Gene Dispersion after Natural Regeneration under a Widely-Spaced Seed-Tree Stand of Pinus sylvestris (L.)

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Summary

For determination of gene dispersion the patterns of certain, rare isozyme markers have been studied within a seed-tree stand of *Pinus sylvestris* in northern Sweden. The stand investigated consists of 18 seed trees per hectare situated in Arvidsjaur, Sweden (lat. 65°29). Marker genes for certain rare isozymes occur in several seed trees. Those markers are overrepresented among seedlings in the vicinity of those trees.

Different seed trees contribute with their genes to the next generation in different proportions. In the seed-tree stand, many seedlings do not originate from seed trees in the neighbourhood. It seems likely that much of the natural regeneration originates from felled trees or sources outside the seed-tree stand.

The study suggests that approximately one quarter of the seedlings within 5 m from a seed tree has that seed tree as seed parent.

Key words: Pinus sylvestris, natural regeneration, isozyme marker, gene dispersion.

Introduction

Natural regeneration was the dominating method for reforestation in Sweden until 1950. Due to rationalization of the reforestation work and introduction of new methods involving planting of selected and improved material, transferred provenances, etc. the proportion of forest land regenerated naturally has decreased. Natural regeneration today is employed on roughly 25% of the yearly reforestation area in Sweden, or about 60,000 hectares per year.

Natural regeneration involves a chain of events in the stand generation cycle, from initiating of flower buds to establishment of the seedlings.

The climate has a decisive influence on the stimulation of flowering and on fertilization and on seed quality (MORK, 1957; BERGMAN, 1976). A high seed fall of good quality in *Pinus sylvestris* requires proper weather conditions under several years (HAGNER, 1965). In northern Sweden in relatively mild years, good seed production for *Pinus sylvestris* occurs every 4th year and in the south every second year. A rich seed fall is not enough for good germination and sufficient plant establishment, however — proper weather conditions and a favourable micro-environment are also required. Death of plants is greater during the establishing phases. Therefore a considerable number of years of seed fall may be needed before a new regeneration has the chance to get established. From a forest genetic point of view, natural regeneration may be considered as one way to preserve genetic variation of natural forests.

The genetic structure of a forest tree population is changing continously during a stands life cycle. The selection processes are repeated in every generation of forest trees. This means that the rich variation in the beginning gradually is reduced during successive developmental stages in the stands life (LUNDKVIST, 1985). There is a continuous selection and adaptation of a stands genetic structure to the local environment. The selection intensity is very high, from millions of seeds per hectare to the final felling comprising some few hundred stems. Understanding of the genetic selection process and its relation to site-related environmental factors has high relevance for studies of ecological adaptation and for forest silvicultural practices. Isozyme technique has been used for studying the genetic constitution of the stand after natural regeneration. The results demonstrate that in natural regeneration a certain proportion of the plant material is produced by selfing. Such embryo and plants will to a larger extent be eliminated from the population than is the case for outcrossed material (YAZDANI *et al.*, 1985). In this study isozyme markers are used to investigate the gene dispersal pattern after natural regeneration in a stand located in the harsh climate of northern Sweden. The results indicate that in low density seed stands, the contribution from each seed tree to the regeneration is low, and therefore significant fractions of the regeneration does not originate from the seed trees at all.

Material and Methods

2.1 Material

This study was carried out on material from a seed-tree stand at Gårdstjärn, Arvidsjaur (lat. 65°29', long. 19°17' and alt. 400 m, *Fig. 1*) (RUDIN et al., 1977). The number of seed trees per hectare varied between 10 and 18 on two test plots, 1 and 2. The age distribution of plants on an average was 50% below 10 years, 35% between 10 to 20 years and 15% above 20 years. Significant differences have been found in plant regeneration in different parts of the seed-tree stand. On the average, plant establishment close to seed trees did not differ very much from the area between seed trees. The average number of plants around seed trees were 2467 and between seed trees 2920 per hectare. Under seed trees and within a radius of 20 meters plant numbers are unevenly distributed. A slightly higher number of plants is found within the area 5 to 10 meters from seed trees. The lowest number of plants is more obvious within a radius of 5 meters from seed trees. The seed trees were betwen 80 years and 100 years old. The seed-tree stands were established in 1960 and the seed trees were felled during 1982. Cones were collected from these trees after felling.

Isozyme analyses of endosperm from seeds were made for all seed trees. These analyses were performed in order to attain information on the genetic constitution of each of the seed trees.

