Strategies for the collecting of wild species

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Introduction

The human race relies on a relatively small number of crops for its survival and, as world population increases, ever more pressure is placed on these limited resources. Plant breeding is a continuous search for new sources of diversity (i.e. genes) which can be incorporated into advanced material in an effort to ease this pressure. Lately, demand for new sources of genetic variation has been increasing rapidly. There is keen interest both in developed and in developing countries in novel variation, even completely new species, to be used in breeding or introduced into cultivation.

Due to rapid technical developments in gene transfer in recent years, the diversity of wild species has become more accessible for use in breeding. However, our knowledge of the wild and weedy relatives of most crops is still fragmentary. Basic information on species delimitation, chromosome numbers, distribution and genetic variation is in many cases still imperfect or completely lacking. At the same time, genetic erosion has also increased alarmingly in many parts of the world, and with it national and international concern. There are thus several reasons why systematic collecting of germplasm of wild and weedy species may be done:

- collecting for taxonomic, phylogenetic and biosystematics research;
- collecting for genetic diversity study and conservation;
- collecting for immediate use in a breeding programme.

Collecting and conservation of wild species is generally most effective when there is a clear and specific need for the material, whether for research or exploitation. These different objectives can certainly not be entirely separated from each other but the plant collector needs to

carefully consider the aims of each collecting effort, because, as is described in this chapter, the choice of an appropriate strategy will to some extent depend on it.

The main area of research of the authors is the tribe Triticeae of the family Poaceae, and that is where the majority of the examples used in this chapter will come from. The principles and recommendations are, however, generally applicable to wild crop relatives, and indeed potentially useful wild species in general, for instance forages, of which in fact some species in the Triticeae are examples. The tribe Triticeae comprises about 350 species (Dewey, 1984; Löve, 1984). Generic limits are matters of some controversy (Baum *et al.*, 1987; Gupta and Baum, 1989; Seberg, 1989). Phylogenetic relationships within the tribe are rather complicated (Kellog, 1989; Frederiksen and Seberg, 1992), polyploidy and extensive hybridization being the main factors responsible for the intricate patterns of variation that are observed (e.g. Löve 1982, 1984; Dewey, 1984).

The gene pool concept

Central to the study of genetic diversity in wild crop relatives is the concept of the gene pool (Harlan and de Wet, 1971). This may be considered a broad summary of cytogenetic and biosystematic data and genome relationships in a particular plant group. The value of the gene pool concept has been its direct application in plant breeding. Though higher priority may be given to those species within the gene pool that are most easily used and those that are endangered, it is the gene pool as a whole that is the unit of study and conservation and must therefore be the target of collecting.

The primary gene pool consists of the taxa, including cultivated, weedy and wild forms of a crop, among which there are no sterility barriers and gene transfer is therefore straightforward. The secondary gene pool consists of all taxa that will cross with the crop, but from which gene transfer is difficult. The tertiary gene pool consists of taxa from which gene transfer is very difficult due to strong sterility barriers.

To take the case of cultivated barley, *Hordeum vulgare*, as an example, the primary gene pool comprises ssp. *vulgare* (i.e. breeding lines, commercial varieties and landraces) and ssp. *spontaneum*, which is the progenitor and closest wild relative of cultivated barley (Fig. 6.1; Brown, 1992). There are no problems in gene transfer from subsp. *spontaneum* to cultivated barley. However, immediate use in breeding programmes is not possible, since several unwanted traits of the wild form (e.g. shattering and shrunken seeds) are transferred simultaneously with the desired characters. There must therefore be a prebreeding programme, including some generations of backcrosses to barley and repeated selection of desired genotypes (Lehmann and Bothmer, 1988).
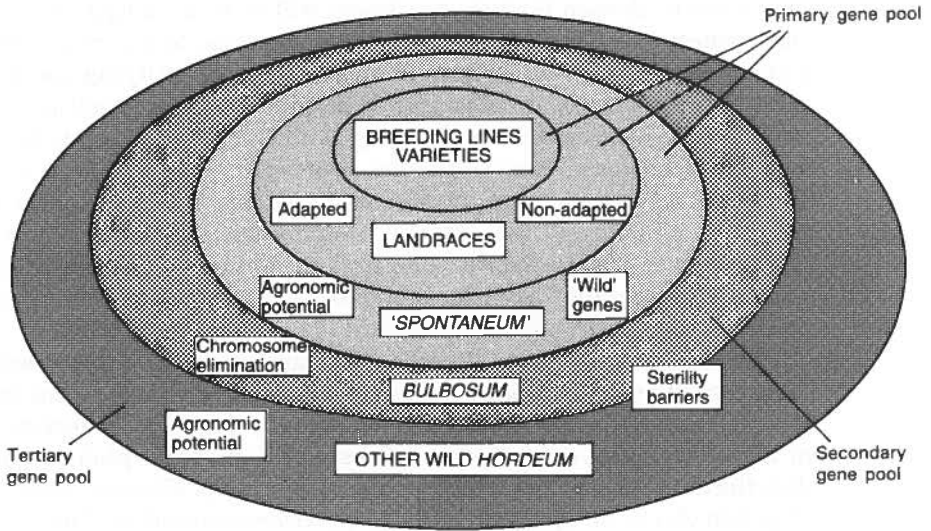
Fig. 6.1. The gene pool of cultivated barley (*Hordeum vulgare*).

