Concepts and requirements in the conservation of forest genetic resources

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Abstract

Hattemer, H. H.: Concepts and requirements in the conservation of forest genetic resources. — Bocconea 7: 329-343. 1997. — ISSN 1120-4060.

The manifold and increasing human influences on the genetic structures of tree populations make it necessary to devise measures for conservation of forest genetic resources. After presenting a definition of gene conservation, the various objectives of forest tree conservation are considered. Because forest trees are long-lived plants and still at a relatively low level of domestication, the conservation of the genetic adaptability of their populations to environmental changes should be emphasized. This implies that the choice among candidate populations for conservation should be based on the results of surveys of genotypes at marker gene loci. In view of the expense involved, and the long-term importance of this step, the quality of gene markers and the information on their mode of inheritance becomes critical. Besides marker gene loci, the expression of genetically controlled adaptive phenotypic traits deserves proper weight. The selection criteria are then estimates of certain parameters, such as the adaptive potential, the genetic meaning of which must be made transparent. Methods of dynamic conservation should be given preference because of the environmental situation of tree populations. Conservation *in situ* requires high sylvicultural skills. The combination of these prodecures with conventional static conservation *ex situ* induces flexibility and is very promising. Problems exist as to how to sample populations and how to measure their variation. Current research needs are indicated.

Forest tree populations

As long-lived sedentary plants, forest trees are exposed to a sequence of different environmental conditions during their life span. Their populations also have to face spatial environmental heterogeneity. In many countries, the majority of forests cover areas which are inadequate for agriculture and human settlements. This means poorer sites and more pronounced variation in environmental conditions. Temporal heterogeneity has also to be viewed in terms of the length of the generation cycle of trees, which considerably exceeds that of their herbivores and parasites. The latter may become adapted to a substrate offered

to them over decades or even centuries. Woody perennials are iteroparous. During their long reproductive period they produce abundant seed. The subsequent heavy reduction of population sizes corresponds to this fertility. It has been demonstrated that a considerable portion of the early mortality is selective (Gregorius & Degen 1994).

Many tree populations possess an efficient system of gene flow through pollen and/or seeds. This counteracts local decay of genetic variation and blurs adaptive differentiation of their populations, as far as this could be inferred from marker studies. Although the greater part of the pollen becoming viable in a given stand of trees might have been produced by that very stand, the trees which contribute viable pollen at reproduction might be much more numerous than the seed trees.

This condition has been tested in anemophilous tree species of the temperate and boreal zones. The distribution of the transport distances of viable pollen in the predominantly zoophilous trees of the lower latitudes may be more erratic and more difficult to estimate.

Woody perennials possess more variation at marker gene loci than short-lived plants. It has been supposed that this is due to the greater probability with which their plant body accumulates mutations. However, this hypothesis awaits testing by surveying genetic polymorphisms of DNA.

The combination of high genetic variation, prevalent cross-fertilization, and subsequent selection is responsible for the fact that degrees of heterozygosity in trees exceeds those of any other organisms hitherto surveyed.

Only a few species of trees are cultivated. With the exception of some major crop trees in horticulture, these may hardly be considered to be domesticated. Therefore, in conserving genetic variation of forest trees the focus is on a multiplicity of species comprising several populations and occupying a range of heterogeneous environments.

The need for gene conservation

The presence of humans has multiple impacts on the genetics of other species, as has been described comprehensively by Ledig (1992). Humans have physically eliminated well-adapted tree populations or species and will continue to do so. The release of domestic animals destroys plant populations or prevents their regeneration. Hence, in many parts of the world the present forests represent the last generation of their respective tree populations. It has been suspected that besides goats, sheep, and cattle, even honeybees have an impact on tree populations by displacing their natural pollen vectors and thus changing their breeding systems. The reduced diversity of artificial forests in the tropics depletes the food basis of the pollinator fauna and may lead to a reduction of its biodiversity.