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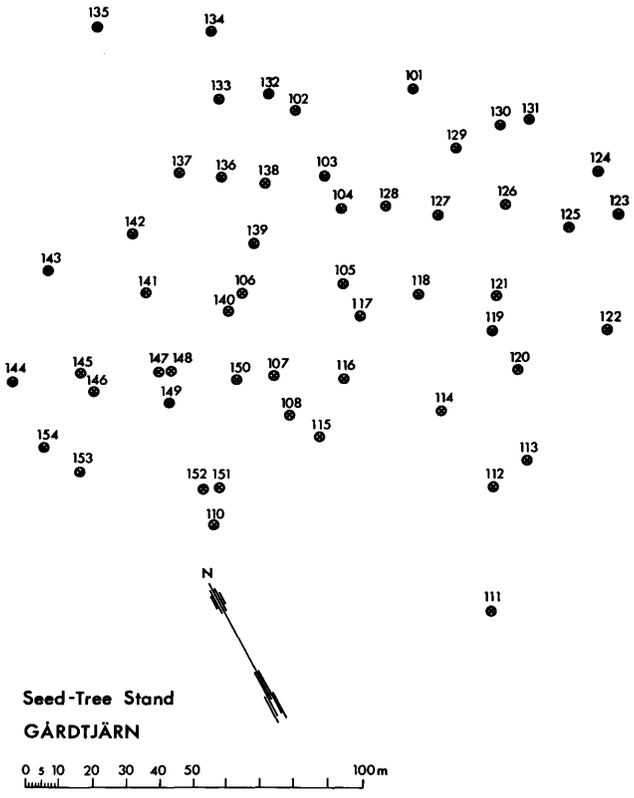

Figure 1. — Map of the position of the seed trees at Gårdtjärn.

For studying the gene dispersal pattern in the seed-tree stand, buds from around 800 plants with age 1 to 20 years were collected during the autumn 1983. These plants surrounded eight marker trees with certain rare isozyme markers. These marker trees were seed-tree numbers 106, 113, 117, 118, 123, 129, 136 and 151 (*Fig. 1*). Every seedling within 10 m from the marker tree was collected. If 100 were not obtained, seedlings between 10 m and 15 m were also collected.

For studying the genotype of a seed tree, at least 7 macrogametophytes were analysed. Ten loci are scored in parental seed trees. These loci were LAP-A, B and GOT-A, B, and F-EST, GDH, MDH-A, B and ADH-A, B. Eight seed trees were found to carry rare alleles for LAP-A, B and GOT-A, B enzyme loci. These eight seed trees are regarded as marker parent trees. For better classification of marker parents offspring relationship additional enzyme systems (PGM-A, B and SHDH-A, B and ACO, 6PGD-A, B and APH) are studied in these eight seed trees. For evaluation of the gene dispersal pattern it is desirable to find plants which carry the marker allele at different distances from marker seed trees. About 800 plants with age intervals (1 to 5, 5 to 10, 10 to 15, 15 to 20 years are sampled within 0 to 5, 5 to 10, 10 to 15 meter distance from eight marker trees. Buds collected from all plants were subjected to isozyme analysis. Two enzymes (LAP and GOT) are analysed in all plants.

Plants carrying rare isozyme markers for these two enzyme systems were subjected to further isozyme analysis. Thirteen loci have been checked in all marker plants to confirm that the marker tree is a parent. These isozyme loci were LAP-A, B and GOT A, B and F-est, GDH and PGM-A, B and MDH-A, B and ShDh-A, B and 6PGD-B. Plants within 15 m distance from seed trees which carry marker alleles are excluded as offspring of a certain marker tree based on parental check.

2.2 Methods of biochemical analyses

Buds for isozyme analyses were collected from plants of different ages and were stored at —20 °C. The macrogametophytes were separated from the embryo in each seed and the tissues were homogenized in tris-glycine buffer pH 8.3 consisting of 0.05 M tris and 0.33 M glycine with the addition of 1% soluble polyvinylpyrrolidon (Mw 40.000).

The buds were ground with the same buffer as above. Starch gel electrophoresis was carried out on 12% starch gels. The following enzyme systems are used for studying gene dispersion.

