adh* polymorphism in the tetraploid *Bromus mollis* (Brown, Marshall and Albrecht 1974) where nullisomic stocks are not available to distinguish homoeologous loci). (3) At least 25 percent of substitutions are detectable, (and this detectability is not immediately related to their biological effect, Marshall and Brown 1975a). (4) The loci which can be studied may approach a random sample of all structural genes irrespective of their level of variation. (However the effect of enzyme function on the extent of variation found by Johnson (1974) indicates that this fourth point should be carefully examined).

Because of the sensitivity of electrophoretic mobility to single amino acid substitutions, and the facility with which an array of loci can be assayed using material in minute quantities and with minimal preparation, the application of electrophoretic screening over the last decade has revolutionised the field of experimental evolutionary genetics. These techniques will also play an increasingly important role in the manipulation of our crop genetic resources (Rick 1976), as the following consideration of sampling strategies, coadapted complexes, environmental matching and population structure will make clear.

2.1 Sampling Strategies

Some rules of sampling which improve the efficiency in terms of maximizing the genetic variation collected per unit of cost and effort can and must often be formulated a priori (Marshall and Brown 1975b). Lacking precise knowledge of the distribution of variation, we must assume at the outset that all populations have essentially similar levels of variation. The optimal strategy that follows from this assumption is (i) to collect 50-100 random individuals per site, (ii) to sample as many sites as possible within the time available, and (iii) to ensure that sampling sites represent as broad a range of environments as possible, within the target area.

First, the assumption that all populations have the same level of genetic variation is a simple and crude one. It is known to be false in the case of *Avena barbata* in California (Clegg and Allard 1972). To what extent does the assumption hold in regions close to centres of diversity of crop species? Second, the above strategy assumes there is between populations considerable differentiation for the kinds of alleles present. In particular it gives priority to alleles which are common (say frequency greater than 10%) in only one localised area and rare or absent from other populations in the target area. If populations lack such differentiation, much larger samples at fewer localities may suffice, with concomitant saving in collection and maintenance costs. Third, it assumes that the differentiation itself is adaptive and ecologically meaningful. Otherwise sampling sites should be chosen not on maximised environmental diversity per se, but randomly, or on other criteria such as population size, degree of isolation, parasite and disease incidence, or environmental unpredictability.

2.2 Variation in Diversity

(i) Macrogeographic Patterns

Since the pioneering work of Valilov, it has been a basic tenet that the world distribution of crop plants differ regionally in their level of genetic diversity. There are some recent data, obtained from the study of world collections in hand, to support this view. For example, in the world collection of safflower, Ashri (1971) identified regions (the Near East, Egypt and Iran) from which disease resistant lines originated at high frequencies. Jain *et al.* (1975) found the greatest diversity for morphological characters in a world collection of durum wheat to be in Ethiopia and Portugal. Variation of flavonoids between barley lines was greatest in the Near East (Iran, Afghanistan) and Ethiopia (Fröst *et al.* 1975). For esterases in barley, Allard *et al.* (1971) found the most variation in entries of the USDA collection from Middle South Asia. The esterases of rice are most diverse in the Nepal entries (Nakagahra *et al.* 1975). Finally Rick and Fobes (1975b) showed that the Ecuador-Peru cultivars of tomato are conspicuously more variable at five isozyme loci than those of other American regions. Whilst these studies are very im-

portant for the use of collections in hand, there are potential hazards in extrapolating back to population variation in the field. Most collections have been assembled from the entries of different collectors. Frankel (1977) has pointed out that the entries are not representative of the original populations because the samples were small, non-random, subject to erosion or duplication, or received from secondary sources.

Knowledge of macrogeographic patterns of variation is one important factor when deciding in which region a particular collection should be mounted, that is in deciding the priorities for the target area. Once this decision is made, sampling strategies then hinge on the distribution properties between populations within the target area. Data at this level are discussed in the next section.

(ii) Patterns Within a Target Area

Unfortunately quantitative estimates of variation in levels of genetic diversity in plant populations, as measured by the isozyme technique, continue to be scarce – especially in land race materials. Table 1 gives some recent estimates in natural populations, summarized from the literature. For each species the table shows the number of populations, the total number of protein loci scored, the number of variable loci (n_v) and diversity statistics based on Nei's (1975) measure of the mean expected panmictic heterozygosity ($H_e = \Sigma\, p_i\,(1\text{-}p_i)$, where p_i is the frequency of the i^{th} allele), averaged over the number of variable loci in the data set. The diversity statistics are the mean over all populations, the minimum and the maximum, expressed as a percentage.

