

Genetic structure of self-pollinating species: the case of wild *Avena*

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Abstract

Pérez de la Vega, M. & García, P.: Genetic structure of self-pollinating species: the case of wild *Avena*. — *Boccone* 7: 141-152. 1997. — ISSN 1120-4060.

The genetic structure of plant populations is determined by several factors, from selection processes to historical events (i.e. bottlenecks). Mating system is one of the most important factors due to its direct influence on the way progeny genotypes are generated and on the recombinational potential. In self-pollinating species, individuals are predominantly homozygous and consequently effective recombination is very low. As a consequence strong allelic disequilibria arise among linked and unlinked loci, in particular if selection is involved, and hence multilocus associations. The distribution of multilocus associations suggests that natural selection could be involved in their maintenance in some habitats, so they could represent coadapted gene complexes. Another critical point for genetic variability and population structure is the polyploidy level, because in self-pollinating polyploids the loci can be maintained throughout generations in an heteroallelic state, which is phenotypically equivalent to the heterozygote. The example of *Avena* is taken to illustrate these points, and the consequences that the genetic structure have when planning the collection and conservation of genetic resources in this species are discussed.

The extent of genetic variability of a population, the way in which this variability is arranged in and among its individuals, and the distribution of genetic diversity in and between plant populations is determined by several factors such as breeding system, pollen and seed dispersal, effective population sizes, selection processes and historical influences. However, the species breeding system influences the level and distribution of the genetic variability in such a strong way that inbreeding and outbreeding species have clearly different genetic structures. In highly or predominantly self-pollinating species, as many major crops and wild relatives are, populations are almost exclusively constituted by homozygous genotypes.

The reproductive system exerts a major influence on the genetic structure in two ways. Firstly, it determines the way in which the zygotes are formed from gametes, and secondly it affects the recombinational potential of a species. Effective genetic recombination requires two premises: sexual reproduction and heterozygosity. Inbreeding reduces the

population frequency of heterozygotes, which entails a great reduction of effective recombination and a sharp increase in allelic disequilibria in self-pollinating species. Experimental data accumulated over the last two decades (mainly from isozyme loci and more recently from DNA polymorphisms) have confirmed that wild populations and non-bred land races of self-pollinating species are usually formed by mixtures of multilocus homozygous genotypes of coadapted non-allelic genes. Heterozygotes in these populations are the results of infrequent, but measurable and recurrent, cross pollination between plants and the subsequent segregation of heterozygous plants. Allelic disequilibria in turn imply that the presence of a particular allele in a locus is not completely independent from the presence of other allele/s in different locus/loci, linked or non-linked. Therefore, selection at any given locus is likely to affect much of the genome, generating non-random multilocus structures with multiple epistatic interactions. As a final consequence, the genetic structure of populations of inbreeders is characterized by one, or more generally, by several multilocus associations. There are many examples showing that these associations includes all kinds of genes, such as loci for morphological characters, storage proteins, isozymes, rRNA or quantitative characteristics; some of them are presented in Table 1.

The meaning of the multilocus associations has been explained in two ways. From one point of view, this pattern is due to epistatic selection leading to the arising of coadapted genes. However, there also exists the possibility that other non-adaptive forces (migration, genetic drift, hitchhiking) could be responsible for the associations. In some cases the existence of well coadapted complexes of non-allelic genes which increase adaptation to a local environment has been demonstrated in several inbreeding species. Mostly the demonstrations were based on indirect data, as for instance the presence of correlations between environmental and genetic factors, but there are also examples in which more direct approaches have reached the conclusion that in fact some multilocus genotypes were coadapted (Allard & al. 1992, Parker 1992).

In diploid inbreeding species favorably interacting complexes are constituted of non-allelic genes, but in polyploid inbreeding species such complexes are made up of genes at homoeologous loci (intralocus variability) and at non-homoeologous loci (interlocus variability). Furthermore, in polyploid species the different homoeologous genome pairs can be homozygous for the same allele (homoallelic genotypes) or for different alleles (heteroallelic genotypes) (see Pérez de la Vega 1997, this volume), so that in polyploid self-pollinating species permanent gene interactions between allelic and non-allelic genes can be maintained in the form of multilocus homozygous genotypes.