Acid phosphatase (APH), Lundkvist (1975); Aconitase (ACO), Guries and Ledig (1978); Fluorescent esterase (F-EST), Yazdani and Rudin (1982); 6-phosphogluconate dehydrogenase (6PGD), Szmidt and Yazdani (1984); Glutamate dehygrogenase (GDH), Vallejos (1983), Rudin (unpubl); Glutamate oxaloacetic transaminase (GOT), Rudin (1975), Leucine aminopeptidase (LAP), Rudin (1977); Malate dehydrogenase (MDH), Rudin and Ekberg (1978); Phosphoglucomutase (PGM), Shaw and Prasad (1970); Shikimate dehydrogenase (SHDH), Linhart, et al. (1981).

2.3 Methods of mathematical evaluation

The total gene distribution is the combined effect of the transport of the pollen from the father to the mother and the transport of the seed from the mother to where the plant is found. When studying rare markers in diploid plants, the impact of the rare markers in the background can not be neglected. Thus, some plants, which isozymatically could be the progeny of a particular tree are not. Parental check improves accuracy, but only to a limited extent. Parental check makes unbiased estimates of the background complicated. By "markers" in this section, markers which passed the parental check are referred to. The following mathematics was applied, basically as outlined by Yazdani *et al* (1985).

An area at a certain distance interval from a point source is studied.

$$G = AdF \quad (1)$$

F proportion of the genes which originate from the source;

G proportion of all genes dispersed from the source ending up in the area;

d density of seed trees (0.0018 trees per m^2);

A $A = \pi (b^2 - a^2)$ is the area limited by concentric circles around the source with radius a resp b. a and b are bounds of a distance interval from the source (m). In this investigation the values of a and b are 5,10 and 15.

The interval is assumed to be so small that F can be regarded as a constant.

To link to observations and correct for background the following formula was used (note that only half of the genes from a marker tree are marker genes:

$$Q = F + (1—F)*2B \quad (2)$$

Q measurable proportion of diploid marker seedlings (= two times proportion of marker genes)

B proportion of background marker genes.

Solving equation (2) for F gives:

$$F = (Q — 2B)/(1 — 2B) \quad (3)$$

Inserting the obtained F in (1) gives G as a function of the measurable Q.

Table 1. — Frequency of seedlings with marker genes/ all analysed seedlings after parental check. GOT-A1, LAP-A1, LAP-B1.

Tree	Marker	Distance 0- 5	5-10	10-15
136	GOT-A1	0/11	1/58	0/ 0
113	LAP-A1	0/17	0/29	1/54
123	LAP-A1	0/15	1/64	0/20
136	LAP-A1	0/11	3/58	0/ 0
113	LAP-B1	0/17	0/29	0/54

To calculate B, we need to know both the actual frequency of the rare allele used as marker in the "background" (p) and the frequency of background gametes carrying such alleles which will not be detected in the paternal check (P). Knowing these values B can be calculated as B = pP.

Results

3.1 Progenies carrying GOT-A1, LAP-A1 and LAP-B1 marker alleles

The results of distribution of marker alleles among progenies are visualized in *table 1* and *figures 2 to 3.*

As so few marker seedlings were identified for GOT-A1, LAP-A1 and LAP-B1, no detailed analyses for the marker plants have been done. The data are not suitable for a quantitative analysis of gene dispersal at different distances considering background, etc. Instead the results are demonstrated in *figures 2* to *3.* However, it may be noted that there are remarkably few marker seedlings. Within 15 m from the marker Q = 6/437 = 0.013. Thus the closest seed tree contributes only something like one percent of the genes in the seedlings.