Quite a range of population genetic structures is evident (as has been frequently expected, e.g. Jain 1975). Nevertheless, there is evidence of some important trends. First, the range of values for H_e in any one data set is influenced greatly by the breeding system. Populations of predominantly inbreeding species tend to show greater

Table 1. The mean, minimum and maximum diversity (H_e) per variable locus per population observed in various natural populations

Species and Region	Breeding System	Number of populations	No. of loci Scored	Variable (n_v)	Diversity ($H_e \times 100$) Mean	Min.	Max	Authors
Oenothera biennis Cook County	I	16	20	1	8	0	50	Levin (1975 b)
Oe. biennis S. Illinois	I	28	20	4	22	0	50	„
Avena barbata California	I	16	5	5	7	0	48	Clegg and Allard (1972)
Hordeum spontaneum Israel	I	28	28	25	11	0	20	Brown et al. (1978)
Lycopersicon pimpinellifolium Ecuador and Peru	I	43	11	11	14	0	27	Rick et al. (1977)
Lupinus subcarnosus Texas	S.C.	8	8	5	12	0	18	Babbel and Selander (1974)
L. texensis Texas	S.C.	10	8	5	43	34	53	„
Hymenopappus scabiosaeus Texas	S.C.	14	8	5	28	19	35	„
H. artemisiaefolius Texas	S.C.	12	8	5	29	21	43	„
Phlox cuspidata Texas	S.C.	10	16	7	11	0	27	Levin (1975 a)
P. drummondii Texas	S.I.	10	16	4	27	15	39	„
Stephanomeria exigua California	S.I.	11	14	8	30	22	38	Gottlieb (1975)
Picea abies Sweden	S.I.	11	4	4	36	32	41	Lundkvist & Rudin (1977)
P. abies Finland	S.I.	10	6	6	40	35	44	Tigerstedt (1974)

I = Predominant inbreeder S.C. = Self compatible S.I. = Self incompatible

variation in their level of diversity than do outbreeders. Second, this range is magnified when the number of variable loci (n_v) that are encountered or reported are few. This might indicate that extreme variation in the level of diversity (as measured by these statistics) might be an artefact due to too few loci in a study. Third, the fewer the number of polymorphisms in inbreeders, the greater is the discrepancy between the mean and the mode in diversity. The distribution tends towards an L-shape with the majority of populations scoring zero diversity, and a few showing high diversity for the loci studied. Inbreeding, recently colonizing species might be expected to continue to display this trend despite the inclusion of yet more polymorphic loci in the study (e.g. *Avena barbata* in California, Miller and Allard 1976). When it is known to the plant collector that this is the case, then the most efficient sampling, storage and evaluation strategy is to concentrate on the variable populations.

Two alternative patterns are exemplified in Figures 1 and 2. Figure 1 plots the distribution of diversity (H_e) values for 28 Israel populations of *Hordeum spontaneum* estimated from 28 loci as a histogram where H_e is expressed as a percentage of all 28 loci. The shape of the curve is bimodal with two populations showing no diversity as one mode. The remainder is convex (platykurtic) around the mean of 9.8%. Figure 2 is a histogram of the values for *L. pimpinellifolium* in Ecuador and Peru (Rick, Fobes and Holles 1977). The distribution is a trimodal one in which the group of populations with mode near 0.22 are from northern Peru and the remainder are from Ecuador and Southern Peru.

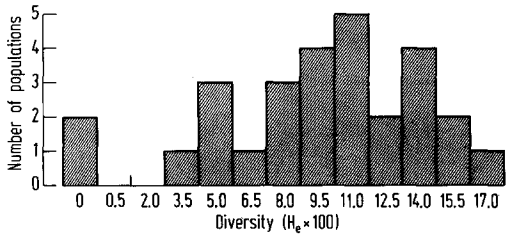

Fig. 1. The distribution of diversity (H_e x 100) values in 28 Israel populations of *Hordeum spontaneum* (Brown *et al.* 1978)

