TREE BREEDING, PRACTICES

Breeding and Genetic Resources of Scots Pine

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Introduction

This article covers Scots pine (*Pinus sylvestris*) genetic resources and breeding, and especially contrasts issues in this species to those in other important species of conifers. The emphasis is on results on northern countries, where this species is very important and has been intensively studied. As most pines are at early stages of domestication, breeding is still largely aided and/or constrained by the natural features of the species. Breeding programs have not advanced beyond F_2 generation seeds and advanced generations are not yet available. Commercially sold improved seed is still based mostly on first-generation seed orchards. Further, the silvicultural production populations are in cultivated forest-like situations rather than intensively managed plantations. In these conditions Scots pine has to grow for the full rotation, even 80–100 years.

Biological Characteristics of Scots Pine

Scots pine's natural distribution ranges from western Scotland to eastern Siberia and from northern parts of Scandinavia to central Turkey or southern Spain. It has the widest distribution of all pine species, and, in relation to its range, the discontinuities in its distribution are minor. The species is wind-pollinated and predominantly outcrossing. Very efficient pollen flow has been documented through direct measurements of radioactively labeled pollen or by measuring seed set in isolated young stands that do not yet produce any own pollen. Early on, it was evident that seemingly functionless (nonadaptive) variation in seed cone morphology is not differentiated across populations, consistent with the high gene flow. Many studies on a wide range of populations have shown that at neutral (or near-neutral) genetic markers, such as isozymes or repeat sequences of DNA (microsatellites), allelic frequencies in populations over the whole area of distribution are quite uniform, with less than 5% of the total variation between populations. The same finding has also been made in initial studies of nucleotide sequence variation. Thus, all available evidence suggests that the whole distribution area of Scots pine functions in many ways as a single enormous population, with very minor differentiation at marginal very isolated populations, e.g., in Spain. Initial studies of maternally inherited markers, in mitochondria, confirm that seed migration is not as efficient, but thorough studies of maternally inherited markers are still lacking. This picture of the enormous population size with efficient gene exchange gains further support from the fact that the levels of variation, e.g., at the standard isozyme loci, have been among the highest in any organism. Further, even if quantification of variation at the DNA level has not been done extensively, it has been rather easy to detect genetic variation for genetic mapping with various DNA techniques.

The broad area of distribution covers a wide range of climatic conditions. Even within Finland the length of the thermal growing season ranges from 120 days in the north to 170 days in the south (latitude 60° N to 68° N). Consistent with the large range of environmental variation, many adaptive traits, such as timing of growth or the development of frost tolerance show steep clines, correlated with the environmental gradients. In common-garden experiments in greenhouses, first-year seedlings of northern populations form the terminal buds and become frost-tolerant several weeks before the southern Finnish strains. Such clines extend also south of the Finnish populations. While populations in different latitudes are highly differentiated, they still contain much genetic variation in these traits, as documented by quantitative genetic studies. The adaptive significance of the variation has been demonstrated by extensive transfer experiments between latitudes and altitudes in Sweden. Results from large-scale provenance trials suggested that 1° of latitudinal transfer northward causes a decrease in survival of approximately 10%, while an increase of 100 m in elevation results in a reduction of survival of approximately 3%. The evidence thus suggests that the patterns in quantitative traits are due to intense natural selection that overcomes the high level of gene flow at the relevant loci, and creates a pattern of detailed adaptation to the climate. In the north the relevant trait is adaptation to the short growing season and survival in cold conditions, in the southern margin (e.g., Spain) traits like drought resistance may be most important. This high differentiation for quantitative traits affecting survival has to be taken into account when breeding the species. Note that the parallel selection for, e.g., early cessation of growth and early frost tolerance results in high correlation of these traits among populations. However, this does not imply that the traits are genetically correlated at the within-population level. In fact, initial quantitative trait locus (QTL) mapping studies have shown that different loci are responsible for growth cessation and the development of frost tolerance.

Many other conifer species are much less sensitive to such transfers between areas, and breeding for specific geoclimatic zones may not be as important, e.g., in *Pinus radiata* or *P. taeda*.