When studying the genetic variability of the natural populations, the usual approach is to analyze it on a locus by locus basis; therefore the collection, evaluation and conservation of the genetic resources is mainly founded on the data on allelic variability in both inbreeding and outbreeding species. It is clear that in the presence of gametic disequilibrium the alleles present at different loci are not randomly distributed. Consequently, any study on the level of genetic diversity and structure of populations should take this fact into consideration and an evaluation of the multilocus allelic complexes should be made. These complexes may play an adaptive role, as has been demonstrated in several cases, thus the goal of a conservation project should change from having a collection in which the different alleles were present to a different one in which the various several multilocus associations (genotypes) were the units to be preserved.

Table 1. Some recent (1980-1995) references on the multilocus structures in predominantly self-pollinating species.

Species	Number of accessions	Type of sample	Loci	Reference
<i>Amphicarpaea bracteata</i>	R. L.	C	Isozymes	1
<i>Avena barbata</i>	>150	S C	Isozymes	2
	48	C	rDNA	3
<i>Avena hirtula</i>	30	R	Isozymes	2
<i>Avena sativa</i>	267	C	Isozymes	4
<i>Hordeum spontaneum</i>	267	R	rRNA	5
	148	R	Esterases	6
	26	C	Isozymes	7
<i>Hordeum vulgare</i>	92	W	rRNA	5
	1358	W	Esterases	6
	158	C	Isozymes	8
	1032	W	Isozymes	9
	cc	W	Isozymes	10
	cc	W	Morphological	10
<i>Lens culinaris</i>	105	S C	Isozymes	11
			Morphological	11
<i>Setaria viridis</i>	164	W	Isozymes	12
<i>Triticum dicoccoides</i>	12	C	Isozymes	13
	23	C	Amylases	14
	12	R	Storage proteins	15
	25	R	Isozymes	16
	25	R	Glutenin DNA	16

R. L.= recombinant line; C= samples from a single country; S C= samples from several countries; R= samples from several countries of the same regional zone; W= world-wide samples; cc=composite crosses.

- 1 Parker 1992.
- 2 Allard & al. 1993, García & al. 1991, Pérez de la Vega & al. 1991a.
- 3 Cluster & Allard 1995.
- 4 Pérez de la Vega & al. 1991b, Pérez de la Vega & al. 1994.
- 5 Saghai Maroof & al. 1990.
- 6 Kahler & Allard 1981.
- 7 Brown & al. 1980.
- 8 Bekele 1983.
- 9 Zhang & al. 1990a, 1993.
- 10 Allard 1988, Allard & al. 1992.
- 11 Erskine & Muehlbauer 1991.
- 12 Wang & al. 1995.
- 13 Golenberg 1989, Nevo & al. 1982.
- 14 Nevo & al. 1993.
- 15 Ciaffi & al. 1993.
- 16 Nevo & al. 1995.

But as Allard (1992) pointed out, the task of managing germplasm at the genotypic level is unfortunately much less straightforward and much more difficult than at the allelic level. This is because having superior alleles in breeding stocks is not enough in itself: superior alleles must be assembled into superior combinations at the intralocus and interlocus levels to exploit favorable interactions within-locus and among alleles at different loci.

Another important point to consider in genetic resources sampling and management is that current statistics assume that populations contain equal amounts of variability, but actually the amount of genetic variation may be very different among populations. This fact is particularly frequent in populations of inbreeding species that show wide variations in the number and frequencies of alleles and genotypes. As a consequence, when computing the variance preserved from the number of populations conserved in a collection, the assumption of similar variability in the populations is only a first approximation (Brown & Schoen 1992).

There is enough experimental evidence to support three premises on the genetic structure of inbreeding species which have to be taken into account in evaluating and conserving genetic variability: 1) genetic variability distribution is commonly organized in the form of multilocus associations; 2) multilocus associations play adaptive roles; and 3) distribution of variability among populations is highly uneven. We propose that these facts are important in determining strategies for sampling and conservation of genetic resources. These points can also be present in outbreeding species, although usually to a lesser extent than in inbreeders. Some examples of outbreeding species showing multilocus associations are *Cunninghamia lanceolata* (Yeh & al. 1994), *Pinus ponderosa* (Mitton & al. 1980) or *Pinus sylvestris* (Semerikov & al. 1993), and there is evidence of epistatic selection in *Pinus contorta* (Yang & Yeh 1993), *Pinus ponderosa* (Epperson & Allard 1987), and *Trifolium repens* (Ennos 1982).

In this paper, data on several inbreeding species will be reviewed, in particular our results on Spanish populations of different species of the genus *Avena*.