The secondary gene pool of barley includes only one species, *H. bulbosum*, a perennial Mediterranean species from which gene transfer to barley through sexual hybridization is putatively possible (Pickering, 1988; Bothmer *et al.*, 1992; Xue and Kasha, 1992). This species has also been of great importance for the production of barley haploids through selective elimination of the *H. bulbosum* chromosomes in the interspecific hybrid (Kasha and Kao, 1970; Lange, 1971; Thörn, 1992).

The tertiary gene pool of barley comprises the remaining species of the genus *Hordeum* (Bothmer *et al.*, 1992). In the near future, however, improvements in the techniques for gene transfer will probably result in the inclusion of the whole of the Triticeae (Bothmer, 1992). As pointed out by Marshall (1990), molecular technologies may in the near future make the gene pool concept obsolete.

Collecting for taxonomic, phylogenetic and biosystematic research

The first step in planning any collecting programme for wild species will be a background study of the taxonomy and distribution of the target group, often called an ecogeographic survey (Chapter 14). Such surveys should be planned for the gene pool as a whole, even if it may only be possible to collect relatively small parts of the distribution area at any one time. Floras and monographs are the primary sources of such

information, though floristic databases will be increasingly important in this respect (Chapter 10). A Flora[1] is an account of the plants found in a specified region, usually providing means of identifying the different taxa. A monograph, in contrast, is a comprehensive treatment of the available taxonomic data pertaining to all the taxa in a specified group (less comprehensive treatments are sometimes referred to as revisions, conspectuses or synopses, in decreasing order of thoroughness (Stace, 1984)). A monograph will usually be more taxonomically rigorous, for example as to nomenclature, than a Flora, but some Floras (often called 'critical') are designed as series of monographs, each treatment being produced by an expert on the group (Stace, 1984; Funk, 1993).

The information in Floras, monographs and other taxonomic literature is only as good as the plant material available to the authors. Distribution maps, for example, sometimes reflect the collecting routes of botanists more than the actual distribution of the plants. Thus, the distribution map of the South American species *Elymus cordilleranus* (Fig. 6.2) shows an aggregation of populations around La Paz in Bolivia, Lima in Peru and along the Panamerican Highway in Ecuador. This is known not to reflect the true distribution of the species or its habitat preferences very faithfully. Similarly, taxonomic treatments go out of date as more material and thus data become available and species concepts change. In many groups, numerous taxonomic names of various

Fig. 6.2. The distribution of *Elymus cordilleranus* in South America from herbarium records.

[1] Editors' note. Notice the upper-case initial letter, and compare with the lower case initial letter of 'flora' referring to the ensemble of plant species found in an area. Following Stace (1984), this convention will be used throughout this volume.

ranks have been proposed, often referring to biologically insignificant morphological variants extracted from a virtually continuous range of variation. Even in an intensively studied group such as the Triticeae many entities remain ambiguous as to correct name and delimitation. Until such taxonomic issues are settled it will be difficult to interpret data on habitat preferences, distribution, etc. An important reason for collecting germplasm of wild species, particularly crop relatives, is thus to clarify their taxonomy and genetic relationships, for example by hybridization, mating systems and cytogenetic studies. This is a prerequisite for the optimal use of genetic resources in breeding and introduction programmes and for making informed decisions on conservation strategy.