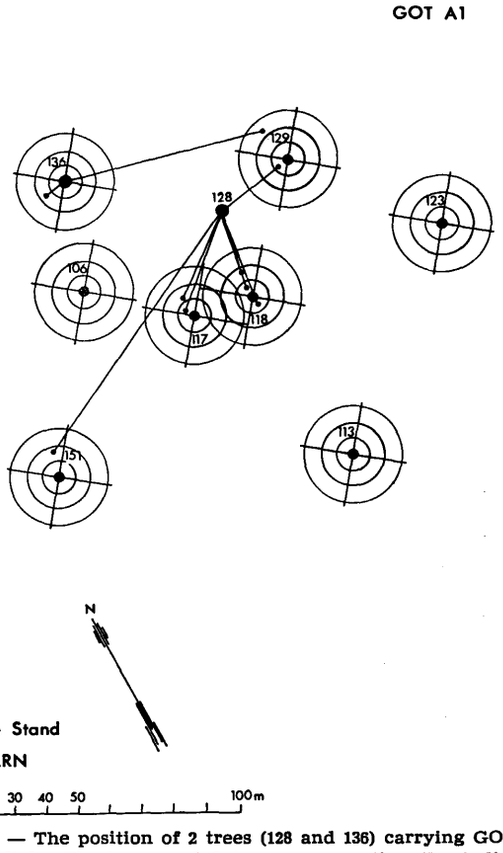

Figure 2. — The position of 2 trees (128 and 136) carrying GOT-A1 marker allele. Plants around 8 trees representing the indicated areas were analysed for isozymes. Positions of plants carrying marker allelles are indicated with small black dots. Marker trees and marker plants whose parent-child relationship could not be excluded based on other enzyme systems are connected.

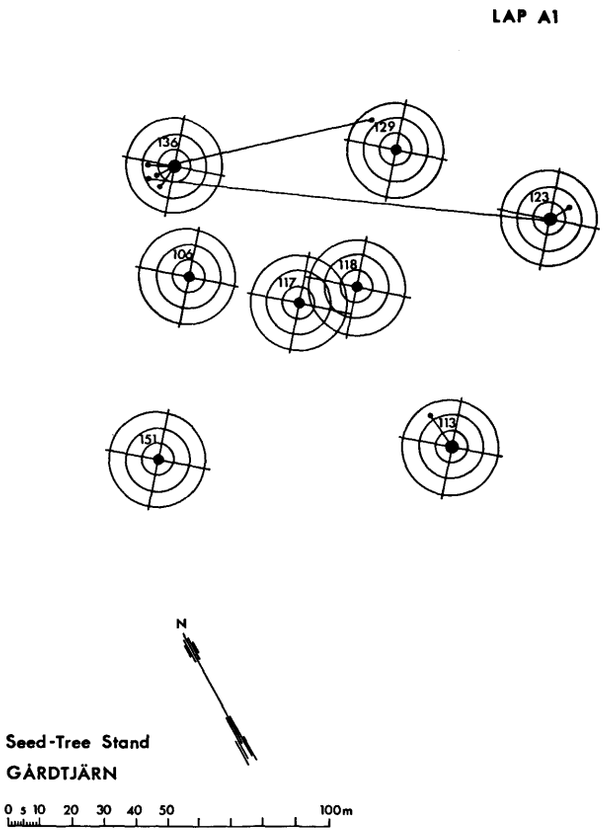

Figure 3. — The position of 3 trees (113, 123 and 136) carrying LAP-A1 marker allele. Marker trees and marker plants whose parent-child relationship could not be excluded based on other enzyme systems are connected.

Two trees appeared with marker allele GOT-A1 — tree 128 and tree 136 (*Figure 2*). 75 percent of plants with GOT-A1 are not excluded after parental check with other isozyme systems. For tree 136 there are too few plants as offspring to allow for meaningful statistics. For tree 128 there were no plants collected within 15 meters distance from the seed tree.

Three marker trees carried LAP-A1 allele — trees 113, 123, 136. *Figure 3* is constructed to show parental trees and plants with marker allele LAP-A1. 75 percent of plants with LAP-A1 remained after parental check. Tree 136 carries both LAP-A1 and GOT-A1 isozymes; this tree seems not to be so bad as a parent.

For marker tree 113 with LAP-B1 there are no marker plants within 50 meters of the marker seed tree, and any marker plants more than 50 meters away might just as well be a result of "background". No figure is presented for LAP-B1.

3.2 Progenies carrying GOT-B1 marker allele

Five seed trees carried GOT-B1 marker allele. These trees are seed trees 106, 117, 118, 129 and 151. The frequency of GOT-B1 markers in plants is more obvious around each of the marker trees 106, 117, 118, 129 and 151 than around non marker trees 113, 123 and 136 (*Table 2*). This supports the theory that most of the marker plants indeed are progeny of the marker trees. It seems all trees with GOT-B1 contribute with their genes to natural regeneration.

