

Sustainable gene conservation of *Pinus sylvestris* in Lithuania

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To cope with future pressure on forests, gene conservation should be based on an evolutionary approach with simultaneous genetic improvement and dynamic gene conservation. In agreement with many recent reports on forest tree gene conservation we recommend *ex-situ* dynamic gene conservation and breeding according to the Multiple Population Breeding System concept. The progeny test plantations established in different natural forest regions during the period 1979-1995 will constitute the core of this conservation. Additional selection and establishment of test plantations will be done for the breeding zones without proper coverage today. This allied gene resource and breeding population will consist of twelve subpopulations of 50 genetic entries each: with two subpopulations in each of the six breeding zones in the country. The new generation of gene resource and breeding subpopulations will be created by artificial crossing of the best individuals selected within each of 50 families constituting a subpopulation. It would also be cost saving when the two objectives, breeding and gene conservation, are fulfilled. The status and functions of the high number of existing conventional *in situ* gene reserves, in which no management is allowed, should be reconsidered.

Key words: *Pinus sylvestris*, *ex-situ* dynamic gene conservation, Multiple Population Breeding System

Introduction

In recent years there has been increasing understanding that sound forest tree gene conservation must be based on evolutionary genetics. As a consequence of this several papers advocating dynamic gene conservation were published (Namkoong 1984, Hattemer and Gregorius 1990, Finkeldey and Hattemer 1993). In a paper on dynamic forest tree gene conservation Eriksson et al (1993) stated "Because of differences in objectives or approaches, some programs conflict with others, even though they all carry the banner of gene conservation". Some programs focus on *in situ* conservation without any management of the gene resource populations while others focus on a dual function of the breeding population for simultaneous genetic improvement and gene conservation. Evidently such a dual function has to be dynamic to cope with the uncertain future. It would also be cost saving if the two objectives, breeding and gene conservation, are fulfilled.

To put the tree gene conservation in a specific country into a global frame we prefer to first address the future pressure on forests and then discuss different objectives of gene conservation.

Long-term pressure on forests and gene conservation objectives

In accordance with a recent projection by FAO (Anonymous 1994) the global demand of wood for pulp and paper for the years 1990-2005 was estimated to rise by 3.1 % annually. The demand of wood for other human utilities is expected to increase by 2,000 million m³ during the same period. This corresponds to a 50 % increase for the same time period. These increases in demand are accompanied by an annual reduction of tropical forests by approximately 15 million hectares (Palmberg-Lerche 1994) and a skyrocketing increase of the human population, presently by 90 million people every year.

Therefore, in a long term perspective with high probability there will be an increased pressure:

1. To raise the production of wood for all types of utilities,

2. On land for all types of human utility production.

Forest tree gene conservation has to work within this time frame and develop gene conservation methods that are maintained even under these pressures.

An obvious consequence of the increased demand for wood in the future is that tree breeding must be given priority. This will be especially important for developing countries and countries with transition economies, like Lithuania. With increasing cost of raw material it becomes more urgent for such a country to be self-sufficient with respect to wood. Therefore, gene conservation ought to be considered jointly with tree breeding and not isolated from tree breeding.

Eriksson et al (1993) strongly argued for a clear formulation of objectives in gene conservation. In agreement with them we have as the prime objective of gene conservation *the creation of good conditions for future evolution*. Within this frame we identify a joint breeding and gene conservation objective of *Pinus sylvestris* in Lithuania as a consequence of the expectation of the sharp increase in the long-term need for wood.

Many conservation programs favour *in situ* conservation. Sometimes this builds on a misconception that the maximum fitness would be obtained in nature. Consequently of this the present genetic constitution is identified as the prime objective of gene conservation. Eriksson et al (1993) carried out a detailed discussion of this matter, and we shall only briefly summarize the main reasons for this misconception. Even if the maximum fitness is not prevailing it is thought to eventually be obtained if natural selection had time enough to act upon the population. This requires that the two conditions below are fulfilled:

1. Total independence of all traits, and
2. Constant environment.

It should be remembered that natural selection acts on the individual as a whole and not on parts of it only. Therefore, progress in one trait may be accompanied by recession in another. The environment has never been and probably never will be constant. Other evolutionary forces such as genetic drift and gene flow among populations may cause large deviations from the idealised state of maximum fitness. Eriksson et al (1993) strongly emphasized that the present-day genetic structure is transient. It should therefore not be regarded as the objective of gene conservation but as

the starting material for gene conservation. Our reasoning should not be interpreted as a denial of adaptation via natural selection rather than natural selection, is one of several evolutionary forces.