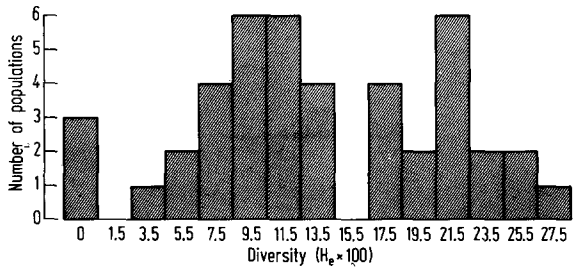

Fig. 2. The distribution of diversity (H_e x 100) values in 43 Ecuador and Peru populations of *Lycopersicon pimpinellifolium* (Rick *et al.* 1977)

The data of Table 1 do confirm that levels of diversity are likely to vary from place to place, nevertheless they do suggest that severe L-shaped distributions may be less common than previously supposed. Whether these conclusions can be extended to variation in diversity between samples of land races collected within a target area cannot as yet be assessed. Preliminary data from the polyallelic β glucosidase locus in Mexican races of *Zea mays* (Stuber *et al.* 1977) indicates that diversity levels do vary, but not inordinately, between samples.

2.3 Localised Common Alleles

We argued (Marshall and Brown 1975b) that the alleles which deserve priority in sampling procedures are those which have a restricted or localized occurrence, but high frequency. At this time evidence was lacking to support the existance of such a class of allozymes in plant populations (Lewontin 1974) unless populations were com- great similarity of allelic frequency profiles across populations (Lewont in 1974) unless populations were completely isolated from one another. Recent studies of various kinds of plant materials, however, indicated that alleles restricted to one or two populations or areas, but occurring there in appreciable frequency (say > 10%), can make up a substantial fraction of the variants at a locus. Table 2 summarizes these recent studies. For each entry is given the type of material (cultivars, land races, wild relatives, other natural species), the regional grouping of the data, the number of loci and the number of kinds of proteins studied. Each allele is classified on the number and distribution of occurrence of frequency > 10% into 5 classes:

(i) COMMON; At least one sample with frequency > 10%
- (a) WIDESPREAD; Common occurrences in more than 2 regions.
- (b) SPORADIC; Common occurrences in 2 regions.
- (c) LOCALIZED; Common ocurrence(s) in only 1 region.

(ii) RARE; Never occurs with frequency > 10%
- (d) WIDESPREAD; in > 1 region
- (e) LOCALIZED; in only 1 region.

The number in each class is reported in the table together

with the percentage of the total number of 'variants' contributed by this class (*in italics*). The percentage was computed by subtracting the number of loci studied from the number of alleles in class (i) (a). This adjustment standardized any differences in the number of invariant loci reported.

When these percentages are averaged over species in this admittedly heterogeneous set of materials and regions, the proportion of variants which fall into each class is 50%, 15%, 20%, 6% and 9% respectively. These figures would suggest that plant populations show quite appreciable levels of population differentiation for allele occurrence. This was found in both the outbreeding and inbreeding examples of Table 2. The effect of mating system on these statistics is much less than it is on the variation in levels of diversity (Table 1). Furthermore, the results of Table 2 substantiate the argument for sampling more populations at the expense of large samples per population, despite fluctuations in the level of diversity. This is because the risk of travelling to a new region of lower diversity can be offset by the chance of finding entirely new variants which are locally common in the new region.

Of course, questions of much wider significance than those concerned with the efficiency of collection can be addressed by studies of genetic variation. Pickersgill (1977) has recently emphasised the role of isozyme data in the analysis of evolutionary relationships of domesticates. The next four sections consider other issues which motivate the study of population genetic structure.

3.1 Rationale of Conservation – Coadapted Complexes

In general, genetic conservation is an open-ended task. There are no clear rules on how far into the future we would consider, (Frankel (1974) has suggested that the time scale is 50 to 100 years), which materials we will need, what characters will be at a premium, and therefore

Table 2. The numbers of alleles with various kinds of distribution (see text), and the percentage of variants in those classes

