

A NOTE ON GENETIC DIVERSITY IN NATURAL POPULATIONS AND CULTIVATED STANDS OF SCOTS PINE (*Pinus sylvestris* L.)

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SUMMARY

Aspects of genetic diversity in Scots pine are reviewed with particular emphasis upon the interactions with silvicultural practices such as the use of natural or artificial regeneration. There is little evidence that greater use of artificial regeneration will result in decreased genetic diversity at the stand level. This result appears to hold whether the planted seedlings are derived from wild stands, from seed stands or from seed orchards. However, in the last instance, the orchard should contain at least 40 clones to provide the heterozygosity expected of natural populations. There is limited knowledge of genetic diversity in adaptive traits such as cold hardiness or drought tolerance. Despite nearly a century of research into the genetics of Scots pine, there is still inadequate knowledge of the desirable level of genetic diversity in forests of this species and the interactions with management practices. However, current European regulations on Forest Reproductive Materials pay due attention to issues such as genetic conservation and matching of seed sources to regions. Therefore, it seems unlikely that current silvicultural practices will limit genetic diversity in Scots pine.

KEY WORDS: Scots pine
Genetic diversity
Regeneration
Seed orchards

INTRODUCTION

The term *genetic diversity* refers to the phenomenon that individuals of a given species do not have the same genotype, and consequently differ in appearance and behaviour. In a pine forest one can observe differences among trees in size, stem form, branching habit etc. All these differences are not due to genetic factors alone. For example, in a mixture of age classes, older trees are usually taller and bear more cones than young ones. Branchiness, stem form, and diameter are strongly modified by the ambient soil fertility and the growing space available.

Genetic diversity within such a widely distributed tree species as Scots pine (*Pinus sylvestris* L.) is a complex issue. As the natural range extends from Scotland to China, and from Norway to Turkey respectively, there must be a considerable differentiation due

to climatic and edaphic factors. This is very clearly shown in numerous provenance experiments (e.g. Wright and Bull, 1963; Giertych, 1979). A complicating factor in discussing genetic diversity is the traditional «free movement of germ plasm» in Central Europe. Large areas of pine plantations were established with non-local material, whose origin cannot be traced.

In most traits the separation between the genetic component of variation and the effect of environment requires rather sophisticated progeny tests and statistical analyses. For some thirty years it has been possible to reveal the genetic variation at the molecular level, initially by means of isoenzymes, and more recently by means of various DNA markers. Numerous studies on the genetic structure of Scots pine have consistently shown large genetic variation in all characteristics studied (see Forrest *et al.*, this volume). In other words, Scots pine contains broad genetic diversity.

The pattern of variation is noteworthy; as a rule the within population (stand) variation comprises more than 90 % of the total genetic variation within a region. In terms of isoenzyme variation, Muona and Harju (1989) reported that more than 98 % of overall variation in Central Finland occurred within stands. In an old study on cone morphology (Koski, 1970), 70 - 80 % of the total phenotypic variance was within stands, in material extending from latitude 40° N (Turkey) to 70° N (Finland).

Most geneticists consider genetic diversity in adaptive traits advantageous to the species, in fact it is understood to be a necessary condition for survival and evolution of a species. Even though this hypothesis is extremely difficult to test empirically, the maintenance of genetic diversity is generally accepted as an unconditional goal of forest management. One can sometimes find the statement that genetic diversity of forest trees can be maintained only by means of natural regeneration, and in extreme opinions only in unmanaged forests. As a consequence, there is a common belief that artificial regeneration would always lead to loss of genetic diversity. Genetically improved reforestation material originating from clonal seed orchards has an especially bad reputation. The purpose of this note is to review this argument with Scots pine, using results primarily from northern Europe.