Table 2. — **Frequency of seedlings with marker allele (GOT-B1) at different distances from the (closest) marker tree/all analysed seedlings at Gårdtjärn seed-tree stand. The quotient Q measures the calculated proportion of seedlings carrying a marker on a certain spot at a certain distance. F = the calculated proportion of genes from the marker tree in seedlings at distances between a and b from the marker tree. G = estimation of the proportion of gene dispersal within different distances, according to the model developed by YAZDANI et al (1985).**

	Distance				
Tree	0- 5m	5-10m	10-15m		
106	3/28	7/69	0/ 0		
117	9/48	3/32	0/ 0		
118	10/46	6/42	0/ 0		
129	10/31	10/49	4/15		
151	4/19	1/23	2/58		
Q:	36/172	27/215	6/73	Assumed 2pP	Estimate
F	.2093	.1256	.0822	.000	Very high
F	.1912	.1056	.0612	.0224	High
F	.1844	.0981	.0533	.0305	Medium
F	.1350	.0434	-.0040	.0859	Low
	0- 5 m	5-10 m	0-15 m		
G	.030	.053	.058	.141	Very high
G	.027	.045	.043	.115	High
G	.026	.042	.038	.105	Medium
G	.019	.018	-.003	.035	Low
Average	.026	.040	.034		

The "low" estimate leads to unreasonable results.

For the non-marker trees the following distribution was found.

Tree	0-5m	5-10m	10-15m
113	0/17	1/29	2/54
123	1/15	2/64	3/20
136	0/11	1/58	0/0
Q:	1/43	4/151	5/74

For GOT-B1, information from five trees is pooled. Information > 15 m is likely to be highly uncertain because of the rather high background of this not extremely rare allele. There were 10 marker plants among the 268 plants > 15 m from any marker tree. 2p = 10/268 = 0.0373 is an estimate. For the five trees pooled there were 2526 plants at distances > 50 m. Of these, 258 were marker plants, and 155 of these could not be excluded based on a parental check. Thus P = 155/258 = 0.601 of background can not be distinguished based on parental check (assuming F = 0 for > 50 m). Assuming F = 0 for > 15 m, 2pP can be estimated as 0.0377 x 0.601 = 0.0224. GULLBERG et al. (1985) found for another material p = 0.0254, corresponding to 2pP = 0.0305. For the investigated seed trees of the present material at Gårdtjärn, 13 of 91 investigated seeds had GOT B1, thus 2p = 0.1429. 2pP = 0.1429 x 0.601 = 0.0859.

The frequency of marker plants with GOT-B1 after parental check is included in *table 2.*

Discussion

4.1 Possibility of improving dispersal studies by parental check

For improving the gene dispersal pattern, a parental check has been performed on plants with marker alleles. Plants are excluded as offspring of certain marker trees based on multilocus. For unique isozyme markers LAP A1, B1 and GOT-A1, the parental check was much easier to perform than for markers with more frequent alleles,

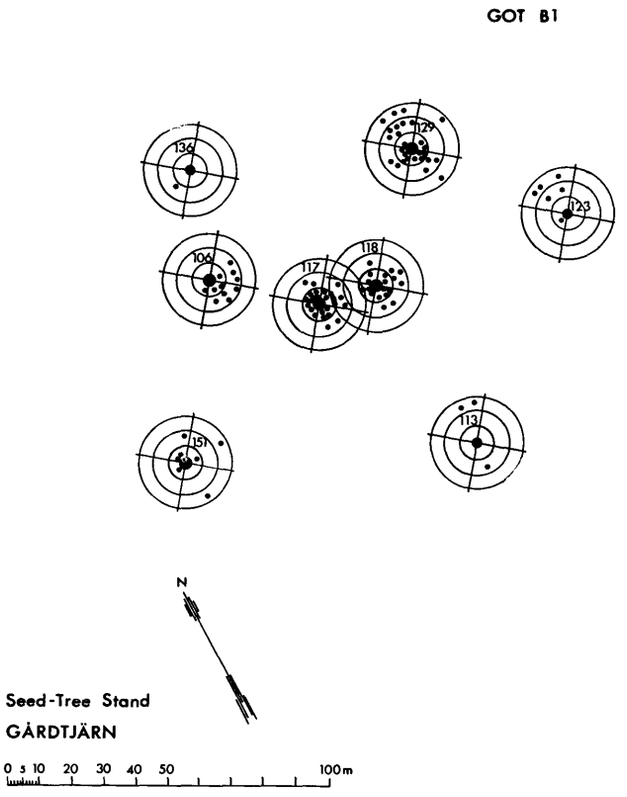

Figure 4. — The position of 5 trees (106, 117, 118, 129 and 151) carrying GOT-B1 marker allele. Since there are many marker plants with GOT-B1, the relationship between marker tree and progeny is not conveniently demonstrated.

like GOT-B1. In the case of GOT-B1, five parents carried this allele *(Fig. 4).* Most of the offspring with GOT-B1 demonstrated correspondence to many marker trees.