Species and items	Samples	Loci	Kinds of protein	Common			Rare		Authors
				Widespread	Sporadic	Localised	Widespread	Localised	
Oryza sativa cultivars	8 S.E. Asian countries	4	1	8 (*40*)	1 (*10*)	1 (*10*)	1 (*10*)	3 (*30*)	Nakaghara *et al.* (1975)
Phlox drummondii cultivars	16 named varieties	19	13	24 (*72*)	1 (*14*)	1 (*14*)			Levin (1976)
Zea mays land races	9 Mexican regions	1	1	10 (*45*)	4 (*20*)	4 (*20*)		3 (*15*)	Stuber *et al.* (1977)
„	38 Guatemala races	1	1	6 (*36*)	1 (*7*)	6 (*43*)	1 (*7*)	1 (*7*)	Stuber *et al.* (1977)
Hordeum spontaneum wild relative	28 Israel popns 7 regions	28	16	45 (*22*)	16 (*21*)	25 (*33*)	6 (*8*)	12 (*16*)	Brown *et al.* (1978)
Avena fatua wild relative	5 California populations	6	2	10 (*67*)	2 (*33*)				Clegg (1969)
Avena barbata wild relative	16 California populations	5	3	10 (*100*)					Clegg and Allard (1972)
Lycopersicon pimpinellifolium wild relative	11 Ecuador-Peru Depts or Provinces	11	3	22 (*28*)	3 (*8*)	9 (*23*)	2 (*5*)	14 (*36*)	Rick *et al.* (1977)
Lycopersicon cheesmanii wild relative	13 Galapogos Islands	8	4	11 (*21*)	4 (*29*)	7 (*50*)			Rick and Fobes (1975a)
Phlox cuspidata wild relative	10 Texas populations	16	9	19 (*42*)	2 (*29*)	2 (*29*)			Levin (1975a)
Phlox drummondii wild relative	10 Texas populations	16	9	21 (*100*)					Levin (1975a)
Stephqnomeria exigua wild plant	11 California populations	14	8	22 (*26*)	6 (*19*)	8 (*26*)	5 (*16*)	4 (*13*)	Gottlieb (1975)
Picea abies forest tree	11 Sweden populations	4	4	10 (*38*)	1 (*6*)	3 (*19*)	5 (*31*)	1 (*6*)	Lundkvist & Rudin (1977)
Picea abies forest tree	10 Finland populations	6	4	12 (*50*)		2 (*17*)	3 (*25*)	1 (*8*)	Tigerstedt (1974)
Average % of variant alleles				*50*	*15*	*20*	*6*	*9*	

which materials deserve priority. Economic trends, development of entirely new domesticates, evolution of new disease strains and environmental changes are unforseeable contingencies. Furthermore, collections require careful maintenance, and depend on both local support and international cooperation.

Therefore the idea that new variants be deliberately created by mutagenesis when they are required, is a very attractive and potentially more efficient alternative to conservation (Brock 1971). Furthermore, rapidly developing techniques for somatic genetic manipulation (Reinert and Bajaj 1977) offer the prospect of far greater immediacy and control of recombination and selection and ultimately, mutation than conventional procedures. As these techniques become part of the plant breeder's repertoire, it could be argued that they will make redundant the materials that have been collected and conserved.

Whilst there is no doubt that mutagenesis and tissue culture increase the genetic diversity at particular loci available for plant breeding, it is much less certain that artificial mutagens can replace natural gene pools as sources of *coadapted gene complexes*. This leads to the question – Just how important are such complexes in the adaptation of natural populations to their environment, and in crop plants for productivity? This question has been an important one to experimental population genetics since the pioneering work of Dobzhansky on *Drosophila pseudoobscura* (1970). It has given rise to an array of notions of 'coadaptation', the major of which are summarized in Table 3. These notions rest on different phenomena. They are obviously neither mutually exclusive nor identical. They cover a range of use of the term 'coadaptation' from the biological particularity of Darwin through the virtually degenerate or redundant usage of Mayr, for whom the mere existence of an allele in a natural population is sufficient proof that it is coadapted to the rest of the gene pool.

The esterase loci in barley (*Hordeum vulgare*), closely studied by Allard and his colleagues (Clegg, Allard and Kahler 1972; Weir, Allard and Kahler 1974) furnish one interesting example of coadaptation at the level of linkage disequilibrium. Three loci are tightly linked, ($B \cdot \overset{002}{\text{——}} A \cdot \overset{005}{\text{——}} C$) and a fourth, *D*, recombines independently. In composite crosses they behave as a complex in that only relatively few specific combinations of alleles at these loci increase in frequency whereas most other combinations become rare. Natural populations of wild barley in Israel also exhibit strong disequilibria at these loci (Brown, Nevo and Zohary 1977). For example, the population at Damun, polymorphic for *A*, *C* and *D*, shows complete association between *A* and *C*, 20% of maximum association between *A* and *D*, and 40% between *C* and *D*. Associations also occur between esterase and other loci, and in most wild barley populations in Israel. They are as a conspicuous feature of the genetic structure here, just as they are in the polymorphic populations of *Avena barbata* in California (Allard *et al.* 1972) and in both wild and cultivated accessions of *Lycopersicon esculentum* (Rick, Zobel and Fobes 1974). The esterases of rice cultivars (Nakagaha *et al.* 1975) constitute another possible example. Thus the finding of examples of coadapted complexes in populations of wild relatives or land races is of key importance in clarifying the need for genetic conservation.