The data on Spanish oats summarized here come from 10 natural populations of the diploid ($2n = 14$) wild slender oat, *Avena hirtula*, and 77 natural populations of the tetraploid ($2n = 38$) wild slender oat, *A. barbata*. Genetic data were obtained by analyzing 14 isozyme loci in a sample close to 100 seedlings in most populations. Also 235 accessions (10 to 15 seedlings per accession) of a collection of Spanish winter land races of the cultivated hexaploid ($2n = 42$) *A. sativa* have been studied for nine isozyme loci in this case. Genotypes are represented as the diploid equivalent (see Table 1 in Pérez de la Vega 1997, this volume and additional data on the genetic nomenclature used has been published by Allard & al. (1993) and Pérez de la Vega & al. (1994)).

Multilocus associations

The first premise, namely the existence of multilocus associations is illustrated by the examples from grasses and other annual inbreeding species. Multilocus associations have been demonstrated in several self-pollinating cultivated species and in their wild relatives – in diploids like *Hordeum vulgare*, *H. spontaneum*, *Avena hirtula*, *A. weistii*, *A. canariensis*, *Lens culinaris*, in tetraploids such as *A. barbata* and *Triticum dicoccoides*, and in hexaploids like *A. sativa* (Allard 1988, Brown & al. 1980, Ciaffi & al. 1993,

Cluster & Allard 1995, Erskine & Muehlbauer 1991, Morikawa & Leggett 1990, Nevo & al. 1988a, b, Pérez de la Vega & al. 1994, Zhang & al. 1990a, 1993). Materials used include experimental populations and regional or world collections. Studies were mainly based on isozymatic and DNA markers (Table 1).

Studies on the population biology of *A. barbata* in Spain and also in California and Oregon, in the Mediterranean Basin and the Middle East, and of diploid *A. hirtula* - *A. weistii* in Spain and Israel, have shown that these species are at present differentiated into a number of ecotypes, each marked by a specific combination of alleles of 15 or more Mendelian loci that code for discretely recognizable morphological, allozyme and, for *A. barbata* also rDNA, variants (see Allard & al. 1993 and Cluster & Allard 1995 for references). Multivariate log-linear analyses of the Spanish oat data set showed that in *A. hirtula* and *A. barbata*, disequilibrium values are high in all populations and accessions and that most, if not all, of the loci studied in each species are tied together through complex networks of overlapping two-locus, three-locus and higher-order epistatic interactions (Allard & al. 1993, Pérez de la Vega & al. 1991, García & Sáenz de Miera unpublished results). In other words, multilocus complexes of non-linked genes are maintained in the populations. In particular, the analysis of a set of 42 *A. barbata* populations representing many ecological areas of Spain showed that the multilocus structure can be extended at least to a seventh-order term of among the nine polymorphic loci considered. This suggests that much of the genome of *A. barbata* has congealed into a multilocus complex in Spain. A similar result was observed in *A. sativa* when multivariate analyses were carried out with subsets and the whole collection of land races, among the seven polymorphic loci scored the same four loci (*Lap*, *Pgm*, *Mdh1* and *Mdh2*) were always associated in multilocus complexes (Pérez de la Vega & al. 1994).

Adaptive roles of multilocus associations

Although the multilocus structure of inbreeding populations could be due to and maintained by non-adaptive processes such as migration or genetic drift, there is enough evidence to demonstrate that in many cases multilocus structure of populations is due to and maintained, at least in part, by epistatic selection favoring different multilocus genotypes in different environments (Table 2). Generally, it is difficult to determine whether the selection is acting on the markers directly or on associate loci responsible for the adaptive properties, but the study of close related species, such as *A. barbata* and *A. hirtula*, and the analysis of the multilocus dynamic through several generations support that inbreeding, even when coupled with tight linkage, does not necessarily hold the genotypes together (Allard & al. 1993, Zhang & al. 1993). Therefore, if the adaptive properties are due to associated loci, for all practical purposes they behave as a virtually indivisible unity with the genetic markers analyzed.

Multivariate analyses indicated that allozyme and storage protein polymorphisms in wild emmer wheat (*T. dicoccoides*) are partly adaptive and differentiated at the multilocus level by climatic and edaphic factors, both micro- and macrogeographically (Nevo & al. 1988a, b, 1993, 1995). Similarly, multilocus analyses in *H. spontaneum* populations suggested that polymorphisms differentiate by micro-niche ecological selection, rather than by stochastic processes (Nevo & al. 1986), or that in a wide collection of this species

allelic state at the two rDNA loci depends not only on the allelic state at the other locus but also on environmental factors (Saghai Maroof & al. 1990).