In the planning process, published information must be supplemented with visits to a number of relevant herbaria, both international and local (Chapter 14). *H. muticum* is a diploid species of northern Argentina, Bolivia and Peru (Fig. 6.3; Bothmer *et al.*, 1991). It has only been possible to establish the northern part of this range through extensive studies of older herbarium material. The southern part of the distribution is rather well represented in germplasm collections, but there is no germplasm conserved from the north. *H. stenostachys* and the hexaploid *Elymus breviaristatus* ssp. *scabrifolius* are both perennial species native to northern Argentina, Uruguay and southern Brazil (Bothmer *et al.*, 1980). These species are far more evenly collected over most of their distribution than *H. muticum*. However, a few restricted areas (mainly in Brazil and Uruguay) still need work. Consideration of the passport data associated with past germplasm collecting in conjunction with other information on distribution often reveals such geographical gaps in existing collections. These will be high-priority areas for conservation collecting. However, though some effort must certainly be invested in finding out about existing collections, experience shows that quite often even areas from which some material is already in gene banks must be re-collected. Reasons range from the unavailability of conserved material to the poor ecological coverage of collections (Chapter 3).

Regions where large numbers of taxa in the target gene pool are concentrated (centres of diversity) will be priorities when collecting to elucidate taxonomy and relationships, though they may not necessarily also show high levels of genetic diversity within taxa. In the Triticeae, examples of regions with relatively large numbers of species are southern South America for the genus *Hordeum*, Central Asia for *Elymus* and southwest Asia for *Triticum–Aegilops*. However, such regions are often the most intensively covered by previous workers. Geographical regions and habitats which have not already been much visited by botanists, and from which information is therefore scarce, will also be important targets.

Special consideration should also be paid to isolated populations, and those on the edges of the ecogeographical distribution of the gene
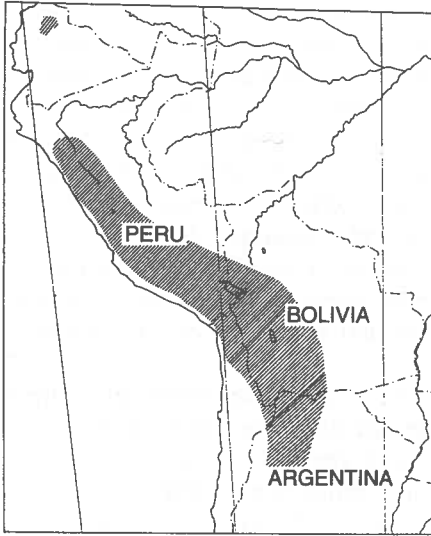
Fig. 6.3. The distribution of *Hordeum muticum* in South America from herbarium records.

pool. Populations in marginal areas or in unusual, distinctive or isolated habitats have a higher likelihood of representing distinct taxa, or at least of harbouring unique traits, though they tend to be genetically more uniform than more central populations. Apparently homogenous regions may reveal species exclusively found in scattered microhabitats, showing an island-like distribution (Hamrick, 1983). Only a very preliminary indication of this may usually be gained from studies of the taxonomic literature and of herbarium material. For example, it will rarely be possible to determine that a taxon is confined to a particular soil type from a Flora or monograph. This may sometimes be ascertained from herbarium labels or the ecological literature, but often it has to be documented by collectors themselves in the field.