It is a matter of probability theory that decreased size of tree populations leads to changes in allelic structures including the loss of allelic variants. Decreased density of tree populations has an impact on breeding contacts between individuals and thus on the breeding systems of the populations. Depending on the major forest type, between 15 and 40 per cent of forest tree species are dioecious. Part of the others are either known to possess a system of prezygotic incompatibility or a system of self-sterility. Other tree species neither have an effective barrier against self-fertilization nor can they eliminate embryos arising from self-fertilization. Hence, low density of flowering individuals means a drastic increase in self-fertilization followed by either rapid selection against seedlings

arising from selfing, or by slowly progressing mortality during the early decades. However, the critical lower limit of population density for both sufficient pollination and adequate cross fertilization varies with species and environments. In some observations, less dense populations produced fewer seeds with higher inbreeding coefficients. In other instances, no such response to reduced density could be observed.

The habitats of tree populations are also fragmented by forest exploitation, by conversion of former forest areas to food production, by road nertworks, and by human settlements. This may mean blockage of gene flow even in tree populations.

Environmental pollution has been shown to induce selection (Scholz & Bergmann 1994). The speed of this environmental change is high in comparison with the generation time of trees. The recruitment of rare genetic variants conferring resistance takes several generations even under very strong selection. Hence, in trees it lags behind environmental change. This holds true also with present predictions of future global warming.

Chemical control of parasites and herbivores introduces planned environmental pollution to those organisms. It can lead to the evolution of resistance against toxic substances. In some insects and vertebrates this resistance is stable beyond the application period of these substances.

A common practice in tree cultivation is the mass-cultivation of only a few genotypes. This may induce or accelerate adaptational processes in parasites and herbivores. A concomitant reduction of the stability of forests has adverse consequences, because forests are not cash crops, and chemical control of herbivores and parasites is too expensive. It is also prevented for ecological reasons (Ledig 1986).

The movement of provenances/races and of species as exotics between continents creates genetic connections among allopatric congeneric species, thus giving rise to problems of species purity (for example, *Populus, Platanus*, and *Larix* in Europe). This movement has also led to the harmful spread of parasites and herbivores on new host species, giving rise to problems in stability (for example pines of the subgenus *Haploxylon; Castanea*).

There are numerous examples of genetic changes in marker gene loci due to sylviculture. Repeated thinning in stands leads to changes in genotypic and allelic structures, which are different from those occurring under natural stand development. The whole process of artificial afforestation, comprising seed collection, seed storage, the production of planting stock and bareland planting at wide spacing, can also affect genetic structure. Some examples are presented further below.

Last, but not least, breeding may cause loss of genetic variation. To avoid such loss careful design of long-term breeding strategies is necessary. Even more than aggressive breeding, a mass-growing of few high-yielding selected varieties (or single clones) may sooner or later become responsible for reduction of their stability.

Motivations for gene conservation

Both ethical and aesthetic considerations call for conserving biological diversity while humans ever more extend their influence over other species. The nature of trees as carriers of the most complex terrestrial ecosystems provides irrefutable ecological reasons for their conservation. After all, forests are efficient carbon binders by production of wood. This wood is used as timber or utilized in industry. In some parts of the world, wood as fuel is

economically most important. The slow accumulation of this wood during long periods of time requires variable populations.

Definitions

In order to prevent inflation of the use of the term 'gene resource', we restrict the definition of a gene resource to biological material possessing particular genetic properties. A gene resource must either be known for or be expected to contain either some specific genetic variation or wide genetic variation (Ziehe & al. 1989). The latter property is somewhat unspecific, as is further elaborated below. It is also essential that genetic variation means the possession of a large number of *not too* rare genetic variants. Gene conservation refers to the preservation of gene resources in a condition allowing for their regeneration and use.

Objectives of gene conservation

In 1926, the Russian geneticist and plant breeder N. I. Vavilov proposed that crop plant improvement should draw from wide genetic variation. He initiated the collection of cultivated plants and their wild progenitors or other relatives from different parts of the world.