Table 3. Notions of coadaptation

	Phenomenon	Author
1.	The coordination at the level of the *individual* of many traits for some single adaptive purpose.	Darwin (1872)
2.	*Heterokaryotypes* of locally occurring chromosomes are more uniformally fitter than those of geographically disparate origin.	Dobzhansky (1970)
3.	*Associations* between particular alleles at loci within a chromosomal inversion, and the particular inversion.	Lewontin (1974)
4.	Switch mechanisms comprising tightly linked gene complexes or *supergenes*.	Ford (1971)
5.	*Repulsion heterozygotes* for polygenes which store a greater level of unexpressed genetic diversity than coupling heterozygotes.	Mather (1973)
6.	The frequency of occurrence of specific *multilocus gametes* differing significantly from that anticipated by the product of the individual allele frequencies ('linkage disequilibrium').	Allard *et al.* (1972)
7.	The set of *gene frequencies* at selective peaks are harmoniously balanced – *epistatic interactions* in fitness of alleles across loci.	Wright (1969)
8.	The *genes* which exist in the local gene pool are coadapted by definition.	Mayr (1965)

3.2 Rationale of Sampling Location and Utilization

The guiding principle of plant exploration for agricultural purposes in recent decades has been that of environmental matching (Hartley, 1963, Rick 1973). This principle states that the most likely source of superior germplasm for a specified need is that population from an environment which is physiographically most similar (homoclimatic) to the local one for which the improvement is sought. This principle is soundly based on Darwinian evolutionary theory and on the wealth of evidence which documents the adaptation of specific populations to their local environment (Briggs and Walters 1969; Bradshaw 1972).

Turning to allozyme variation itself, however, the evidence is that this mode of variability is adaptive, indirect or equivocal (Lewontin 1974; Nei 1975) and very few

studies have been made of natural plant populations. Electrophoretic variants at the alcohol dehydrogenase locus have been shown to differ physiologically in *Zea mays* (Marshall, Broue and Pryor 1973), and *Bromus mollis* (Brown, Marshall and Munday 1976). They also show geographic differentiation according to soil moisture status in wild sunflower (Torres, Diedenhofen and Johnstone 1977). In the case of *B. mollis* the variant with lower enzymatic activity was associated with greater tolerance to flooding but a slower rate of germination. Variation at both *Adh* loci in Israel populations of *H. spontaneum* is summarized in Table 4. Variation was not common as 18 of the 28 populations were fixed for an allele electrophoretically identical with the allele common in cultivars of barley. Five populations in the Hule Valley and Kinneret regions had a variant *S* at the *Adh-1* locus in high frequency. This area was previously waterlogged, indicating a possible link between soil moisture status and *Adh* polymorphism. Since three alleles at each locus have been recovered it is possible to assemble nine homozygous *Adh* genotypes for biochemical and physiological study in relation to problems of excessive soil moisture or germination speed in barley.

The aim of sampling for genetic conservation should be to obtain as much genetic variation at as many loci as possible, in addition to the seeking of a particular disease resistance or a specific adaptation. Therefore, we infer from the principle of environmental matching that one should sample populations from as many distinctly different environments as possible. In this way one would capture any differentiation which has occurred between populations for both currently adaptive alleles (by choosing a diversity of different habitats or climates), and currently neutral alleles (simply by travelling to other partially isolated populations). One example of the merit of sampling from widely different environments is afforded by the phosphoglucomutase (*Pgm*) polymorphism in Israel populations of *H. spontaneum*. Twenty of the 28 populations studied were fixed for a common allele. The population from Mt. Hermon (the most elevated occurrence in Israel at 1400 m) was fixed for an alternative allele which was found nowhere else. Three hundred kilometers south, in four isolated populations of the Negev desert steppes where the annual rainfall is about 100-200 mm, *Pgm* was polymorphic for the common allele and a third allele which also had a remarkably isolated occurrence. Geographic differentiation at the *Pgm* locus is likely to be adaptively meaningful because of the possible regulatory role of this enzyme in metabolism (Johnson 1974). An example of adaptive differentiation in enzymatic properties of populations from different types of climate (although without allozyme analysis) is given by McNaughton (1974) for malate dehydrogenase in *Typha latifolia*.