'Critical' species groups showing complicated patterns of variation (e.g. introgression) need particular attention during collecting. They may be undergoing active differentiation and often there are no sterility barriers or extrinsic isolation mechanisms among the entities, which are sometimes not easily distinguishable even after close examination or crossing experiments, let alone in the field. An example is the *H. patagonicum* group from southern South America, which grows primarily in the steppes of Patagonia. It is a diploid perennial complex, probably of recent origin, which is in a phase of active differentiation in response to ecological and geographic heterogeneity (Bothmer *et al.*, 1986, 1988). Five subspecies are recognized, but the variation pattern is much more complex than is suggested by the taxonomy. Often, 'typical' representatives of two or even three of the subspecies grow together and intermediate forms are frequent. The *H. brevisubulatum* complex is an older group which occurs in Asia with a wide distribution from western

Turkey to eastern China (Bothmer, 1979; Dewey, 1979; Landström *et al.*, 1984; Baum and Bailey, 1991). It is a perennial, polyploid complex with a self-incompatibility system and thus with very heterozygous populations. Five major subgroups, usually treated as subspecies, have been recognized, and these are mainly allopatric. However, in the contact zones between the subspecies, much morphological variation is found. This is obviously the result of hybridization, segregation and introgression. Obviously, the different subspecies have not developed any intrinsic sterility barriers.

Zones of primary and secondary intergradation are of particular importance for collecting living material for studies of speciation. There is also evidence for higher frequencies of rare alleles in hybrid zones (Barton and Hewitt, 1985). During both the fieldwork and the subsequent investigations, the different morphotypes should be kept separate as far as possible, though collectors should be careful not to spend valuable time collecting what may be nothing more than malformed individuals. Bulk seed samples of each variant should be collected separately, each accompanied with a voucher herbarium specimen, but samples need not usually be large (ten individuals). Vegetative samples of each morphotype would provide a useful comparison with the seed samples. Variation may be cryptic, i.e. confined to anatomical or cytological features not readily observable in the field. For example, *H. bulbosum* occurs in diploid and tetraploid cytotypes which cannot be easily separated morphologically. If such cryptic forms as chromosome races are known to be sympatric and likely to coexist at a site, collecting can be on an individual plant basis. Collecting should be concentrated in areas and sites where the taxa meet and where transitional types occur, but it is also important to make a series of reference collections from areas and populations where the different entities are not in contact.

It is very important in all wild species collecting to supplement each germplasm sample with a herbarium specimen. The most frequent source of confusion in follow-up work with material raised from seeds is incorrect field determinations. This confusion can often be avoided, or at any rate diminished, if a herbarium specimen of the original population is made at the time of collecting. Often, good herbarium material cannot be collected at the optimal time for seed collecting. Two-stage visits may be necessary in such cases. Painted wooden stakes, coloured plastic tags and the like can be used to mark target populations. Particularly careful note should also made of the exact location of sampled populations.

Documenting the environment at the collecting site is important in all germplasm collecting, but particularly in wild species collecting. Related taxa are sometimes separated more by differences in habitat preferences than in morphology, and the ecology of the collecting site may thus hold clues as to the identity of the material. Such passport data as latitude, altitude, soil type and pH, drainage and associated species will also help future users screening collections for particular

adaptive traits. Population information should also be documented in the field. The different parameters of importance (e.g. population size, pattern) are discussed in Chapter 19. Such information may be used in the assessment of the conservation status of the target species. The conservation status (number of populations, size of population, risks faced) of most species in the secondary and tertiary gene pools of crops is at best incompletely known. When we do have such information, it may be based on old records. The status of many species and populations may have changed considerably, for example due to urbanization, changes in agricultural systems or land use, deforestation or pollution.

It is an important obligation of collectors to gather information about any changes in the frequency and geographical extent of taxa, and the reasons for such changes. Thus, there is little doubt that *H. secalinum* has declined during the last 80–100 years due to changes in farming practices, in particular the increased use of fertilizers. In Sweden and parts of Denmark it has become very rare and threatened (Bothmer and Jacobsen, 1980). *H. intercedens*, which has been recorded from southern California in the USA to adjacent Baja California in Mexico, was formerly rather abundant in the vernal pool habitats which were very characteristic of this area. Due mainly to urbanization the species has decreased markedly in California in the last 50 years, but it is still fairly abundant in Baja California (Bothmer and Jacobsen, 1982; Bothmer *et al.*, 1982; Baum and Bailey, 1988a). A similar example is *H. arizonicum*, native to a very small area in the southern USA and northern Mexico. The species has now disappeared from most of its former localities. When canals with concrete walls were built for irrigation the species lost its natural habitat, the banks of small ditches or creeks, and it is now very close to extinction (Bothmer and Jacobsen, 1982; Craig and Fedak, 1985; Baum and Bailey, 1988b). This kind of information is essential in evaluating the different possible actions that could be taken to ensure the adequate conservation of the gene pool, and the active collector is often the person best placed to obtain it.