In forestry we recognize three major goals of gene conservation measures (Ziehe & al. 1989). This formulation is similar to that of Ledig (1986). The objectives have a direct bearing on the criteria used in selecting materials as gene resources:

(1) Yield potential, i.e. the genetic potential for conferring desirable phenotypic characters. Selection criteria for gene resources would then be the expression of these traits. One must not forget that the utility of given material is determined by contemporary interests and needs, which are subject to change. Finally, under both regional air pollution pressure and global environmental change it is uncertain whether the conserved potential will be expressed in a comparable way in future. Since economic value is seldom constant over time, only fairly general component of yield rather than financial revenue can be considered.

(2) Genetic adaptability, i.e. the ability of populations to survive and reproduce even in a changed environment. Adaptability relies critically on the availability of both common and rare alleles. Multiplicity and diversity at marker gene loci may therefore serve as selection criteria (see section 6). The parameters measuring adaptive potential have to be combined with the expression of adaptive phenotypic traits (see section 7).

(3) Conservation of as wide a range of variation as possible.

The ranking of (1) through (3) depends on predictability of the environment. Many authors have given goal (2) highest priority in the context of woody plants and forest ecosystems (Gregorius 1991a).

Genetic markers

Genetic markers allow one to infer the genotype of individuals (seeds or complete plants) at one or several Mendelian gene loci from their variable phenotypes (e.g.

electrophoretograms of proteins, particularly enzymes, or of DNA fragments). Hence, they represent invaluable tools in the framework of gene conservation measures. The prerequsite for the unambiguous inference from phenotype to genotype is the appropriate analysis of their mode of inheritance. Unless this has been established, genetic statements based on findings gained with the help of markers are only preliminary.

For several reasons, classical crossing experiments are difficult to carry out in trees. Gillet and Hattemer (1989) devised a method for analysing the segregation among the progeny of a seed tree with given phenotype after open pollination. It allows for testing genetic hypotheses on more than two alleles but not on linkage. With regard to the one-locus case, the Mendelian experiment represents but a special case of this approach. However, efficient use of the method is based on the assumption that the alleles at the controlling gene loci are codominant (with the possible exception of a recessive null allele).

Codominance is widespread at enzyme gene loci. This condition has been responsible for the wide use of isoenzymes as marker gene loci, because codominance is a necessary prerequisite of genetic markers employed in characterization of genetic inventories. The simple reason is that allelic structures can be estimated, and genetic variation and differentiation of biological populations be measured, only if the genetic variant can be detected also in heterozygous condition. Almost all insight into the dynamics of genetic structures of plant populations has been achieved with the aid of isoenzyme markers.

According to numerous statements in the literature, isoenzymes have frequently been considered to be adaptively neutral. The more populations of more species that have been surveyed at gene loci showing both minor or major polymorphisms for the same prevalent alleles, the less likely it has become that this interpretation is true (Gregorius & Bergmann 1995). The greater part of the existing variation at enzyme gene loci resides within populations, which usually possess similar allelic profiles. Even in view of fairly large population sizes, this must reflect the effect of selection towards similar genetic structures in spite of the heterogeneous conditions encountered in the distribution range. On the other hand, Bergmann (1978) and Bergmann & Gregorius (1993) have found adaptive differentiation among conifer provenances. Rothe & Bergmann (1995) were able to present enzyme kinetic reasons for differential viability of spruce genotypes under pollution stress. The frequent reports on consistent viability selection (Müller-Starck 1993) or change of genotypic structure in general (Konnert 1991) present ample evidence that enzyme gene loci may have more to do with the genetic control of longevity than assumed previously.