GENETIC ASPECTS OF NATURAL REGENERATION

Entirely natural or spontaneous regeneration can take place only in unmanaged protected areas, such as national parks or nature reserves. In commercially managed forests, man's impact always has some effects on the genetic composition. Natural regeneration results in good stands and large diversity, if, and only if, regeneration fellings are carried out in an appropriate way. Official guidelines recommend leaving a sufficient number (> 50 stems per hectare) of good seed trees and to use adequate site preparation. In a correctly managed silvicultural system using natural regeneration, the seed trees represent the dominant trees of the former stand, which maintains the genetic adaptation as well as the genetic diversity. The seed trees are not lost; the economic return from these trees is only delayed by a few years. However, one can sometimes see that natural regeneration per se is considered the decisive factor. The very heavy pressure to maximise economic profits may lead to harvesting of all valuable stems and neglecting of site preparation. Small trees with suppressed canopies

are left as future seed trees. This kind of exploitation with the pretext that «natural regeneration» will make good any loss in genetic quality is not acceptable. Such negative selection may result in a decline in the status number (see below) and a probable genetic erosion if repeated over generations.

Natural regeneration is not successful in all cases. The quality of the existing stand may be poor, circumstances of germination and seedling development can be too harsh, or in case of afforestation, there are no seed trees. Mechanisation of logging favours clear cut openings and increases the need for cultivation. Simultaneously the availability of genetically improved stock and expectation of higher yield and higher quality have encouraged greater use of artificial regeneration.

Scots pine has been cultivated widely in Europe for many tree generations. Mostly the results have been satisfactory, but the small proportion of failures has caused serious doubts on artificial regeneration as such. Unsuitable origin was one reason for poor quality or low survival. Many provenance trials in the Nordic countries have shown that mortality of southern origins is very high in relation to the local origin (e.g. Eiche and Andersson, 1974; Persson and Ståhl, 1990). Provenance research was originally targeted to detect superior seed sources for each region.

The tentative results of international provenance trials of Scots pine were not published until 1930, and current knowledge has been gradually accumulated during several decades (e.g. Giertych, 1979; Persson, 1994). In Central Europe, transfers over long distances and the use of unknown origins scarcely caused any loss of diversity or high mortality, but at high northern latitudes reforestation sometimes totally failed.

Currently the importance of a proper seed source is recognised, and the EC-Directive requires a certificate of identity on each set of reproductive material. Because movement of reproductive material must not be restricted within the EU, foresters need to be aware of suitable seed sources for their regions. Classification of reproductive material has been subject to change for many years, and a new EC Directive on the marketing of forest reproductive material was accepted by the EC Commission on 22 December, 1999. The new categories will be: 1. Source identified, 2. Selected, 3. Qualified and 4. Tested.

Artificial regeneration of Scots pine is always based on seed. The genetic composition of the cultivated stand essentially depends on the characteristics of the seed source. Source identified, i.e. regional seed is collected from several unselected stands, selected seed from approved seed stands, and seed orchard seed from seed orchards. In the past, cones of Scots pine were sometimes collected from a few «easy to collect» trees, but that kind of method was exceptional. In addition to heavy branches and bad stem form, such material might transfer rather narrow genetic variation into the stand in question.

As regards Scots pine, all approved seed sources provide as large genetic diversity as natural regeneration. Clonal seed orchards are alleged to produce genetically uniform material. Plus trees were selected for their superior phenotype in growth and stem form. Grafts of plus trees are used in clonal seed orchards, but the seed produced originates from out-crossing of numerous parents. The normal practice has been to group clones originating from plus trees growing in a range of localities to each seed orchard. Even though the exact pattern of spatial consanguinity in natural stands may be uncertain, I feel that the artificial composition of genotypes from distant sources characteristic of seed orchards should not decrease genetic variability.