Some basic population genetics

All gene conservation programs aim at conserving genes above certain frequencies. Varela and Eriksson (1995) graphically illustrated the number of individuals that should be sampled to save genes of varying frequencies. From their graph it is evident that an effective population size (N_e) of 500 would be enough to save genes in frequencies down to 0.01 in several loci. Since N_e is usually less than the census number, 1000 individuals would be enough to conserve genes at reasonable frequencies. Genes at low frequencies (<0.01) as well as genes at high frequencies (>0.99) do not contribute much to the additive variance. This means that neither natural selection nor breeders will be able to raise low frequency genes since additive variance is a prerequisite for progress by selection. Even if they are equipped with a considerable amount of fitness their existence in the population is dependent on chance only. At random sampling the remaining fraction of additive variance can be estimated by the formula $1 - 1/2N_e$. This formula indicates that the remaining additive variance is almost the same whether 500 or 1000 individuals are sampled. Thus, from a population genetics point of view there is no need to have populations of tens of thousands of individuals in the gene resource population. Other reasons, such as risks for fire and other calamities might call for larger populations than 1000 trees. If gene conservation of associated species is included as a conservation objective this will also require larger populations than 1000 trees.

Dynamic or static gene conservation?

Eriksson et al (1993) strongly argued in favour of a dynamic gene conservation. The main reasons for this are:

1. The continuous change of the environment including silvicultural techniques,
2. The uncertainty about future values of different traits.

We have declared that creation of good conditions for future evolution is the main objective of the joint gene conservation and tree breeding of *Pinus sylvestris* in Lithuania. A dynamic gene conservation is therefore proposed.

In Fig. 1 we have schematically illustrated one difference between dynamic and static gene conservation. Static gene conservation is widespread as regards agricultural crops. It should be realized that the objective of gene conservation of crop species is mostly to have a material ready for breeders who want to transfer a major gene into a high yielding variety. This can be accomplished by 7-8 generations of back crosses; a technique that is beyond the possibility for long-lived forest trees. Even foresters may have the objective of preserving the present genetic constitution as a reference for future comparisons. In such a case *ex situ* storage of seeds or other propagules is the most efficient method.

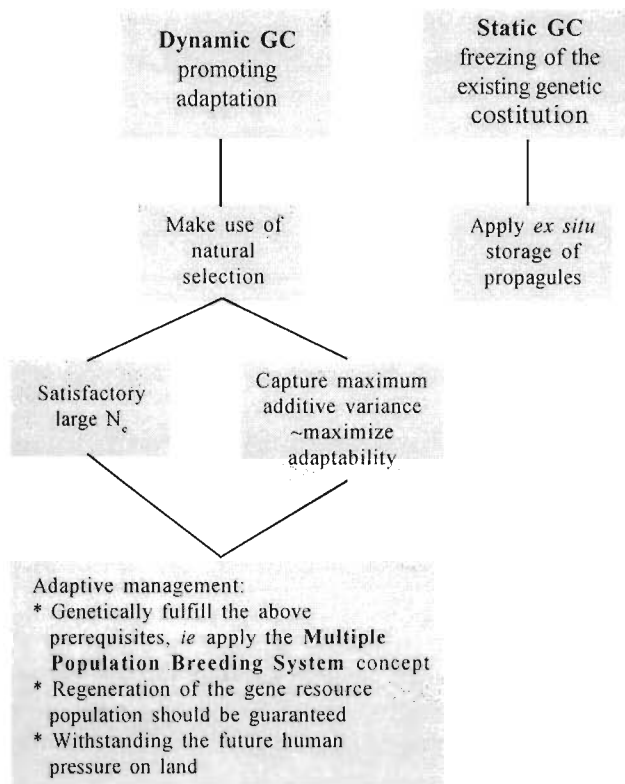


Fig. 1. Schematic illustration of dynamic and static gene conservation

The essence of dynamic gene conservation is to promote adaptation by exposing the gene resource population to natural selection. It should be noted that this might be obtained both by *ex situ* and *in situ* methods (cf. Eriksson et al. 1993). A prerequisite for natural selection to be operative is that the gene resource population is large enough to avoid genetic drift. Since additive variance is another prerequisite for natural selection, the sampling should be carried out such that maximum additive variance is captured. To achieve such

a sampling without any genetic knowledge, educated guesses about existing genetic variation must guide the sampling. If successful we shall be close to maximum genetic adaptability. Genetic adaptability is defined as the potential of a population to respond to changed environmental conditions by change of gene frequencies.