The ways of verifying the inheritance mode of DNA markers have been explained by Gillet (1991). The present debate on the relative merits of isoenzymes vs. DNA variants in general will hardly yield much clarification, because the two groups of genetic markers possess specific advantages in specific situations (Gillet 1993). The identification of ubiquitous codominant nuclear DNA markers with multiple 'allelic' variants will hopefully enrich greatly our future methodology of genetic inventory. During recent years, the survey of polymorphic organelle DNA has led to critical findings about species' distinction and the descent of populations within species.

Inventories of genetic markers indicate which are the most variable among many populations. They are at least as useful in detecting remnants of autochthonous populations and in studying the genetic system of target tree species.

Adaptive traits

There are good reasons for advocating the selection of gene resources on the basis of both genetic markers and the phenotypic expression of adaptive traits, because it is hardly possible to explore the adaptive values of many allelic variants in different environments. The adaptive importance of traits such as the spring and fall phenology of trees in regions with strong variation in temperature is self-evident. In this instance, coming into leaf late means a higher probability of escaping the damage of late spring frosts. In other traits it is not quite clear which expressions indicate greater viability under which environmental conditions. In view of expected global warming, certain ecophysiological tests may help to find out which trait expressions may be advantageous in the future (Larsen & Mekié 1991). The need for considering future rather than past environmental conditions is stressed by Matyks (1995).

It is difficult to judge whether certain traits are under genetic control, to what extent the environment modifies their expressions, and how they are inherited. Planned experiments have been devised for direct comparison among provenances, families, or clones that have received equal pretreatment. Eriksson (1995) discusses reasons for the superior differentiation of populations in phenotypic traits in comparison with single gene loci. Unless trait expressions are stable during ontogeny, such as certain phenological traits, these experiments take much time and effort and are valid for past environmental conditions only. Inheritance studies involving adult trees and their progeny produced in the forest may deserve more confidenee, because they yield results on genetically controlled differences between families and allow inference on the subsequent generation.

Gregorius (1989) devised a method of studying the degree of genetic control of traits in the field. Pairs of trees with contrasting phenotypes are chosen following a certain procedure. Subsequently, the genetic distance between phenotypic groups measured at marker gene loci indicates whether trait expression has something to do with genotype or is independent of it. This indirect method has led to plausible results in the analysis of pollution effects on tree populations (Scholz and Bergmann 1994). Only consistent pair comparisons made simultaneously in different populations permit statements on whether the assayed marker gene loci are themselves involved in the genetic control of tolerance. The method possesses importance wherever genetic control of phenotypic traits has to be studied in *adult trees under forest conditions*. In this situation, stands may have already undergone several thinning operations which may have reduced their variation. This procedure can be followed in sampling trees for ecophysiological tests in the laboratory.

Selection criteria for genetic resources

The choice among candidate materials is based on genetic diversity and multiplicity as the primary criteria. Diversity is only little influenced by rare alleles. A rare allele may become favorable, but still possess only little importance because of the long time required for its recruitment. Therefore, Finkeldey (1993) has proposed measuring adaptive potential by $M(\alpha)$, the effective number of genetic variants with frequency above a certain threshold such as $2 \leq \alpha \leq 3$ per cent. Since the effective number of variants or genetic diversity is only little influenced by (locally or globally) rare alleles, the difference to $M(\alpha)$ is mostly

moderate. α can be derived from the causal interpretation of the variation pattern of populations in terms of mutation, gene flow, genetie drift, and selection.

Genetic variation as such at a gene locus is not always indicative of its adaptivity, and superior genetic variation in a population is not necessarily indicative of its superior evolutionary potential. On the basis of an analysis of frequency profiles of gene loci, Gregorius (1995) proposed methods of separating prevalent and rare genetic variants because of their different role and different behaviour in adaptation processes.

Low genetic differentiation of a certain candidate population from the lumped sum of all other eligible populations indicates that this population well represents the species (Gregorius 1985). On the other hand, wide differentiation of a population from the others may point to its adaptive specialization or its different descent.