From the viewpoint of reproductive biology and mating pattern, there is no reason to expect significant differences between the genetic diversity of natural seed sources and seed collected for artificial regeneration. On the other hand, the difference in the density of seedlings between natural seeding and planting can be striking. The Finnish practice, for instance, is to plant no more than 2000 seedlings per hectare, whereas spontaneous seedling density may be 50 000 per ha (Lönnroth, 1925, cit. Muona and Harju, 1989). Seed germination and early growth of seedlings takes place in much more favourable environment in nursery than in forest. Inbred seedlings may survive through the nursery stage, but not in harsh field conditions. The mortality of natural seedlings is very high, the probability to grow up to a parent of next generation being less than 1 %. Even if one assumes that the elimination is mostly at random, differences of genetic diversity may occur between spontaneous regeneration and artificial stands.

A survey of reproductive processes and subsequent genetic composition of regeneration material in natural regeneration and cultivation of Scots pine stands does not identify factors that would cause reduced genetic diversity in cultivated stands. It is, however, necessary to test this hypothesis with available methods of population genetics.

ESTIMATING GENETIC DIVERSITY

Estimates of the effective population size (N_E) or status number (N_s) (Lindgren *et al.*, 1997) describe the number of unrelated parents in a panmictic population that would produce the same genetic diversity as that measured in the field. Several simulation studies suggest that the minimum number of unrelated clones in a panmictic, hypothetical, seed orchard should be 20–30 in order to contain the genetic diversity of natural stands (Giertych, 1974; Bondeson and Lindgren, 1993; Roberds and Bishir, 1997). In real seed orchards, a value of $N_s = 20$ corresponds to an actual number of clones (census number) c. 40, due to fertility variation among clones, unequal numbers of ramets per clone and spatial distribution of clones. Most seed orchards of Scots pine contain at least 40 clones, and, in Finland there are on average over 100. Hardly any seed orchard is perfectly isolated from immigrating pollen clouds. Exact measurement of the effect of pollen contamination on the effective population size would be extremely laborious, but would certainly show it to be significant.

Estimates of expected heterozygosity can be obtained by means of studies with isoenzymes. Muona and Harju (1989) used 13 loci in their comparison of two small seed orchards with three natural stands. The differences in expected heterozygosity were negligible, even though the orchards comprised only 25 and 28 clones respectively. The above mentioned pollen contamination, was estimated to be 30 %, which explains the high diversity. Harju (unpublished results) has recently calculated expected heterozygosity (H_e) for natural populations, for a seed orchard with 82 clones, and its predicted seed crop, using five polymorphic allozyme loci. The results showed no difference in expected heterozygosity between the natural stands and the product of the orchard (Table 1). When she plotted H_e against increasing number of parents, Harju found that the level expected in natural populations was reached at around 40 clones. The conclusion is again that 40 clones in a seed orchard are enough to produce sufficient genetic diversity in the seed crop.

TABLE 1
COMPARISON OF EXPECTED HETEROZYGOSITY (H_E) BETWEEN A SEED ORCHARD AND TWO NATURAL STANDS IN FINLAND (AFTER HAJU, UNPUBL. DATA)

Comparación de la heterocigosidad esperada (H_e) de un huerto semillero y dos rodales naturales en Finlandia (Haju, datos no publicados)

Object	Sample	He
Seed orchard 141	Clones	0.432
	Pollen cloud	0.408
	Seed crop	0.419
Southern natural stand	164 trees	0.436
Northern natural stand	109 trees	0.395

Phenology and growth rhythm could perhaps be revisited with respect to diversity. They are, at least in northern Europe, directly adaptive traits and often coupled with resistance to pests and disease. Artificial freezing tests of families originating from clonal seed orchards are used when screening reproductive material for harsh environments (e.g. Andersson, 1992; Pulkkinen, 1995). The variation in the timing of hardening among progenies from one and the same seed orchard is very broad, which supports the theory of high genetic diversity of seed orchard seed. The northern seed orchards represent an extreme situation, where natural selection for hardiness should be strong. The plus trees were selected for superior growth and quality, but nevertheless their offspring display wide variations in adaptive traits. So far there are hardly any comparative studies on the diversity of adaptive traits, even though they would have more practical significance than markers with unknown influence.