A fulfillment of these requirements is a part of adaptive management. As regards natural populations that have been designated as gene resource populations it is obvious that a dynamic gene conservation requires that the gene resource population is regenerated. We have seen too many cases where a gene resource population is being replaced by a population of another species owing to a *noli me tangere* attitude to gene resource populations. Such an attitude means that the designated gene resource population is a dead end. Thus, active measures should be taken whenever there is a need for maintenance of the gene resource population over generations. We have stressed that there will be a strong pressure on forest land in future. Therefore, it is important to include an evaluation whether or not a potential gene resource population has its long-term existence threatened before it is designated as a gene resource population.

One elaborate form of gene conservation is the multiple population breeding system (MPBS) concept first developed for breeding (Namkoong 1976) and then extended to joint breeding and gene conservation (Namkoong 1984). The MPBS means that the allied breeding/gene resource population consists of approximately 20 subpopulations, each with an effective population size (N_e) of 50 genetic entries making a total gene resource population of 1000 entries. These figures are based on the probability of saving genes at frequencies above 0.01 and of avoiding severe inbreeding in the subpopulations. With an effective population size of 50 individuals the rate of inbreeding will be 1% per generation ($1/2N_e$) which might be regarded as satisfactorily low. As stated above this is also the rate of loss of additive variance. The most intensive form of MPBS includes planting of regular progeny trials. Less intensive forms may be utilized for species not included in any breeding program (cf. Eriksson et al. 1993, Varela and Eriksson 1995).

Purpose of this paper

The purpose of this paper is to scrutinize whether the existing breeding population of *Pinus sylvestris* in Lithuania satisfies the needs for gene conservation of this species within Lithuania.

Existing breeding population

The breeding population of *Pinus sylvestris* in Lithuania consists of generative and vegetative progenies in archives and different experimental plantations: 1) clonal archives, 2) provenance or population archives, and 3) progeny test plantations. To understand the incentives of distribution of the test plantations as well as the collection of material for the breeding population we shall first address the zonation done for *Pinus sylvestris* in Lithuania.

The overlapping ecoclimatic gradients of various kinds such as photoperiod, temperature sum and season-

al temperature variation, amount of precipitation, site fertility, and humidity have created a complicated geographical pattern of ecoclimatic conditions in Lithuania. Based on ecoclimatic characteristics, predominating site types, and forest vegetation, Lithuania was subdivided into six natural forest regions (Karazija, 1969, Fig. 2). These ecoclimatic gradients resulted in different growth rate, phenology and frost damage. This has promoted a differentiation among populations within the country. The results of our studies on *Pinus sylvestris* and *Picea abies* gene ecology confirm the existence of significant differences in growth and adaptedness of Lithuanian



Fig. 2. Scots pine (*Pinus sylvestris* L.) joint breeding and gene resource populations in Lithuania

populations (eg. Ramanauskas et al. 1993, Pliūra & Gabrilavičius, 1993, Pliūra 1995). Therefore, that geographical ecoclimatic pattern is considered to be of crucial importance in forestry, forest regeneration, tree breeding, and gene conservation. For the time being, laws and regulations in Lithuanian forestry are based on this forest zonation (Lithuanian forestry act 1995).

Clonal archives of *Pinus sylvestris* were established in order to collect grafted plus tree clones as well as to conserve and test them. A total of 133 (128 survived) clones from 46 populations were selected. Of them 56 clones grow also in test seed orchards in different forest enterprises. Apart from seed production these seed orchards of *Pinus sylvestris* are used as clonal archives and test plantations of clones. There are 205 clones from different populations collected in 8 such orchards. Some of the plus tree clones are represented a few times in clonal archives and different test seed orchards. A total of 265 plus trees are conserved *ex-situ* in clonal archives and test seed orchards which make up about 55% of all national *Pinus sylvestris* plus trees. During the period 1976 -1985, 478 plus trees of *Pinus sylvestris* were selected, of which 384 remained in forests in 1993. These plus trees are to be replaced by new plus trees in coming years. Each population is represented in clonal archives

and test seed orchards by different number of genotypes (1-9). Half-sib progenies of almost all these 265 plus trees clones are under testing in 8 progeny test plantations (No.12-19) in the two natural forest regions 2B and 4B (Table 1, Figure 2).

Archives of provenances (populations). Three provenance archives - test plantations were established in natural forest regions 2B and 4B in 1961-1973 (No. 1-3, Table 1, Figure 2). Open pollinated progenies from 21 population (11-16 populations in each archive) were collected. The populations which are conserved and/or tested in different test plantations are presented in Figure 2. The progenies from individual populations in form of bulked seed from one stand are planted in large rectangular plots consisting of 300-900 trees without replications.