Table 4. Population differentiation at the *Adh* loci of *Hordeum spontaneum* in Israel

		Location and No. of Populations						
Locus	Allele	Various		Hule Valley, Kinneret		Bar-Giora	Jerusalem (Talpiot)	Atlit
		18	2	3	2	1	1	1
Adh-1	F						0.52	
	M	1.0	0.89	0.62		1.0	0.48	1.0
	S		0.11	0.38	1.0			
Adh-2	F						0.52	0.08
	M	1.0	1.0	1.0		0.02	0.48	0.92
	S					0.98		

There exists already a number of reports of clinal variation in allozyme frequencies. In *Avena barbata*, Hamrick and Allard (1972) studied a cline for alleles at five enzyme loci which they related to increasing aridity. Levy and Levin (1975) found latitudinal or longitudinal clines at 4 loci in *Oenothera* species. Bergman (1975) reports a case of correlation between allele frequency at one acid phosphatase locus in *Picea abies* and latitude and/or altitude. Rick *et al.* (1977) found three kinds of clinal structure for allozyme variants in *L. pimpinellifolium*. These were (i) simple regional clines, in which one allele characteristic of one extremity of the linear distribution is gradually replaced by an alternative from the other extreme, (ii) single-peaked clines in which an allele predominates in some region within the range and (iii) double-peaked clines in which an allele exhibits two widely separated centres of concentration.

Jain (1976) reviews some of the difficulties that arise in the interpretation of geographic patterns, especially when founder effects and migration become superimposed on an overall patchwork. Despite the difficulties, however, indeed while fully cognisant of them, the testing for relations between ecological variation and allelic occurrence, or between ecological variation and levels of diversity (Nevo, 1978) must remain an important task. Once such relations are demonstrated, and when they can be further supported by analytic biochemical or physiological studies (Koehn 1969), the case for the adaptive significance of the variants is compelling.

3.3 Rationale of Genetic Structure for Productivity and Reduced Vulnerability

Recently there has been increased awareness of just how vulnerable our highly selected, advanced cultivars are to new pest and disease attack (National Academy of

Sciences 1972; Day 1977). This has raised again the question of whether or not genetically heterogeneous populations might offer an added safeguard (Marshall 1977). Not only is the constantly evolving biotic environment of our crops to be considered, but also there are problems over wide areas in the physical environment. Examples of these are the extension of plantings into more marginal areas, rising soil salinity, increasing costs of fertiliser and energy, and soil loss by erosion (Brink *et al.* 1977). There is also the possibility that certain heterogeneous populations may be marginally more stable for yields in fluctuating environments (Marshall and Brown 1973).

The variation within a genetically diverse cultivar may be structured in any of three distinct ways –

(i) as heterozygosity, such as in a hybrid variety,

(ii) as a small number of distinct genotypes, e.g. a varietal mixture,

(iii) as many independently segregating polymorphisms so that every individual in the population is essentially a distinct genotype, e.g. a composite cross.

With this in mind, it is interesting to examine the genetic structure of natural populations, particularly those of species which are related to a crop. Are such populations generally more heterozygous than expected? Are they polymorphic in particular situations? Are their genotypes structured into multilocus associations? Are there consistent differences between marginal vs central populations in their genetic structure?

However we cannot assume uncritically that the genetic structures observed in natural populations provide a paradigm for the way to structure genetic variation within a crop to achieve optimal performance. Yet equally we cannot be confident that the high productivity of today's genetically pure cultivars will be maintained in the face of environmental challenges. The genetic structure of natural populations might provide a tentative lead on how to meet these problems. A survey of relevant examples (most of which are inbreeders) in the literature (Table 5) shows that local genetic structures vary dramatically within the geographic range of the species. Edaphic and climatic variables will therefore have to be considered when predictions for crops are made. As yet the data are too few to indicate which environmental parameters are likely to be the key ones in making predictions.