However, care must be taken as a species may change in frequency and in extent of distribution for entirely natural reasons. In comparatively well-known areas like northern Europe it may be quite easy to judge whether a species is declining for natural reasons or not, as in the case of *H. secalinum*. In other cases it may be more difficult. In species like *H. erectifolium* and *H. guatemalense*, only recently described and known from only a single population or a very restricted area, it may not be possible to judge whether the populations are declining or increasing (Bothmer *et al.*, 1985; Bothmer and Jacobsen, 1989). Populations of such a species should be carefully monitored, and possibly *in situ* conservation efforts instituted to complement *ex situ* activities.

Collecting for genetic diversity study and conservation

The material chosen for long-term storage in a gene bank should be representative of the genetic variation within each species within the target gene pool. Populations within a species may diverge genetically to different extents, possibly resulting in ecotypes and/or clines. Geographical areas and individual populations themselves may be genetically homogeneous or heterogeneous. If a population or species is in immediate danger of severe genetic erosion or even extinction, it will not be possible to await detailed scientific investigations indicating what should be preserved. The only thing to do is to sample as quickly as possible following the basic strategy set out in Chapter 5. Ideally, however, a detailed analysis of the amplitude, partitioning and ecogeographic pattern of distribution of genetic diversity should precede germplasm collecting. This will allow the sampling effort to be directed as efficiently as possible, for example at the within-population level at the expense of the between-population level (or vice versa), at particular geographical areas of high overall genetic diversity, and perhaps at some species more than others.

Genetic diversity studies may be carried out using a variety of morphometric, biochemical and molecular methods. Isozyme electrophoresis has been the technique most widely used in studying the organization of genetic diversity in plant species (Schaal *et al.*, 1991; May, 1992). For example, Crawford's (1991) admittedly not exhaustive list records isozyme work on some 38 genera between 1983 and 1987 alone. Variation in seed storage proteins has also been used to study genetic diversity, the process of domestication and homologies among genomes (Gepts, 1990). However, advances in molecular techniques, including the perfecting of the polymerase chain reaction (PCR), have meant that DNA sequencing, restriction fragment length polymorphism (RFLP) analysis and randomly amplified polymorphic DNA (RAPD) analysis are becoming increasingly easily accessible and popular (Schaal *et al.*, 1991; Aquadro *et al.*, 1992; Hoelzel and Green, 1992). With the advent of these molecular methods in recent years, the range of species being covered has been increasing rapidly. Clegg (1990) and Schaal *et al.* (1991) compare and contrast isozyme and molecular methods for the detection and study of genetic variation.

It will clearly be worth searching the literature before embarking on a new genetic diversity study (Chapter 13). It may be that some genetic diversity data on the target species have already been published, though the study perhaps covered only a portion of the range and/or a limited number of traits or markers. If published data are lacking or inadequate, the collector may decide to carry out an exploratory, fairly coarse-grid genetic diversity survey of the target species prior to detailed germplasm collecting. Two types of approaches are possible. In one, germplasm is collected (either seeds or vegetative organs), grown out near the laboratory and material then harvested for analysis. The other approach

involves the sampling of leaves directly from the field, and the transport of these samples back to the laboratory for analysis. The methodologies may be summarized as follows:

1. (a) Seeds are collected from a number of individuals in each population, keeping the seeds from each individual separate. Seeds can then be tested directly (e.g. for seed storage proteins) back at the laboratory and/or germinated and the seedlings tested for isozymes and/or molecular markers. (Morphological traits may also be measured on the adult plants.) At least ten seeds should be collected per plant, if possible, to allow the determination of the maternal genotype with reasonable statistical confidence (e.g. Brown and Allard, 1970).

(b) Whole plants or appropriate plant parts (i.e. organs of vegetative spread or perennation) are collected and transported to the laboratory alive. There, they are transplanted, grown out and maintained for later use, or sampled and tested immediately, or samples are taken and freeze-dried or stored in liquid nitrogen. Appropriate temporary storage methods for vegetative material are discussed in Chapters 21, 22 and 23.