Table 2. Some references to the evidence that biochemical and/or molecular polymorphisms are, at least partly, adaptive in inbreeding crop species and their wild relatives.

<i>Avena barbata</i> Isozymes, rDNA	Allard & al. 1993, Pérez de la Vega & al. 1991, Cluster & Allard 1995 (and previous works by Allard and co-workers) (*).
<i>Avena hirtula</i> Isozymes	Allard & al. 1993, Pérez de la Vega & al. 1991 (and previous works by Allard and co-workers) (*)
<i>Avena sativa</i> Isozymes	Pérez de la Vega & al. 1994 (*)
<i>Hordeum spontaneum</i> Hordeins Isozymes Isozymes rDNA rDNA	Nevo & al. 1983 Nevo & al. 1979 Nevo & al. 1986 Saghai Maroof & al. 1990 (*) Zhang & al. 1990a
<i>Hordeum vulgare</i> Isozymes Isozymes, rDNA Isozymes, rDNA rDNA	Zhang & al. 1990b (*) Allard 1988 (*) Allard & al. 1992 (*) Saghai Maroof & al. 1990 (*)
<i>Triticum dicoccoides</i> Amylases Isozymes Isozymes rDNA	Nevo & al. 1993 (*) Nevo & al. 1988a (*) Nevo & al. 1988b (*) Flavell & al. 1986

(*) multilocus analyses

These authors concluded that natural selection plays a major role in the development and maintenance of observed patterns of molecular and genetic organization of rDNA variability. The most important sets of experimental data demonstrating the differential adaptedness of multilocus genotypes have been obtained in cultivated barley by Allard and co-workers and summarized in recent papers (Allard 1988, Allard 1990, Allard & al. 1992, Zhang & al. 1990b, 1993). Some of their conclusions are particularly relevant to demonstrate this differential adaptedness: 1) statistically significant non-random associations among alleles of different loci develop within a few generations in each of the

experimental populations they studied, 2) the internal structure of two- to five-locus associations frequently changed over generations, often in concert with shifts in environmental factors, 3) different populations grown at the same locality soon developed similar multilocus structure, 4) subpopulations of the same experimental population soon developed different multilocus structures when transferred to different environments. Likewise, Zhang & al. (1990a) regarded selection as the major factor in explanation of the differences in the observed multilocus structures among regions of a world barley collection.

Associations between multilocus structure and environmental factors have been also demonstrated in Californian and Spanish populations of *A. barbata* (see Allard & al. 1993, Cluster & Allard 1995, Pérez de la Vega & al. 1991a for references). A preliminary multivariate analysis of 42 Spanish populations indicated a clear association between multilocus genotypes and annual rainfall and mean temperatures, a result similar to the ones obtained previously with Californian populations. Analyses of additional sets of Spanish populations have also indicated the association between multilocus genotypes and environmental factors (unpublished results). In particular the study of additional Spanish populations revealed a clear association between a four-locus genotype (*6Pgdl 13, Lapl 23, Prxl 11, Estl 57*) designated Northern, and an ecological zone in the Northern Meseta characterized by more than 80 days of frost per year and a relatively cold spring. The frequency of this multilocus genotype changes abruptly between this and adjacent areas, and temporal frequency changes of the Northern association in a specific population correlate with temporal climatic variations. This and further studies with Spanish populations of *A. hirtula*, the diploid ancestor of *A. barbata*, reinforced the conclusion that it is difficult to account for the intricate patterns in which specific multilocus genotypes are distributed in space other than in terms of natural selection favouring specific multilocus genotypes under specific sets of environmental conditions (Allard & al. 1993). Multilocus genotypes of Spanish *A. sativa* land races also showed a clear association with climatic factors. In particular the four-locus association designated Badajoz was predominant in the warmer climatic areas (southern Spain) and was absent or relatively infrequent in the colder areas, while the complementary association, designated Soria, was frequent in the colder parts of Spain but was almost absent in the warmer areas (Pérez de la Vega & al. 1994).