Table 5. Descriptive summary of genetic structures of natural populations of some economically important inbreeders or their relatives

Species and Region	Polymorphism in local populations	Heterozygosity observed Vs expected	Authors
Avena barbata California	Variable extensive MM* regions, others PM	Sometimes in excess with aridity	Marshall & Allard (1970) Clegg & Allard (1972) Jain (1976)
Israel	Polymorphism increases with altitude and/or 'humidity index'.	Very low	Kahler *et al.* (1977)
Bromus mollis Australia, California	Frequently PM	Tends to excess with aridity	Brown *et al.* (1974) Jain (1976)
Hordeum spontaneum Israel	Varying from zero to high levels	As expected	Brown *et al.* (1978)
H. jubatum	Frequently PM	As expected	Babbel and Wain (1977)
Lycopersicon esculentum South America	Peruvian region PM, Others MM	Absent	Rick *et al.* (1974)
L. pimpinellifolium Ecuador – Peru	North Peru extensively PM, declines N and S to MM populations	Often in excess	Rick *et al.* (1977)
L. cheesmanii Galapogos Island	Tiny populations MM, PM within islands	None found	Rick *et al.* (1975)
Glycine spp. Australia	Most populations MM, few PM	None found	Broue *et al.* (1977)
Avena fatua California	Extensively PM	Often in excess	Clegg (1969)

* MM = monomporphic PM = polymorphic

3.4 The Relative Importance of Various Sources of Diversity. Comparative Diversity of Advanced Cultivars, Land Races and Wild Relatives

There are in the literature a few attempts to compare the diversity in the various kinds of gene pools for protein markers. Table 6 summarizes some examples divided into two kinds of data. In the first, the comparisons are drawn at the level of a large sample. The studies with rice and tomatoes have emphasized that cultivated forms are generally markedly depauperate in the alleles to be found in wild species. The reverse conclusion for barley is probably due to the limited sampling of *H. spontaneum*, as the authors point out. It may also reflect the possibility that variation at esterase loci may not be representative of the genome.

Comparisons at the population level can be made in three studies; for barley, *Phlox* and French beans. In *Phlox*, the average self incompatible wild population contains about 70% of the total variation, as measured by H_e. Domestication has changed the breeding system to self compatibility and reduced the diversity within cultivars. However between cultivars there has been an *increase* in diversity with domestication, presumably due to man's predilection to cherish newly arising varieties. In barley, the evidence points to an alternative conclusion. The average Israel population of *H. spontaneum* is about 50% more variable than Suneson and Wiebe's (1962) famous composite cross XXI, measured at the 17th generation for the same loci. This composite cross was originally synthesized from 6,200 barley accessions in the world collection. Variation would likely have been higher in the first generation. For example, between the parents of composite cross II, the diversity of the 4 esterase loci is 48% per locus (Kahler and Allard 1970). These loci show a diversity per esterase locus in C.C. XXI F_{17} of 35%. This indicates that about 1/4 to 1/3 of the variation has been lost, probably in segments with poor adaptive value in the composite cross. The studies in *Phaseolus* are preliminary as they are based on a limited sample of loci with some results lacking. The two species showed contradictory trends in the comparison of land races to wild species, probably due to the small sample (3) of *P. vulgaris* cultivars.

Overall, the present meagre evidence indicates that populations of the wild relatives of cultivated plants do contain large amounts of genetic variation. This supports the case for the representative collection, evaluation and

Table 6. Comparative genetic diversity of gene pools

Comparison	Basis	Result	Authors
(i) Total sample data			
Hordeum vulgare (297 lines)	3 Esterases	42% in *H.v.* only	Allard *et al.* (1971)
H. spontaneum (20 lines)	43 alleles	40% shared	
		18% in *H.s.* only	
Lycopersicon esculentum	10 loci	2% in *L.e.* only	Rick and Fobes (1975b)
47 Peruvian cvs	49 alleles	37% shared	Rick *et al.* (1977)
44 wild accessions		61% in *L.p.* only	
(*L.e.* var. *cerasiformae*)			
43 *L. pimpinellifolium* popns			
Oryza sativa and *perennis*	2 Px + 3 Acph	53% shared	Pai *et al.* (1973)
	17 alleles	47% in *O.p.* only	Pai *et al.* (1975)
	Esterase	78% shared	Shahi *et al.* (1969)
	9 bands	22% in *O.p.* only	