2. Samples of vegetative tissue (usually leaves or leaf fragments) are collected in the field and transported to the laboratory for analysis. Like method 1(b), this recovers the actual genotypes present in the population, rather than the genetic 'potential' represented by seeds. How the tissue samples are stored during transport will to some extent depend on whether proteins or DNA are to be analysed. Proteins are more temperature-labile, but contamination is more of a problem with DNA (May, 1992). Tissue samples should be stored moist and cool while in the field. If the laboratory is nearby, the samples can be taken there in an ice chest and then frozen in liquid nitrogen, pulverized and stored at $-70°C$ until they need to be used. If the laboratory is at some distance, a better option may be to freeze-dry samples at some kind of base nearer the collecting site (Hamrick and Loveless, 1986b). It may be possible to carry liquid nitrogen containers into the field. For DNA, if refrigeration is not possible the best method of tissue preservation is rapid drying in individual vials containing anhydrous $CaSO_4$ or silica gel (Pyle and Adams, 1989; Chase and Hills, 1991; Milligan, 1992). Rogstad (1992) suggests preserving leaves in a saturated solution of NaCl and hexadecyltrimethylammonium bromide (CTAB). Excessive temperatures and ultraviolet (UV) light (sunlight) should still be avoided. It is best to harvest the freshest material available.

Hamrick and Loveless (1986b) compare collecting leaf material and collecting seeds for genetic diversity surveys. An important advantage of leaf material is that collecting may be done over a longer period. With leaves, it is also easier to sample the entire population in an unbiased way, including all age classes and, in dioecious species, both sexes. Finally, fewer analyses will be necessary, as maternal genotypes can be

determined directly, rather than having to be estimated (at least ten progenies are usually required to determine the genotype of a fruiting individual). Collecting seeds of course has the advantage that some will remain as a germplasm sample which it will be possible to store and use, whether in biosystematic studies or a breeding programme. A certain number of seeds from each individual could be bulked to constitute a population sample for conservation, though it is unlikely that such samples will be sufficiently large for immediate long-term storage, or represent enough individuals. Alternatively, a complementary population sample of seeds for conservation can be collected at the same time as the individual plant samples are gathered for genetic diversity studies.

Nei (1987) discusses how many loci and how many individuals per locus should be studied to accurately estimate genetic parameters such as average heterozygosity and gene diversity in a population (see also Hoelzel and Dover, 1991). His recommendation is that as large a number of loci be examined as possible. As few as 20 individuals per locus should be sufficient to keep the bias of the heterozygosity estimate reasonably low, as long as large numbers of loci (30–70) are investigated. If the number of loci that can be investigated is limiting, or when heterozygosity is high, sampling more individuals will improve the precision of estimates of genetic parameters such as diversity and distance. If the number of loci is about 25, the number of individuals should certainly not be less than 20. This compares with about ten (bulked) for taxonomic studies (as already suggested) and 50 or more (again bulked) for conservation collections (Chapter 5). To accurately reflect the frequencies of alleles in the source population, rather than simply being 95% certain of the presence of a copy of all common alleles in the sample (the basis for the figure of 50), Marshall and Brown (1983) suggest that sample size should be about 200 individuals. More detailed, special-purpose genetic studies may require even larger samples. For example, in order to test whether or not two diploid populations with estimated allele frequencies of two alleles of 0.50/0.50 and 0.55/0.45 differ at the 90% confidence level, one would have to sample 2081 individuals.

This discussion has somewhat begged the question of what constitutes the population. Delimiting the population is much more difficult for wild species than for crops, which are usually grown in fairly well-defined fields. In most wild species germplasm collecting, the population is usually pragmatically taken to be all the individuals of a particular taxon found in a particular, fairly ecologically homogeneous place at a particular time. In genetic diversity studies, however, a stricter definition may be necessary, i.e. a local group of individuals of a particular taxon within which free exchange of genes is occurring in nature. This is sometimes referred to as the 'gamodeme' (Briggs and Walters, 1984). The spatial extent of gamodemes can be estimated from studies of pollen movement and seed dispersal (Levin and Kerster, 1974). The general

impression that has emerged from such studies is that 'geneflow is quite restricted in plants and gamodemes are therefore small' (Briggs and Walters, 1984). Thus, species occurring in more or less continuous vegetation types over vast areas, such as the Russian steppe or the Argentinian pampas, may nevertheless be organized in relatively small gamodemes isolated by distance. Sampling of such species can be done in a systematic way, say every so many kilometres.