Two further aspects of genetic diversity may be mentioned, even though there are hardly any empirical results to refer to. Firstly, quantitative traits are most concrete and interesting from the practical point of view. Secondly, genetic load, which is the concealed part of genetic diversity, is crucial from the viewpoint of evolutionary genetics.

Quantitative traits, such as height and diameter, respond strongly to environmental factors. Therefore, the total phenotypic variation is only partly due to genetic differences. The proportion of genetic variance (V_G) of the total phenotypic variance (V_P) is called broad sense heritability (H^2)

$$H^2 = V_G/V_P$$

The proportion of additive genetic variance (V_A) out of total phenotypic variance is called narrow sense heritability (h^2).

$$h^2 = V_A / V_P = V_A / V_A + V_D + V_I + V_E$$

where V_D is the dominance component, V_I is the interaction component, and V_E is the error component of variance. Narrow sense heritability estimates are more frequent in tree breeders' reports. They are around 0.2 for height and c. 0.4 for wood density, rather low

as such. Such a variance ratio is quite abstract, and therefore a coefficient of additive genetic variation ($CV_A = 100 \times SD_A / \text{mean}$) is used. I have not found any reports on comparisons of the coefficients between progenies of random sample trees from natural stands and those of seed orchard clones.

Genetic load is most prominently manifested as inbreeding depression after mating between close relatives. In Scots pine the very low proportion of sound seeds after selfpollination is a well-known example. Inbreeding depression is a major selective force favouring out-crossing, which in turn through recombination enhances large genotypic variation. Genetic load consists of deleterious or lethal recessive alleles. Geographic as well as individual differences have been detected in the number of embryonic lethals of Scots pine (e.g. Kärkkäinen *et al.*, 1996). Inbreeding depression is furthermore expressed at later age of trees (Koelewijn *et al.*, 1999). In addition to its crucial role in evolutionary genetics, genetic load may have significant coupling with the variability of adaptive and quantitative traits.

CONCLUSIONS

Scots pine, *Pinus sylvestris*, is one of the most thoroughly investigated conifers as regards the reproductive systems and genetic factors. Nevertheless, our knowledge is insufficient to draw firm conclusions on the desirable level of genetic diversity in pine forests and the impacts of human activities on this. On the other hand, there is no reason to postulate that artificial regeneration per se would systematically erase genetic diversity. Both in managed natural regeneration and in artificial regeneration the genetic quality of the reproductive material is decisive. Current national and international guidelines take genetic values into account. The same holds true at advanced tree breeding programs, where long term breeding populations are being established for the maintenance of variation.

RESUMEN

Nota sobre la diversidad genética en poblaciones naturales y plantaciones de pino silvestre (*Pinus sylvestris* L.)

En este trabajo se revisan distintos aspectos de la diversidad genética de pino silvestre, con un énfasis especial en las interacciones con tratamientos selvícolas, como el uso de regeneración natural o artificial. Existen pocas evidencias de que un mayor uso de la regeneración artificial produzca una disminución de la diversidad genética al nivel del rodal. Parece que este resultado se mantiene si los brinzales se derivan bien de rodales naturales, de rodales semilleros o de huertos semilleros. Sin embargo, en el último caso, el huerto debe contener al menos 40 clones para proporcionar la heterocigosidad esperada de las poblaciones naturales. Existe un conocimiento limitado sobre la diversidad genética de los caracteres adaptativos tales como la tolerancia al frío o a la sequía. A pesar de casi un siglo de estudios sobre genética del pino silvestre hay un conocimiento inadecuado de los niveles deseables de diversidad genética en los bosques de esta especie y las interacciones con la gestión selvícola. Sin embargo, las actuales leyes sobre los materiales forestales de reproducción prestan una especial atención a temas como la conservación genética y la adecuación de las fuentes semilleras a las regiones. Por tanto, parece poco probable que las técnicas selvícolas actuales puedan limitar la diversidad genética del pino silvestre.

PALABRAS CLAVE: Pino silvestre
Diversidad genética
Regeneración
Huertos semilleros

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