(ii) Population data			Sample size	Alleles per locus	100 x H_e	
H. vulgare	C. Cross XXI F_{17}	28 loci	100	1.39	6.7	Brown (unpub)
H. spontaneum	within popns.		42	1.48	9.8	Brown *et al.* (1978)
Phlox cultivars	– within cvs	17 loci	56	1.12	4.1	Levin (1976)
	– total		16	1.41	13.3	
P. drummondii	– within popns		28	1.25	6.3	Levin (1975a)
	– total		10	1.29	8.9	
Phaseolus	– 8 indigenous cvs	4 loci	22	1.14	3.9	Wall & Wall
coccineus	– 5 wild popns		22	1.25	10.0	(1975)
P. vulgaris	– 3 indigenous cvs		23	1.50	15.9	Wall & Wall
	– 5 wild popns		22	1.15	5.4	(1975)

distribution of such material; factors which have restricted its use in breeding (Harlan 1976).

It remains to point out two important considerations which affect the interpretation of population genetic structure and the issues above.

4.1 Genetic System

An appreciation of the genetic system (Mather 1973) of a species is fundamental to understanding differences between species in the organisation of its genetic variability. The components of the genetic system include the breeding system, karotypic structure, level of ploidy, life cycle features, and the level of recombination, together with temporal and spatial variation in these components. For most species meriting genetic conservation, these components are known in broad outline beforehand. Thus, for example, a mating system with predominant self pollination will, in relation to the preceding sections,

(i) affect the definition of population structure and markedly reduce neighbour size (Wright 1969; Jain 1976), – section 1.2,

(ii) increase the scope of spatial variation in selection to produce population differentiation – section 1.2,

(iii) increase the degree of linkage between loci and assist in the building of coadapted complexes (Allard 1975) – section 3.1,

(iv) reinforce the strategy of sampling more populations and fewer individuals per population – section 3.2,

(v) reduce the level of heterozygosity expected for neutral genes, and thus alter the basis of comparison of genetic structures – section 3.3,

(vi) indicate that fewer samples per line and more lines should be used in the evaluation of wild relatives (Rick 1976) – section 3.4,

(vii) reduce the likelihood of pollen contamination during the rejuvenation of collections (Rick 1976).

4.2 The Neutrality Controversy

There has been a considerable debate recently, concerning whether allozymic variation in natural populations is selectively meaningful or whether it is just 'evolutionary noise' (Lewontin 1974). Likewise, it might be argued that the assay of population genetic structure by means of electrophoretic procedures and the scoring of allozyme variants may be of little practical interest if such variation is in fact adaptively neutral. This is another way of stating that this variation is of little direct interest to the genetic conservationist *per se*, because its correlation with agronomic characteristics is obscure. This objection harkens back to the points made in the earlier section (1.3). There it was stressed that we are scoring a quasi-random sample of the genetic variation as closely as possible to the DNA level (Rick 1976). All topics discussed above benefit considerably from electrophoretic analysis because to date this is the only technique which can on a modest scale score gene numbers. Conversely, the data will indirectly permit a judgement on the selective importance of the bulk of this variation. Furthermore, it is increasingly clear that single loci cannot be studied independently of closely linked loci (Marshall and Allard 1970; Clegg *et al.* 1972; Lewontin 1974). Thus inferences drawn on such data apply more properly to the segments marked by such loci, rather than just the marker genes themselves.

4.3 Conclusions

Population genetic studies have made substantial contributions towards the exploration (i.e. sampling), conservation and use of plant genetic resources. Significant among the recent contributions are those which stem from isozyme studies of a variety of plant populations. The isozyme method is currently the most convenient available for detecting genetic differences close to the DNA level. First, concerning sampling, the evidence verifies the substantial degree to which plant populations are differentiated genetically, both in kinds of alleles and in levels of variation. This evidence supports those sampling and preservation strategies which stress the choice of more sites with fewer individuals per site. Second, concerning conservation, the allozyme data demonstrate an abundance of variation in both the land races and the wild relatives of crop plants. If the sources of such variation are in danger of extinction (as they are for land races and some relatives), measures to conserve samples should be vigourously encouraged. Third, concerning evaluation and use, isozyme markers readily monitor the comparative diversity of various kinds of genetic resources, the extent of coadaptation and the degree of allozyme – environment correlations. Such data foreshadow the use of these variants in plant breeding programmes to an increasing extent.

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