Adaptedness can be estimated by measurements of adaptive phenotypic traits. The estimation of adaptive potentials can be supported by expressions of adaptive traits in field tests or planned-environment tests (Holzer 1978). Survival, particularly under conditions of stress, is simple to assess, but it still requires careful statistical design.

The suitability of stands for reproductive purposes according to Article 5 of Directive 66/404/EEC ('selected material') or Article 5b of Directive 75/445/EEC ('improved value for use') hardly reflects their adaptive capacity. It is a triviality that large tree dimensions as a criterion for approval according to the Directives also reflect vegetative adaptedness. More relevant for adaptation is probably growth rhythm (Eriksson 1995). The consideration of quality traits, which is also being practiced in the selection of genetic resources, is justified only in pursuing goal (1) of genetic conservation. These traits should be given second priority in the choice among otherwise eligible populations.

Highly variable non-adaptive DNA markers are indispensable in reconstructing the descent of populations, because they shed light on which populations may be considered equivalent for conservation purposes due to their common descent (Gillet 1993). They may also be useful in finding autochthonous populations. These are not necessarily more adaptable, but they may serve as a valuable referencey

Conservation methods

Static conservation attempts to preserve the *status quo*. It is usually achieved by *ex situ* preservation when the population is endangered in its natural habitat. However, slight genetic changes due to manipulation are inevitable. In forest trees, this approach to conservation was taken when foresters had become aware of forest decline due to air pollution and wanted to evacuate tree populations until the atmosphere became as clean as before.

Storage of seeds is possible in many tree species such as European conifers, with the exception of firs (*Abies*). In certain angiospermous genera, such as *Fagus* and *Quercus* it does not seem to be possible for long periods of time without considerate losses in germination. Many tropical trees possess extremely short-lived seed. Only recently ways of inducing a short dormancy in species of dipterocarps were able to be developed (Villanueva & Linares 1995). Decreases in germination percentage have been shown to be accompanied by selective change in the gene pool. Melchior (1985) and Gallo (1991) have shown that the seed of aspen (*Populus tremula* and *P. tremuloides*) can be successfully stored over several years. However, the longevity of the seed is under rather strict genetic

control. The resulting differential losses in families during storage induces a process of family selection for longevity, leading to the decay of genetic multiplicity as observed at several enzyme gene loci. Since aspen seed rapidly loses its power of gemination under natural conditions, the intensity and direction of the adaptation to the climate of the cold store are supposedly determined by the length and the conditions of storage.

The necessary precondition of viability selection is the loss of germination percentage. Therefore, artificial selection is avoided by avoiding losses of seeds. The multitude of forest tree species with very different physiological properties in their seed make the management of gene banks in the lower latitudes complicated. The recently established forest seed centres, with their research in methods of handling seed and producing planting stock, have a very important function in the genetic conservation of trees in that part of the world.

Therefore, the need for *ex situ* conservation over longer time periods leads to the establishment of conservation stands. The mortality in these stands introduces a dynamic element into this approach to conservation, which contradicts the believe that this type of conservation is static. Conservation stands are therefore addressed further below in the context of dynamic conservation.

Genetic change is also implied by the operations during seed collection. The appropriate mode of sampling the seed produced by a population is rather complex. The classical way of arriving at a representative sample would be to collect all seeds, mix them thoroughly and then select a sample. In reality, problems arise due to the collection of only part of the seed production from part of the trees, and the lack of genetic homogeneity of that seed. Given that the (majority of) the extranuclear genetic information in angiosperms is transmitted by the seed parent, this meant collecting seed from many trees. The number of trees can be reduced, since this information has low variability. In any event, the quantities collected from every tree should amount to roughly equal proportions of the actual seed produced by those trees. The trees themselves should be identified by a grid system.