The progeny test plantations were designed to test both populations and families. Test plantation No. 5 was established in 1979 (Table 1, Figure 2). Each of 4 populations was represented by 10-19 half-sib families (totally 51 families). All test plantations established after 1979 are designed with replications.

Six test plantations of 4th series were established in regions 1, 2B, 3, and 4B in 1983 (Nr. 6 -11). In each of six test plantations 7 populations were represented by 20 half-sib families. Test plantations No. 7 and No.10 are in the same

Table 1. Scots pine progeny test plantations in Lithuania

Series	No ^a	Forest enterprise	Forest district	Year of establishment	Area, ha	Number of populations families	Number of replications	Forest natural region ^b	Site type ^c	Site index ^d H ₁₀₀
1	1	Švenčionėliai	Žeimena	1961	2.5	11/bulk	1	2B	Nab	25
	2	Veisiejai	Stalai	1973	6.5	16/bulk	1	4B	Na	21
2	3	Švenčionėliai	Januliškiai	1973	5.8	14/bulk	1	2B	Nb	27
	4	Dubrava	Vaišvydava	1973	1.0	19/210	1	4A	Lb	29
3	5	Dubrava	Kuras	1979	2.3	4/51	4	4A	Nb	27
	6	Druskininkai	Latežeris	1983	1.5	7/140	6	4B	Na	21
4	7	Ignalina	Ignalina	1983	1.5	7/140	5	2B	Na	20
	8	Veisiejai	Kapčiamiestis	1983	1.5	7/140	5	4B	Nab	24
	9	Šilutė	Juknaičiai	1983	1.5	7/140	5	3	Nb	27
	10	Telšiai	Telšiai	1983	1.5	7/140	5	1	Nc	30
	11	Nemenčinė	Purviniškiai	1983	1.5	7/140	5	2B	Nbc	29
	12	Ignalina	Ignalina 1	1987	1.5	n/88	5	3B	Na	21
	13	Ignalina	Ignalina 2	1988	2.5	n/131	5	3B	Na	21
5	14	Ignalina	Ignalina 3	1989	1.5	n/55	5	3B	Na	21
	15	Veisiejai	Kapčiamiestis	1989	1.8	n/137	5	4B	Na	23
	16	Ignalina	Dūkštas	1990	2.0	n/110	5	3B	Na	21
	17	Veisiejai	Ančia 8	1990	2.0	n/133	5	4B	Na	23
	18	Veisiejai	Ančia 7	1991	2.0	n/54	5	4B	Na	23
	19	Veisiejai	Ančia 4	1992	3.2	n/42+32+97 ^e	5	4B	Na	23

^a - Numbers of test plantations are marked on the map (Fig. 2)

^b - forest ecoclimatic regions - after Karazija (1969)

^c - Humidity and fertility of the soil - after Vaičys (1972)

^d - Mean height of stand at mature age - after Kuliešis (1995)

^e - Full-sib families

forest natural region 2B, on sites of contrasting fertility (Table 1.). Test plantations No. 6 and No. 8 are in the same region 4B on slightly different sites. Test plantations No. 9 and No. 10 are in forest natural regions 3 and 1, respectively. They were established on fertile sites. However, there were no test plantations established on poor sites in regions 3 and 1, and there were no test plantations established in the forest natural regions 2A and 4A.

Test plantations that were established later (in the period 1985-1995) were designed to test progenies of plus trees - open pollinated half sib families from grafted clones of plus trees in seed orchards. These test plantations of 5th series (No. 12-19) were established in two ecoclimatic regions of Lithuania where *Pinus sylvestris* is dominating species (forest natural regions 2B and 4B). There are 54-171 families being tested in each of these test plantations. These families do not represent pure populations owing to intermating of trees from different populations in the seed orchards. In test plantations established in 1989 and 1990, most of the families are tested in two test plantations at the same time in two different forest ecoclimatic regions. In some test plantations the same families from different years of harvesting, from different seed orchards, or from different ramets are tested in order both to evaluate families in general and to find out the effects of the latter three factors. However, some test plantations in the forest ecoclimatic region 3B (test plantations 12, 13, 14, 16) are of poor status because of damage by ungulate animals or shortages in management. Totally there are over 700 genetic entries being tested in different types of test plantations.

Does the existing breeding population satisfy the long-term dynamic gene conservation objectives?