Breeding System

Another important trait of Scots pine is its breeding system. There is no self-incompatibility system, and wind pollination results in considerable proportions of self-pollination and self-fertilized zygotes. Direct measurements at the zygote stage have not been made, but it can be inferred that the primary selfing rate must be often at least 20%. In the mature seed, however, there are usually only a low percentage of selfs. There seems to be little occurrence of other kinds of inbreeding. Several studies have shown that adult Scots pine populations do not have any selfs. Thus, between the zygote stage and the adult tree stage the selfs are preferentially eliminated by usually severe inbreeding depression. Much of the elimination takes place very early, during seed development. At the age of a few years natural populations show little evidence of inbreeding. When selfs survive in experimental conditions, they show a cumulative decline in relative survival with lowered fitness over several decades due to poorer growth, which results in size-specific elimination of selfs by competition. The inbreeding depression is presumably due to a large number of deleterious recessive genes in the Scots pine genome. The average tree is heterozygous for 8–10 so-called embryo lethals, which means that Scots pine is among the species with the very highest genetic loads. Scots pine reproduction is exclusively sexual. Note that the generation time is very long; the trees do not become fully reproductively mature before the age of 20. Not only is there no asexual reproduction in the natural populations, but Scots pine has also proved a very recalcitrant species for various modes of vegetative propagation.

Natural versus Artificial Regeneration

A large proportion of Scots pine forests is still naturally regenerated. In some areas, e.g., in Russia, the huge annual requirement for Scots pine seed still exceeds the supply of genetically improved seed. Artificial regeneration is either with seedlings or through sowing of unimproved seed. In Scandinavia, natural regeneration has an established role in forest management practices even in areas with ample breeding material available. Natural regeneration is preferred over artificial regeneration mainly because of the lower cost. Moreover, problems in the development of planted seedlings have resulted in decreased use in some areas. Furthermore, problems in producing seeds adapted to the intended area (owing to problems with background pollen contamination in seed orchards) have slowed the use of improved material in northern areas. This has forced foresters to use natural regeneration and/or seeds from natural seed-tree stands in artificial regeneration in the northern areas.

Breeding Goals

The boreal flora with only a few tree species is challenging for tree breeders: seedlings of Scots pine may be planted, e.g., on dry and poor or relatively moist and nutrient-rich soils. The rotation age of Scots pine is much longer (80–100 years) than in some other conifers, e.g., loblolly pine in the southern USA (25–28 years). Enough genetic variation must thus be maintained in Scots pine populations to meet the requirements of spatially and temporally heterogeneous environments.

When breeding for northern environments, survival is the most important trait. In line with the large effects on survival of provenance transfers, several breeding zones have been established for Scots pine in northern countries: 11 in Finland and as many as 24 in Sweden where altitudinal variation is much more important than in Finland (**Table 1**). Growth rate has also been an important trait in breeding programs. Furthermore, stem and wood quality, crown shape and size, and pest resistance are other traits that have been taken into consideration in most breeding programs of Scots pine.

Breeding programs of Scots pine in Finland are national and use only domestic material. However, several international large-scale provenance studies have been established and maintained by the International Union of Forest Research Organizations (IUFRO). The first IUFRO trial was established as early as 1907, and it was followed by several other international trials. These experiments have documented the ample genetic variation that exists within and among populations in many quantitative characters. Recently, international provenance trials and progeny tests of Scots pine have been used for studies on resistance to air pollution and adaptation to climate change.

Scots Pine Breeding and Seed Production

Genetically improved material in Scots pine is produced by sexual reproduction. No clonal material

Table 1 Breeding activities of Scots pine in Finland and Sweden; estimates were obtained with permission from Haapanen and Mikola (2002)

	Finland	*Sweden*
Agency running the program	Finnish Forest Research Institute (Metla)	Forestry Research Institute of Sweden (Skogforsk)
Basis of subdivision of program	Mainly latitudinal gradation	Latitudinal and altitudinal gradations
Number of breeding populations	11	24
Proportion of regeneration of Scots pine served by breeding program	50% (proportion varies between areas being high in the southern parts and very low in northern parts of Finland)	66%
Number of planted seedlings in 1999	60 million	124 million
Seed orchards, ha	2000	924

for regeneration purposes is currently available. Cloning by somatic embryogenesis, which succeeds only with immature seed embryos, is difficult in Scots pine, which lowers the efficiency of cloning as a breeding tool in this species. The material of the production populations is generated in seed orchards, where genetically and/or phenotypically superior trees intermate and produce seed. These seeds are used in nurseries to produce seedlings to be planted at 1 to 2 years of age.

Actual breeding takes place in breeding populations. Selection of phenotypically superior "plus" trees has taken place in virtually all countries cultivating Scots pine. These phenotypes have been vegetatively propagated by grafting to establish the first-generation seed orchards. Several criteria have been used in selecting plus trees. Growth rate has been one of the major criteria, others including stem and wood quality and self-pruning. The first-generation seed orchards have been in full seed production already for several decades, and the genetic gain over wild seed has been estimated to be about 5–10% in growth and somewhat lower in quality characteristics.