At least half of the nuclear genetic information contained in the seeds of a given tree represents its allelic constitution. However, the spatial distribution of the genotypes in the field may be clumped (except after sowing or planting without subsequent natural selection) and distances of pollen transport may be short. The allelic contributions of the pollen parents to the seed produced by different trees are therefore not likely to be homogeneous. The total genetic differentiation among the seed trees and the genetic differentiation of their effective pollen clouds add up to the differentiation among the individual seed lots (Finkeldey & Gregorius 1994). These are of different size due to female fertility selection.

The complexity of this structure is scarcely reduced by picking the seed off the ground rather than collecting it from the trees. In beech, *Fagus sylvatica*, two out of the many conceivable approaches to seed collection were compared by Ziehe & al. (1996). Samples of 200 trees in each of three old stands were genotyped at 10 enzyme gene loci according to a grid system. During a full mast, seeds fell either on to nets (method I) spread under heavily fruit-bearing portions of the stands, in accordance with a widely adopted commercial procedure. This quantity was sampled after cleaning and mixing the seeds. The number and size of the nets was large enough to collect many thousands of beechnuts per stand. In addition, equal numbers of beechnuts were picked one year later by hand

(method II) under each of the 200 trees identified in the field. About 300 (method I) or 200 seeds (method II) were also genotyped at the same gene locus. As expected from other studies, both seed samples differed from the respective adult stands. Depending on allelic diversity at the respective gene loci, seed lots I and II differed considerably. However, in terms of allelic structure seedlot II resembled the allelic structure of the adult stand more closely. This close similarity was partly due to the fact that the seeds were picked just below the sample trees used in estimating the stand's genotypic structure. In one of the stands, genetic distances between samples I and II were most pronounced at a particular highly variable gene locus. This could be explained by clumped occurrence of trees that were homozygous for a given allele. Collecting the seeds in nets evidently failed to account for these clumps. The genetic distances between samples I and II were close to 10%, i.e. for an average of the ten gene loci, about one tenth of the alleles were not shared by the two samples. The genetic distances induced by different procedures of seed collection are possibly inflated by the effect of different seed years but they are of the same order of magnitude as the differentiation among 50 beech stands in the respective regions (Ziehe & al. 1996). This result demonstrates both the complexity and the importance of appropriate sampling of the seed population. It also has a bearing on population genetic studies in general and on provenance experiments.

Minimum sample sizes of seeds can be derived by the method published by Gregorius (1980). If the total seed is properly mixed, a sample size N can *a priori* be computed that warrants that all genes with a frequency of at least α are contained in the sample with probability $1-\Phi(\alpha)$.

Kim & al. (1994) devised an empirical method of choosing a sample size and presented examples from enzyme gene loci in three tree species. They genotyped 20 to 50 trees within each of 25 populations of *Pinus densiflora*, 13 populations of *P. thunbergii*, and 8 populations of *P. koraiensis* in South Korea. The trees stood at least 30 m apart. In this material they studied the minimum number of seeds required for detecting almost all alleles at 18 enzyme gene loci common to the three species. By starting out with a random tree and adding more trees in random order, the resulting non-decreasing detection curves were used for retrospective statements about the appropriate sample size. Beyond a critical point these curves flattened out to become parallel to the abscissa. In *P. densiflora*, for instance, an average of 18 randomly selected individuals already contained 96 per cent of the alleles encountered at the 18 gene loci in the respective population; and 22 trees contained 99 per cent. They concluded that in searching for candidate populations, genotyping this number of random trees was sufficient for getting an idea about the variation present in a population. One may conclude that a moderate number of seeds collected from each of these trees might contain almost all alleles at the 18 gene loci of the female contribution to the embryos. Probably more alleles are contained in the pollen contribution.

Storage of pollen of anemophilous species such as conifers is possible in a partial vacuum. This is unfortunately not possible in the bulk of tropical zoophilous species with their short-lived pollen. In any event, its regeneration requires receptive female flowers.