From the previous section it is evident that the number of genetic entries in the existing breeding population satisfies the "magic number" of 500 trees or genetic entries advocated above. Moreover, we argue for active breeding which means that artificial crosses are carried out in the breeding population. This means that all trees make up the effective population. The problem of decisions on the ratio between census number and N_e is avoided.

We suggest two allied gene resource and breeding subpopulations in the form of test plantations in each of 6 breeding zones (forest natural ecoclimatic regions). In each zone these test plantations are located on *Pinus sylvestris* sites with different site indices. Each subpopulation should consist of at least 50 genetic

entries. The total gene resource/breeding population will amount to 600 genetic entries ($6 \times 2 \times 50$). The new generation of gene resource and breeding subpopulations will be created by artificial crossing of the best individuals selected within each of the 50 families constituting each subpopulation. Considering the small size of Lithuania, six breeding zones must be regarded as highly ambitious.

Based on the six breeding zones identified for Lithuania the question still remains if all zones are appropriately covered by the existing breeding population. Since there is no or limited representation in zones 1, 2A, and 3 supplemental sampling of *Pinus sylvestris* populations should be carried out. There are no test plantations established in zone 2A, and there are no test plantations on poor sites in zones 1 and 3 (Figure 2). Therefore, two test plantations, on poor and fertile sites, should be established in zone 2A and two test plantations on poor sites in zones 1 and 3. Since this species covers a wide range in neighboring countries we see no problem for its gene conservation as long as judicious breeding is carried out.

Many *Pinus sylvestris* stands have been designated as *in situ* gene resources. From a population genetics point of view they seem to be redundant. In case of limiting financial support for breeding and gene conservation their status should be reconsidered. One possibility is that they are utilized as approved seed production stands.

In agreement with the suggestion of Varela and Eriksson (1995) we suggest that one large conservation area (200-300 hectares) is established in each of zones 1-4 for conservation of associated species. These gene resource populations should be selected and managed to maximize habitat diversity within and among them. This means that all tree age classes, including overmatured trees, should be represented in such gene conservation areas. This type of gene resource will be useful for the majority of plant and animal species but will never be satisfactory for the large mammals. However, most of the latter will continue to dwell in vigorous populations in forests with various degrees of management.

Conclusions

The existing *ex situ* breeding population, after some complementary selections and establishing of test plantations, will satisfy the gene conservation objective of creation of good conditions for future evolution of

Pinus sylvestris in Lithuania. This breeding population will satisfy both gene conservation and tree breeding. The large number of *in situ* gene resource stands ought to be converted to seed-production stands. The gene conservation of associated species can be done by creating large gene conservation populations, 200-300 hectares, in each of the four main ecoclimatic zones in Lithuania. These gene resource populations should be managed to create maximum habitat diversity within the gene resource population.

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СТАБИЛЬНОЕ СОХРАНЕНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ СОСНЫ ОБЫКНОВЕННОЙ (*PINUS SYLVESTRIS*) В ЛИТВЕ

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Резюме

Для преодоления негативных нагрузок на лес сохранение (консервация) генетических ресурсов должна быть построена на эволюционной основе и при совмещении с динамическим сохранением

генетических ресурсов. На основе многочисленных новейших публикаций о сохранении генетических ресурсов лесных древесных мы предлагаем *ex-situ* динамическое сохранение генов и практическую селекцию осуществлять на основе концепции "Многопопуляционной селекционной системы". Экспериментальные лесные культуры по испытанию потомств, заложенные в разных лесорастительных регионах Литвы в период с 1979 по 1995 год, будут служить основой сохранения генетических ресурсов. Дополнительный отбор и закладка испытательных культур будут производиться в лесорастительных регионах, которые недостаточно репрезентированы. Объединенная популяция генетических ресурсов и селекции будет состоять из 12-ти субпопуляций, каждую из которых составляют 50 генетических единиц: по две субпопуляции в каждом из трех лесорастительных регионов (селекционных регионах) страны. Новая генерация субпопуляций генетических ресурсов и селекции будет создаваться при помощи искусственных скрещиваний между лучшими индивидами, отобранными внутри каждой из 50-ти семей, составляющих субпопуляцию. С целью экономии средств две цели – практическая селекция и сохранения генетических ресурсов должны быть совмещены. Статус и функция многочисленных обычных *in situ* лесных генетических резерватов и заказников, в которых хозяйствование очень строго лимитирована, должны быть пересмотрены.

Ключевые слова: *Pinus sylvestris*, *ex-situ* динамическое сохранение (консервация) генетических ресурсов.