4.2 Difference between GOT-B1 and other markers

It is remarkable that considerably fewer marker seedlings were found in the three trees of *table 1* compared to the five trees of *table 2.* The likely explanation is that the former trees just by chance happen to produce fewer gametes. This is supported by the low number of seedlings found within 5 m in *table 1* (11, 17, 15) compared to the corresponding number of seedlings in *table 2* (28, 48, 46, 31, 19), which indicates a difference in the rate of seed fall. Large differences between different genotypes in seed production are not unexpected, as this has been found in seed orchards (ERIKSSON *et al.*, 1973) as well as in stands (LINDGREN and LINDGREN, 1977).

4.3 Frequency of plants which have a close seed tree as parent

A certain seed tree is the closest over an area of 10 000/18 = 556 m² (18 seed trees per hectar corresponds to 1 in 556 m²). This area corresponds to circle with radius 13.3 ($13.3^2 \pi = 556$). Thus, on average, a seed tree is the closest to plant growing within 13.3 m from the tree.

Within 5 m, *table 2* indicates F = 0.18, while based on *table 1* a value of the magnitude F = 0.01 is calculated. Weighted average for number of trees with 5 and 3 values, respectively, gives F = 0.12 as a reasonable estimate. It seems thus likely that 1/4 of the seedlings close to a seed tree has that seed tree as a mother. With the methods used in this study it is impossible to conclude if an individual

plant got its marker genes from the father or the mother. However, for plants within 5 m from a parent it seems likely that the common relationship is that the parent is seed parent. For the moment selfing is disregarded. The average distance to the pollen parent is expected to be longer than that to the seed parent as the former is the sum of the flight of the pollen grain and the flight of the seed. It is likely that at least some seeds fall more or less directly to the ground, while pollen has to travel to another tree first to crossfertilize. Only in the unlikely case that the seed happen to "fly back" towards the pollen parent, a close tree will be the pollen parent. Selfing means that the same tree is seed parent as well as pollen parent. Thus for the selfed plants, the distance to both parents are the same, while for the outcrossed plants the distance to the pollen parent is expected to be considerable larger than to the seed parent. There are very few plants which have more than a single tree within five meters. Most plants originate from outcrossing. Thus the most common relationship for plants to parents within five meters will be that they are seed parents.

4.4 Frequency of genes from a seed tree as a function of the distance

The proportion of genes from a marker tree in seedlings drops rather fast in relation to distance (see *Table 2*). The frequency of offspring from the marker tree is roughly twice as high at 0 to 5 m compared to 5 m to 10 m, and seems to drop again by a factor of two when distance is increased to 10 m to 15 m. A possible explanation may be that the seed fall close to the individual marker tree is relatively much more important in a seed-tree stand with 18 trees per hectare than one with 37 or 123 (YAZDANI et al., 1985, 1989).

It seems as if less than 10 percent of the genes of the plants on area within 15 m from seed trees originate from those trees (*Table 2*). These estimates are surprisingly low. If the genes of the regeneration really originate from the seed-tree stand, 90% of the genes must travel more than 15 m from the source, but still stay within the stand. This seems unlikely.

It is possible that a large fraction of the genes in the regeneration does not originate from the seed trees in the stand. These genes may come from already existing trees, through seeds which were preserved on the ground or seeds originating from outside sources which were transported to the stand by different means. However, if so, we would not expect to find a co-variation between total number of plants close to seed tree and the percentage of plants originating from the seed tree, and as discussed above, such a reationship seems to exist.

Yet seeds from the marker trees contribute to less than 1/4 of the plants immediately below these trees.

A comparison of the gene dispersion observed in this study with that of the earlier one (YAZDANI *et al.*, 1984, 1989) demonstrated that twice as many genes were dispersed in the Svartberget and Stenträsk stands within a 15 meters radius from the seed trees. This is probably due to better climatic conditions and greater density of seed trees per hectare in these stands. The extremely low gene dispersal from seed trees in Gårdstjärn points out the difficulty which exists with this regeneration method at high latitudes and under the harsher climatic conditions in Norrland. There could be several factors involved in such a low frequency of gene dispersal in this stand. One important factor could be a high selfing rate which causes elimination of embryo and plants in different stages of the life cycle (YAZDANI *et al.*, 1985).

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