The performance of plus trees has been evaluated by progeny tests, and the proven best parents with respect to, e.g., hardiness, growth, and stem and wood quality have been selected to establish 1.5-generation seed orchards. This work has started, for example in Finland and Sweden, and some of the 1.5-generation seed orchards are already functional. In Sweden, crosses between most superior trees have already started to produce elite trees for new-generation seed orchards.

The long-term breeding programs of Scots pine try to maximize genetic gain, avoid inbreeding in production populations, and maintain enough genetic variation for potentially changing environments. In Sweden, a multiple-populations breeding approach has been adopted, where double-paired matings produce material for within-family selection experiments. In Finland, breeding will proceed with a rather similar approach where double- and multiple-pair matings produce material for among- and within-family selection experiments. The genetic gain in growth may be as high as 20–24% in the second-generation seed orchards.

Because of the long generation times, these breeding populations are still at a very early stage of development compared to those of *P. radiata* and *P. taeda*.

Genetic Functioning of Scots Pine Seed Orchards

High genetic gain and maintenance of genetic diversity are two demands that clonal Scots pine orchard designs should meet. These aspects have been studied empirically and theoretically by Scots pine breeders.

The loss of genetic variation is not a major problem in present seed orchards of Scots pine. The first-generation seed orchards of Scots pine consist of many clones with a few ramets per clone. In Finland the average clone number in a Scots pine seed orchard is 121 and in Sweden 80. The number of clones will be substantially lower in advanced-generation seed orchards. Furthermore, variation in fertility among clones lowers the effective population size, thus reducing the expected genetic variability in the seed crop. Fertility variation is most pronounced in young seed orchards, reducing effective population size to half of the actual (census) number of clones. However, the loss of genetic variability due to fertility variation is much less serious in seed orchards with full pollen and seed production. Furthermore, the numbers of clones in present-day orchards, and also in the planned advanced-generation orchards, should be high enough to maintain much genetic variability.

The major factor reducing capture of genetic gain of seed-orchard material is background pollination. Orchards producing seed should be spatially isolated from surrounding Scots pine forests. In Finland, the orchards producing seed for northern areas were established in more southern areas to improve seed set, and thus it was expected that they should be genetically isolated from local forests, not only due to spatial separation but also due to temporal separation because of the earlier flowering of northern seed-orchard clones compared to local populations. Long-distance pollen dispersal, however, has been observed to be much more common than was earlier believed. The estimates of background pollination in Scandinavia vary between 6% and 75%, being as high as 25–35% in many old seed orchards with full pollen and seed production. In southern orchards, background contamination reduces the genetic gain expected from the seedlot. The situation is much worse in northern seed orchards, where background pollination (north × south crosses) makes seed lots unsuitable for regeneration in planned areas due to low hardening and high mortality. The area suitable for regeneration with seed orchard material has been estimated to be between the origin location of clones and location of a seed orchard. Suitable areas for different seedlots have also been estimated by freeze tests of the seedlings. Attempts have been made to solve the problem of background pollination with various methods, e.g., by supplemental pollination or selective seed collection, but the problem persists without a clear solution.

Genetic Resources and Gene Conservation

Although large natural populations of Scots pine maintain ample genetic variation, some measures have been taken to assure the maintenance of genetic variability for potentially changing environments, for instance, in planning long-term breeding programs. The Swedish multiple-populations breeding scheme is considered to serve both breeding and gene-conservation purposes. In Finland, several gene-reserve areas of Scots pine have been established in addition to breeding populations. In gene-reserve stands only natural regeneration is allowed, and seed from these stands may also serve as natural-population control material in genetic studies.

Future Aspects

The impending climate change will be an important issue for Scots pine breeding. The core of Scots pine breeding will be the production of seed material that survives for 80 years in the forests. Breeding for climate change has also already started. For instance, all testing is done in several breeding zones, in order to detect genotypes that are preadapted to the future conditions. Recently, more emphasis has also been placed on understanding and breeding for wood physical and chemical properties.

See also: **Genetics and Genetic Resources**: Genetic Systems of Forest Trees; Propagation Technology for Forest Trees. **Silviculture**: Natural Stand Regeneration. **Tree Breeding, Principles**: A Historical Overview of Forest Tree Improvement; Breeding Theory and Genetic Testing; Forest Genetics and Tree Breeding; Current and Future Signposts.

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