Sampling of individuals should be at random within the spatial extent of the gamodeme, or stratified random in the case of obvious environmental heterogeneity (Chapter 5). A minimum distance should be kept between sampling points, ideally dictated by consideration of the average size of clones in vegetatively spreading species and the leptokurtic pattern of dispersal of seeds and fruits.

If it is not possible to carry out preliminary genetic diversity surveys prior to germplasm collecting, and no previous work has been done on the target species, generalizations based on reviews of isozyme studies can provide rough predictions of the genetic structure of unstudied plant species (Hamrick et al., 1991). Many such reviews are available, assessing the influence of different parameters of life history, reproductive biology, and distribution on overall genetic variation within species, and how this is partitioned within and among populations (e.g. Hamrick et al., 1979, 1981, 1991, 1992; Hamrick, 1983, 1989; Loveless and Hamrick, 1984; Hamrick and Loveless, 1986a; Govindaraju, 1988; Hamrick and Godt, 1990; Schoen and Brown, 1991; Loveless, 1992). On average, inbreeding species show more variation among populations than outbreeders, and annuals more than perennials. On the other hand, individual populations of outbreeders are usually more variable than populations of inbreeders, and those of widespread species more than those of narrow endemics. Schoen and Brown (1991) suggest that preliminary surveys of genetic diversity will be more important for inbreeders than for outbreeding species, where there is comparatively less variation among populations in gene diversity.

There are some problems with using such generalizations to develop germplasm sampling strategies (Hamrick et al., 1991). First, the kinds of life history and other parameters considered typically only explain less than 50% of species-to-species variation in genetic diversity parameters. Species-specific factors are thus equally important. However, it seems clear that in order to effectively study the genetic variation of a species its breeding and pollination systems must be known. These should certainly be investigated before large-scale collecting is started (e.g. Brown, 1990). Some work may already have been carried out, of course. Fryxell (1957), for example, catalogues the mating systems of some 1200 plant species. Richards (1986) is a more up-to-date review. Crane and Walker (1984) provide information on the mode of pollination of crops. If the literature is no help and a special study is not possible, a qualified guess may in some cases be possible, for example based on the size and morphology of the reproductive organs. Thus, in the

Triticeae and other grasses, long-anthered species with large styles are usually outbreeders whereas short-anthered species with small stigmas are inbreeders.

It may not be possible to use the results of isozyme and molecular studies to predict variation in other traits, in particular quantitative morphological traits. Though results often correspond, this is by no means always the case (Schaal *et al.*, 1991). Large-scale clinal and ecotypic variation is sometimes not revealed by isozyme studies (Falkenhagen, 1985). Morphologically heterogeneous species are sometimes quite uniform at the biochemical or molecular levels and vice versa. As Marshall (1990) points out, there is nothing unexpected or unusual about this. There are good reasons, for example, why the kinds of morphometric differences used to distinguish infraspecific taxa, in particular domesticated forms from related wild taxa, often develop prior to divergence at isozyme loci (Crawford, 1991).

Hamilton (1994) argues that quantitative genetic variation and the structure of genetic correlations should be given at least as much importance, if not more, in formulating a sampling and conservation strategy as biochemical and molecular markers. While agreeing that quantitative traits are important, Marshall (1990) points out that the argument will be irrelevant to most collectors, who usually have almost no information at all on the population genetic structure of their target species.

> In such cases it would appear reasonable to suggest that the most effective strategy would be to spend only sufficient time at each site to ensure the collection of common genes, and to visit as many sites as possible, so as to maximise the opportunity of sampling those populations that are variable for each class of character or gene.

This is the basis of the collecting strategy set out in Chapter 5. Of course, genetic variation often follows environmental variation, though the proportion of genetic variation regarded as explicable by ecogeographic factors rarely exceeds 50% (Chapman, 1989). In the absence of genetic diversity information, climatic, physiographic and edaphic factors can be used to delimit areas which are different from each other but relatively homogeneous internally. In first-stage exploration for conservation, as many as possible of these areas should be visited and sampled. If environmental variation is clinal, sampling should be done systematically along the gradient. In the case of two-stage collecting, Marshall (1990) advises the prudent approach of ensuring that the most variable populations for each class of characters are sampled.