Clone banks are instruments that allow for genotype conservation by avoiding recombination. If properly designed as seed orchards, they have an important function in restoring mating contact in relict populations of low density. Organ cultures are reliable

for gene resource conservation in tree populations only inasmuch as *any* genotype can be regenerated *in vitro*. They are often used for pursuing goal (1).

Static conservation implies a risk of accumulation of a genetic load as a result of decoupling from a dynamic environment.

In static conservation *ex situ* replicates should be included in order to reduce the risk of loss (fire, wind, epidemics, domestic animals, construction).

Dynamic conservation does not preclude evolutionary processes but allows for adaptational and other genetic change in a changing environment. Standard procedure is the maintenance of populations *in sítu* under careful sylvicultural treatment in combination with natural regeneration. In contrast to resource conservation in crop plants, this method of conservation is most important in forest trees and range species. Its precondition is the ability of the resouree to reproduce also under changed environmental conditions.

Dynamic *ex situ* conservation or off-site maintenance also causes adaptation to the environment during evacuation. Conservation stands should be established by sowing so as to allow for adaptation to the new environment from the very beginning. Alternatively, planting with narrow spacing may serve the same purpose, if the species cannot be efficiently sown in the field. Conservation stands *ex situ* create the possibility of designing gene resources. The conservation environment must be heterogeneous with respect to both sites and stand structure in order to maintain diversifying selection and associated adaptive differentiation. In order to prevent local erosion of variation, the parts of the resource should be only partly isolated or otherwise enriched by planting.

Dynamic conservation must be given preference. It is possible in production populations if their establishment was not a founder event, and if their treatment did not enhance the loss of genetic variation or induce major changes in mating systems or adaptational processes. The management of gene conservation stands raises complex sylvicultural problems. Slowly progressing natural regeneration of stands might best secure the transmission of all genetic information to the subsequent generation, because with increasing length of the period of stand regeneration an increasing number of trees might contribute their shares to the progeny.

The repeated reproductive cycles of trees in combination with their variable reproductive behaviour (Müller-Starck 1985) supports genetic polymorphisms in their progeny (Gregorius 1991b). Furthermore, the genetic differentiation between the seeds produced in different years tends to increase the effective number of alleles in the subsequent generation.

Much has been published on the appropriate size of parts of a larger population, that are to be conserved *in situ*. When considering the results, it must not be forgotten that in deriving minimum numbers of individuals, only the alleles at a single gene locus are considered. However, in practice one has to consider the whole genome. This leads to much larger minimum numbers which easily demonstrate that very rare variants can never be conserved with a very high degree of security (Krusche & Geburek 1991).

Referring again to the pragmatic approach taken by Kim & al. (1994), in the materials mentioned above in the context of *in situ* conservation, these authors a *posteriori* studied the size of the area of pine stands required for detecting a large portion of the allelic diversity found at 18 enzyme gene loci. Starting with a randomly chosen population, they detected a certain number of alleles. After adding a second, third and more populations,

the resulting non-decreasing graph was used to arrive at a minimum number of populations required for detecting the essential, widespread, rare and sporadically occurring alleles (Brown 1992). In *P. densiflora,* for instance, a number of 14 random populations was sufficient to cause a flattening of the curve. By choosing the population with carriers of private rare alleles and adding a few more populations, only 7 populations were needed to detect 95 per cent of the genic diversity. 14 populations selected in this way contained 97 per cent of the alleles, and 21 populations contained 99 per cent of the total genic diversity found at the 18 gene loci surveyed. Since the minimum distance among the sample trees was 30 m, they were distributed over two to three hectares. This area contained several thousand trees, a number that might be sufficient to conserve the greater part of the genetic variants. As regards static *ex situ* conservation, the conservation of a total of $\geq$ 14 populations $\times$ 14 trees each, i.e. c. 300 individuals would contain almost all of the genetic multiplicity of South Korean *P. densiflora.* It is clear that not all allelic variants are found in every population. However, the large total number of trees in conjunction with rare and exclusive alleles must have helped to detect all but negligibly rare variants at the gene loci surveyed. The validity of these results is probably confined to isoenzyme gene loci with their low interdemic differentiation. Some DNA markers with a different variation pattern (Liu & Furnier 1993, Vornam & Herzog 1996) may have given different conclusions.