Uneven distribution of genetic variability

Over the last twenty-five years, there has been a large number of papers dealing with genetic diversity in inbreeding species. These data indicate that self-pollinating species can maintain as much diversity at their polymorphic loci as outcrossing species. However, the quantity of genetic variability is very unequal among the populations. This uneven distribution of genetic variability can also be illustrated with the examples of oat species. Among the Spanish oat populations three categories of populations can be recognized: 1) Low-variability populations in which one to three relatively frequent multilocus associations are present. (This group represent an example of small-size genetic populations in spite of a high census of individuals; one or a few well-adapted genotypes in populations with a normal number and density of individuals). 2) Intermediate populations in which several multilocus genotypes are present but only a few of them are

relatively frequent. 3) Highly variable populations in which several to many low-frequency multilocus genotypes are present. This variability is geographically distributed in a non-uniform way. The number of *A. hirtula* populations studied is too low to draw conclusions, but the data obtained so far indicate the same uneven distribution as found in *A. barbata*. On the other hand, in California Allard and co-workers demonstrated that the genetic variability of *A. barbata* is distributed according to a clear cut pattern and in a simpler way than in Spain: most Californian populations belong to the first above mentioned class, and multilocus genotypes are distributed according a clear ecological pattern, thus there exists a remarkable precise and consistent association between specific alleles and specific habitats on both macro- and microecogeographical scales. The greater majority of populations of the hot summer zone are monomorphic, or very nearly so, for a specific multilocus combination of alleles designated 'xeric' (approximately 80% of Californian populations). In contrast, most populations of the cool summer zone of California are monomorphic, or nearly so, for a complementary set of alleles designated 'mesic' (approximately 10%). Where semiarid and mesic habitats interface, polymorphic populations occur; then about a dozen multilocus genotypes can be found in addition of the 'mesic' and 'xeric' genotypes which frequencies vary in a microgeographical scale correlated with amounts of available moisture. Although association between multilocus genotypes and available moisture has also been demonstrated in Spanish populations (Pérez de la Vega & al. 1991a), the pattern of variability distribution in Spain is much more complicated since genetic variability is higher in and between populations and genotypes are distributed in a patchy way, probably reflecting the greater habitat diversity in Spain, without a clear association between a single multilocus genotype and a ecogeographical area, except in the previously mentioned zone of the Northern Meseta. However, when relatively wide areas are compared, a different pattern of distribution, with a clear diminution of variability from south to north, can be observed: while the average of multilocus associations per population in southern Spain is 13.2, the mean in Central Spain is 11.4, and it decreases to 6.8 in the northern part of Spain (García & al. 1989).

The uneven distribution over different areas of genetic variability in inbreeding species indicates that sampling strategies are very important when samples of wild populations and of land races are collected or when forming core collections – if most of the multilocus associations present are to be included. In order to estimate the expected results with several gathering methods, we have carried out a computer-aided repeated sampling process (100 repeats each time) with the data from 72 populations of *A. barbata* collected in 21 Spanish provinces and the 386 multilocus genotypes determined for the five most strongly associated loci (*Acph1*, *Est1*, *Lap1*, *Prx1* and *6Pgd1*) in the multilocus genetic structure of the Spanish populations. In each process 10, 20 or 50 seedling were sampled from each of 21 populations (30%) chosen at random, or from 21 populations obtained by selecting at random one per province, or only 10 seedling were sampled from all the 72 populations. In all cases most of the multilocus genotypes were lost (Fig. 1). The best result was obtained when only 10 individuals from each population were sampled (57.6% of multilocus genotypes were lost). Certainly many of the genotypes lost are rare or very infrequent in the whole population and probably not well adapted to any particular environmental condition. Fig. 1 also summarizes the results when only genotypes close to or higher than 1% (frequent genotypes) are taken into consideration. This frequency in our

collection means that the 31 genotypes are present in several provinces or are locally frequent.