Collecting for use in a breeding programme

Breeders and plant introduction workers often have very specific requirements for particular traits. For example, there may be an urgent need for a source of resistance to an abiotic stress (e.g. salinity, drought,

frost, etc.) or to a pest. One possible way of obtaining desired traits is to systematically screen available gene bank holdings of the target species and related taxa in the primary and secondary gene pools. If the traits are not available in existing collections, collecting missions deliberately aimed at searching for them in suitable areas will have to be considered. Even if target material is already present in collections, however, further collecting in the areas where the desired trait(s) or gene(s) is (are) most likely to occur may be justified. For example, a wider range of genetic backgrounds may be sought. Gaining first-hand experience of the variation within and among populations in the field is also an important consideration.

Characterization and evaluation data may be available for particular collections (or parts of collections), but the distribution of desired traits in the gene pool is often not known in detail. However, their presence can often be predicted with some confidence from consideration of the ecogeographical passport data associated with the accession. If material adapted to particular climatic conditions is being sought, only those ecogeographic regions satisfying these requirements will be targeted (homoclime strategy). Searching for germplasm with tolerance to abiotic stress should start in areas where the species has been exposed to the stress factor for a considerable period. Thus, possible sources of salt tolerance in *Hordeum* would be the *H. patagonicum* complex in South America and *H. bogdanii* in Central Asia.

Similarly, in the case of pest resistance, collecting will be focused on areas where there is a long documented history of coexistence of the host and the pest. Anikster *et al.* (1976) and Moseman *et al.* (1990) found a correlation between the occurrence of resistance to *Puccinia hordei* in wild barley (*H. vulgare* subsp. *spontaneum*) and the distribution of *Ornithogalum* species, which are the alternate hosts for the pathogen. However, it should be remembered that resistance may occur anywhere (Harlan, 1978).

Some very detailed investigations have been carried out of the distribution of disease resistance with wild crop relatives. For example, Moseman *et al.* (1985) screened 687 accessions of wild emmer (*Triticum dicoccoides*) from Israel for resistance to leaf rust (*Puccinia recondita* f.sp. *tritici*) and found a clear spatial pattern in the distribution of resistance over the country. The geographic distribution of resistance to stripe rust (*P. striiformis*) in wild emmer is described by Nevo *et al.* (1986). He explained the pattern with reference to rainfall, evaporation and temperature. Several genes were found to be involved in the resistance reactions. Resistance to stem rust (*P. graminis* f.sp. *tritici*) in wild emmer was found to be negatively correlated with two ecological factors, namely altitude and number of hot dry days (Nevo *et al.*, 1991). Accessions collected in marginal habitats where wild emmer was growing poorly and had lower grain weight were more susceptible to powdery mildew than were accessions collected at more optimal *T. dicoccoides* sites (Moseman *et al.*, 1984). The authors recommended collecting wild

barley (*H. vulgare* ssp. *spontaneum*) as well as wild emmer from optimal habitats in areas where the powdery mildew pathogen is prevalent.

Spatial variation may occur even within populations, as shown by the distribution of resistance genes to powdery mildew in *H. vulgare* ssp. *spontaneum* (Segal *et al.*, 1987). There was a correlation between the occurrence of different hordeins (storage proteins) and resistance genes. Such correlation may facilitate the rapid screening of large collections. Epperson (1990) gives guidelines for the sampling of populations with a view to investigating the spatial pattern of genetic variation within them.

In summary, when collecting for specific traits, the target area should be chosen after studying the available ecogeographic and pest information (including information on the distribution of alternate hosts), analysing existing characterization and evaluation data and/or screening gene-bank accessions for the desired (or correlated) trait. Relatively small areas should be selected for careful, intensive sampling. Comparatively large random samples should be collected from populations likely to harbour the desired trait (Chapter 5). In addition to a population sample, a selective sample (e.g. of healthy individuals in an infested field) can be collected if the expression of the target trait is obvious and stable.

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