A decision in favour of dynamic conservation has some impact on the choice between candidate populations. The respective stands must possess syilvicultural stability as well as a density and demography that allows natural regeneration. Under given conditions of density, the breeding contact between trees and the reproduction-effective population sizes may be considerably decreased in tree species occurring as rare and dispersed mixtures in stands of other more abundant species.

Planned avoidance of inbreeding may be applied in small relict populations, such as the Sicilian *Abies nebrodensis.*

Probably the most difficult problems of *in situ* conservation are those raised by the conflict between genetic needs and the demands of the local populations (Ledig 1986, Finkeldey & Hattemer 1993).

Outlook

(1) The choice between several possible materials has to be made on the basis of rational criteria. Surveys of genetic markers should be combined with measurements of adaptive phenotypic traits. In view of the costs and of the importance of the relevant inventories, appropriate measures of variation and differentiation should be used. Mapping of these parameters is useful.

(2) Conservation of a single genetic resource of the size indicated above is hardly an adequate means of conserving a species. Isolated populations have a smaller chance of persisting than those connected by genetic links. Only a large number of genetic resources of this minimum size possesses the potential to continue evolving. The concept of the metapopulation very useful in the context of dynamic gene conservation.. A species is considered as a network of partially isolated populations in heterogeneous environments. They have a tendency towards differentiation due to selection and drift. Their gene pools are interconnected by gene flow. Local losses of trees are compensated by re-immigration from adjacent populations. This raises the need to consider the design of genetic resources

(Finkeldey & Gregorius 1994) both during selection and subsequent conservation. The design of genetic resources is also important in artificial conservation stands. These are several advantages in maintaining or introducing a differentiated substructure of demes, for instance, single tree progenies (Finkeldey 1992, Finkeldey & Gregorius 1994) or the contents of nets used in collecting seeds on the ground (Turok 1955).

The persistence of a metapopulation rests also on demographic conditions (age and sexual structures) and adaptability. The identity of a species is maintained in spite of and in reaction to exogeneous processes (Gregorius 1995).

(3) Considering target species as the objects of conservation is less promising than the concentration of efforts on their respective ecosystems. The human impacts described above are more severe on whole ecosystems than on single species. The close ties between tree species and their symbionts and mutualists require the inclusion of the latter in conservation measures. For instance, the majority of tree species in the tropics and subtropics are zoophilous and are scarcely able to reproduce in the absence of pollinators, many of which may be highly specialised. Species diversity is also required for maintaining closed nutrient cycles. Further arguments in favour of ecosystem preservation are discussed by Ledig (1988) who also pointed out that the success of species conservation strongly depends on the areas reserved for this purpose. Conversely, decisions on the choice among populations of target species are then no longer possible, so that genetic conservation becomes rather unspecific.

(4) Since the identification of marker gene loci and large-scale inventories of genetic and phenotypic characteristics is hardly possible in many species, the implementation of both biological theory and findings from other ecosystems and species gain importance.

(5) Growing numbers of seed centres and gene banks are being established; they provide both seed and information on methods of preservation and regeneration *ex sítu*. Today, such measures are largely the reponsibility of research stations that were formerly devoted exclusively to tree breeding (Melchior & al.. 1989, Kleinschmit 1994). Research is needed on methods of seed collection, on handling and storing seeds, and on appropriate silvicultural methods (defining criteria of stability and modes of natural regeneration).

Acknowledgements

I wish to thank Jozef Turok for valuable discussions and Elizabeth M. Gillet, Hansrolf Grecorius and Martin Ziehe for their critical review of the manuscript and pointing out several shortcomings.

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