

CONCEPTS AND REQUIREMENTS IN THE CONSERVATION OF FOREST GENETIC RESOURCES

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ABSTRACT

Manifold human influences on the genetic structures of tree populations give cause for measures of conserving genetic resources. After presenting a definition of gene conservation, various objectives are considered. In view of the genetic systems of trees as long-lived plants at a low level of domestication, the conservation of the genetic adaptability of their populations to environmental changes is emphasized. This implies that the choice among candidate populations for conservation be based on the results of surveys of genotypes at marker gene loci. In view of the expense and the long-term importance of this step, the quality of gene markers and the information on their inheritance mode is critical. Besides marker gene loci, the expression of genetically controlled adaptive phenotypic traits deserves proper weight. The selection criteria are then based on 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 silvicultural skill. The combination of these procedures 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. Present research needs are indicated.

Key words: human impacts on genetic structures, gene conservation, selection of genetic resources, methods of conservation.

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 in like quality 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 effective in a given 'stand' of trees might have been produced by that very stand, the trees which contribute effective pollen at reproduction might be much more numerous than the seed trees.

This condition has been tested in anemophilous tree species of the moderate and the boreal zones. The distribution of the transport distances of effective 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 cormus accumulates mutations. However, this hypothesis awaits testing by surveying genetic polymorphisms of DNA.

The combination of high genetic variation, prevailing cross-fertilization, and subsequent selection is responsible for the fact that degrees of heterozygosity in trees exceed 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.

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

DEFINITIONS

In order to prevent inflationary 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 information or extensively variable genetic information (ZIEHE *et al.* 1989). The latter property is somewhat unspecific, as will be further elaborated in section 6. 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 VAVILOV proposed that crop plant improvement should draw from wide genetic variation. He initiated 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 *et 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 expressions of 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 and is therefore 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 components of yield rather than financial revenue can be considered. (2) Genetic adaptability is the ability of populations to survive and reproduce even in a changed environment. Adaptability relies critically on the supply of *both major and minor genetic variants*. 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 much 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 inference of the genotype of individuals (seeds or complete plants) at one or several Mendelian gene loci from their variable phenotypes (*e.g.*, electropherograms of proteins, particularly enzymes, or of DNA fragments). Hence, they represent invaluable tools in the framework of gene conservation measures. The precondition 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, the classical crossing experiment is difficult to carry out in trees. GILLET and HATTEMER (1989) devised a method of 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 in the search for marker gene loci, because codominance is a necessary prerequisite of genetic markers employed in genetic inventories. The simple reason is that allelic structures can be estimated and genetic variation and differentiation of biological populations be measured if and only if any 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 conventionally 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 largely possess identical 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 and GREGORIUS (1993) have found adap-

rare genetic variants because of their different role and different behavior in adaptational 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, strong 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 experimental 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 do 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 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 reference.

CONSERVATION METHODS

Static conservation attempts to fixate the *status quo*. It is usually achieved by preservation *ex situ* when the population is endangered in its natural habitat. However, at least 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. It does not seem to be possible for longer periods of time without considerable losses in germination in certain angiospermous genera, such as *Fagus* and *Quercus*. Many tropical trees possess extremely short-lived seed.

Only recently could ways of inducing a short dormancy in species of dipterocarps be developed (VILLANUEVA & LINARES 1995). Decreases in germination percentage are proven to be accompanied by selective change in the resource. 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 of 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 germinability 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 of their seed make the management of gene banks in the lower latitudes complicated. The recently established forest seed centers 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 conservation *ex situ* 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 draw a sample. In reality, problems arise due to the collection of only part of the seed production of part of the trees and the genetic inhomogeneity of that seed. Given that the (majority of) the extranuclear 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 sound seed produced by those trees. The trees themselves should be identified by a grid system.

At least half of the nuclear information contained in the seeds of a given tree represents a more or less representative sample of its allelic constitution. How-

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 resource to reproduce also under changed environmental conditions.

Dynamic conservation *ex situ* or off-site maintenance also causes adaptation to the environment during evacuation. Conservation stands should be established by sowing in order to allow for adaptation to the new environment from the very beginning. Alternatively, planting at narrow spacing may serve the same purpose, if the species cannot be efficiently sown in the field. Conservation stands *ex situ* create the possibility for designing gene resources. The conservation environment must be heterogeneous with respect to both sites and stand structures, in order to maintain diversifying selection and associated adaptive differentiation. In order to prevent local decay 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 no founder event, and if their treatment did not enhance losses of genetic variation or induce major changes in mating systems or adaptational processes. The management of gene conservation stands raises complex silvicultural 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 iteroparity of trees in combination with their variable reproductive behavior (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, which are to be reserved for conservation *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 application 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 (NAMKOONG *et al.* 1980, KRUSCHE & GEBUREK 1991, HATTEMER *et al.* 1993, loc. cit. p. 228).

We consider again the pragmatic approach taken by KIM *et al.* (1994). In the materials mentioned above in the context of conservation *in situ*, these authors a *pos-*

teriori studied the size of the area of pine stands required for detecting a large portion of the allelic diversity encountered at 18 enzyme gene loci. Starting out with a randomly chosen population, they detected a certain number of alleles. After adding a second, third etc. population, 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 detection curve. By picking the population comprising carriers of private rare alleles and adding a few populations more, only 7 populations served the purpose in order to detect 95 per cent of the genic multiplicity. 14 populations selected in this way contained 97 per cent and 21 populations contained 99 per cent of the total genic multiplicity encountered 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 comprised several thousand trees. This number might be sufficient for conserving the greater part of the genetic variants. Making reference to static conservation *ex situ*, the conservation of a total of ≥ 14 populations \times 14 trees each \sim 300 individuals would contain almost all of the genetic multiplicity of South Korean *P. densiflora*. It is clear that *not all* allelic variants are detected *in every* population. However, the large total number of trees in conjunction with the rarity of private 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 led to different conclusions.

A decision in favor of dynamic conservation has some impact on the choice among candidate populations. The respective stands must possess silvicultural stability as well as a density and demography allowing for later natural regeneration. Under given conditions of density, the mating contact between trees and the reproduction-effective population sizes may be considerably decreased in tree species occurring as rare and dispersed admixtures in stands of more abundant other species.

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

Probably the most difficult problems of conservation *in situ* are raised by the conflict between genetic necessities and the demands of the local populations (LEDIG 1986, FINKELDEY & HATTEMER 1993).

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