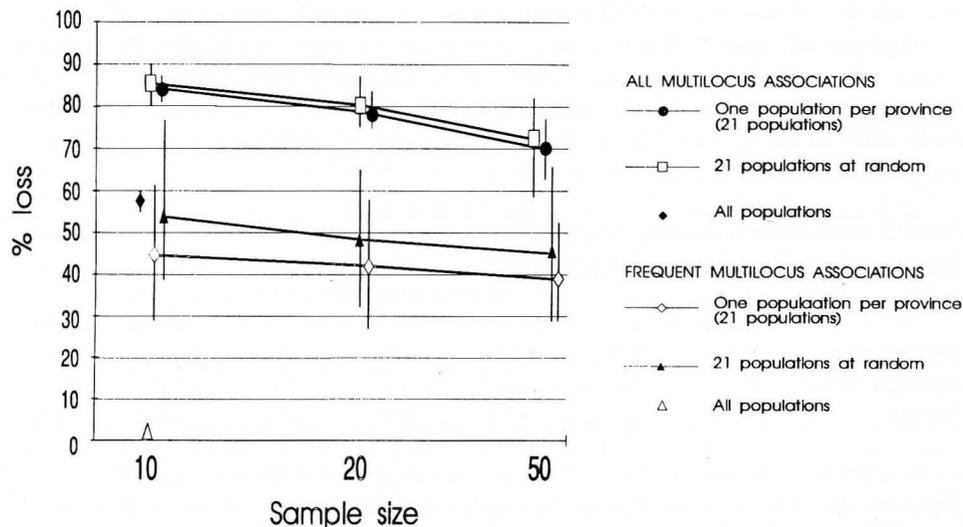


Fig 1. Loss of multilocus genotypes in the Spanish collection of *A. barbata* (the loss range is represented by the vertical bars).

When only 21 populations were sampled between 40% and 50% of the multilocus genotypes were lost, although with a very wide range among replicates; only when all the populations were sampled even with a small sample of 10 seedlings, was the loss of genotypes negligible (0.5%). This indicates that, for species with a distribution of variability such as *A. barbata* in Spain, it is a better strategy to sample as many populations as possible in different ecogeographical areas even with a reduced sample size than to collect a smaller number of larger samples. This strategy seems to ensure that the locally frequent genotypes, in addition to generally frequent ones, will be present in the collection. This is probably equivalent to all the multilocus genotypes which are adapted to many or some of the different habitats.

Since most of the methods used to estimate genetic variability conservation after a sampling process are based more on allelic frequencies than on genotypes, we estimated the loss of alleles among the 10 different esterase ones. Again the best strategy was to sample all the populations with a low sample size, on average less than one allele was lost (Table 3) following this strategy, although it is worth mentioning that in all sampling procedures no allele with frequency >0.02 was lost.

According to Allard (1988): (1) Multilocus analyses based on canonical correlation, log-linear, and cluster analysis procedures showed that highly significant associations developed among alleles of different loci in all predominant self-pollinating populations studies. (2) Patterns of ecogenetic differentiation that developed under predominant selfing were found to be fine-scaled overlays of environmental heterogeneity. (3) The incorporation of increasing numbers of favourably interacting alleles into large synergistic

complexes was accompanied in inbreeding populations by increases in adaptedness to the local environment and also by striking ecogenetic differentiation among local populations that occupy different habitats. (4) Selfing appears to promote the development and maintenance of adaptedness within populations and at the same time to facilitate the development of spatial differentiation. According to these postulates, in inbreeding species, it is not only important to collect and conserve alleles which contribute to adaptedness, but also collect the multilocus combinations of alleles which interact with each other in an epistatic favorable way, therefore forming multilocus genotypes of superior adaptedness in particular habitats.

Table 3. Loss of esterase alleles in the Spanish collection of *A. barbata* depending upon the populations sampled and the sample size.

Sample size	Sampled populations						
	One per province (21)			21 populations at random			All
	10	20	50	10	20	50	10
Average loss	2.5	2.3	2.0	2.3	2.0	1.8	0.7
Range	1-4	1-4	1-4	0-4	0-4	0-4	0-1

In order to achieve this task we propose that an effective strategy is collections of as many samples from ecologically diverse sites as possible, even when the samples are small in size. This is a better strategy than collection of fewer accessions formed by sampling more individuals. A similar conclusion was proposed by Nevo & al. (1982) for wild emmer wheat, a self-pollinating species, when stating that a collection of smaller samples in as many localities as possible was desirable. Our results with *A. barbata* demonstrate that samples of 10 individuals are enough to detect all the genetic variability probably relevant for adaptedness in the numerous and diverse habitats of Spain: all the relatively frequent (>0.02) alleles and practically all the widespread and all locally frequent multilocus genotypes to date known in Spain would be collected following our third sampling strategy. The main conclusion for *in situ* conservation is that in order to maintain variability, when it is distributed in multilocus genotypes in such a diverse way as in *A. barbata* in Spain, the number of ecologically diverse sites to preserve should be as numerous as possible. For annual and short-lived perennial species a few hectares could be a large enough area to sustain small samples of a large number of populations.

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