

# Reproductive systems in conifer seed orchards. I. Mating probabilities in a seed orchard of *Pinus sylvestris* L.

By G. MÜLLER-STARCK

Lehrstuhl für Forstgenetik und Forstpflanzenzüchtung der  
Georg-August Universität, 34 Göttingen, West-Germany

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## Summary

Mating probabilities are described in terms of the probabilities of self- and cross-fertilization. They were studied by analysing enzyme gene markers in viable seeds of a Scots pine seed orchard. The genetic control of the applied enzyme system was elucidated. The genotypes of the clones were identified by analysis of endosperm and/or needle tissue. One of the clones carries a unique allele and thus functions as the marker clone.

Probabilities of self-fertilization were estimated by means of identification of those open-pollinated seeds from the marker clone which contain the pollen contribution from this clone. Average values for three flowering periods ranged between 0.115 and 0.139; values for the individual trees of the marker clone varied between 0.063 and 0.267.

Probabilities of cross-fertilization were estimated by detection of the pollen contribution of the marker clone in the seeds of neighboring individuals from other clones. Results from two flowering periods indicated that cross-fertilization in this case did not depend on the distances between the respective trees.

Mating probabilities are illustrated in the form of three-dimensional plots. A comparison is made between the results of calculations based on hypothetical values and on approximations to the experimental data. Selection against descendants from self-fertilization can thus be documented.

The female and male gametic contributions of the marker clone, including descendants from self-fertilization, were determined in all-orchard seed samples from two flowering periods.

The experimental results were used to calculate the expected average coefficients of inbreeding and kinship in the offspring populations for the actual seed orchard layout and, in general, for varying numbers of clones in seed orchards.

**Key words:** *Pinus sylvestris*, enzyme gene marker, clonal seed orchard, mating probabilities, self/cross-fertilization, genetic structure, inbreeding.

## Zusammenfassung

Paarungswahrscheinlichkeiten werden als Wahrscheinlichkeiten für Selbst- und Fremdbefruchtung beschrieben. Diese wurden mittels Analyse von Enzym-Genmarkern in viablen Samen einer Kiefern Samenplantage untersucht. Die genetische Kontrolliertheit des verwendeten Enzymsystems wurde überprüft. Die Genotypen der Klone wurden aus Endosperm- und/oder Nadelgewebe identifiziert. Einer der Klone ist Träger eines einzigartigen Alleles und fungiert daher als Markerklon.

Die Wahrscheinlichkeiten für Selbstbefruchtung wurden ermittelt durch Identifizierung derjenigen aus freier Abblüte stammenden Samen des Markerklones, welche dessen eigenen Pollenbeitrag enthielten. Die Durchschnittswerte für drei Blühperioden variieren zwischen 0.115 und 0.139; die Werte für einzelne Individuen des Markerklones liegen zwischen 0.063 und 0.267.

Die Wahrscheinlichkeiten für Fremdbefruchtung wurden ermittelt durch Identifizierung der Pollenbeiträge des Markerklones in den Samen von benachbarten Individuen anderer Klone. Die Ergebnisse aus zwei Blühperioden zeigen, daß die Fremdbefruchtung in diesen Fällen nicht von der Entfernung zwischen den betreffenden Bäumen beeinflusst war.

Die Paarungswahrscheinlichkeiten werden in Form dreidimensionaler Plot-Diagramme veranschaulicht. Die auf hypothetischen Werten basierenden Ergebnisse werden mit Approximationen an die experimentellen Werte verglichen. Auf diese Weise kann Selektion gegen Nachkommen aus Selbstbefruchtung dokumentiert werden.

Die weiblichen und männlichen gametischen Beiträge des Markerklones wurden einschließlich seiner Nachkommen aus Selbstbefruchtung aus Plantagensaatgut von zwei Blühperioden ermittelt.

Die experimentellen Ergebnisse wurden verwendet, um die zu erwartenden mittleren Inzucht- und Abstammungskoeffizienten für die Nachkommenschaften der gegebenen Samenplantage sowie generell für variierende Klonanzahlen in Samenplantagen zu berechnen.

## Introduction

In forest tree breeding, a predominant objective is the achievement of phenotypic similarity between the selected parental trees and their offspring. This can be expected to occur as a consequent of conformity between their genetic structures and require mating conditions which are valid for populations in panmictic equilibrium. Such conditions should be realized in the case of all traits, including those under artificial selection. Panmictic conditions thus can be considered to represent any expectation of genotypic and possibly also phenotypic conformity between parental and offspring populations.

In the present paper, components of the mating system of a specified clonal seed orchard of Scots pine (*Pinus sylvestris* L.) are characterized by studying mating probabilities and comparing the results with the panmixia hypothesis. In the case of a monoecious, optionally self-fertilizing species such as Scots pine, mating probabilities include the probabilities of self- and cross-fertilization:

Self-fertilization will be described in terms of the probabilities with which descendants originating from the fusion of gametes of one and the same genotype can be expected among the offspring of this genotype after open pollination. In the case of a seed orchard, one genotype (clone) is represented by several genotypically identical individuals. Therefore, when applied to a specified clone, the given definition includes self-fertilization per se with respect to a single individual of this clone as well as the particular cross-fertilization between two individuals of the same clone. That is, descendants originating from self-fertilization contain gametic contributions either of one and the same individual or of two different but genotypically identical ones.

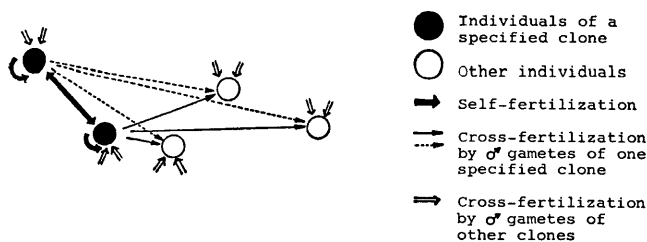


Figure 1. — Schematic presentation of the studied mating types.

Cross-fertilization will be studied in terms of the probabilities with which descendants originating from the fusion of gametes of different genotypes can be expected among the respective individual offspring after open pollination. It is assumed that the clones have different genotypes when all gene loci are considered, which, of course, does not imply genotypic diversity at single loci. In particular, the probabilities will be described with which descendants containing the male gametic contribution of a single specified clone can be expected among the offspring of the neighboring individuals of different clones. In addition, cross-fertilization by male gametes of the other unspecified clones can be studied by means of their different genotypes at the analyzed gene loci. For illustration see Figure 1.

Because seed analysis was performed, in this study the terms "descendant" and "offspring" refer to viable seeds with fully developed embryos. This implies that the obtained results also reflect the effects of any genotypic selection occurring in the early embryonic or any pre-embryonic stage.

It is the aim of this study to describe mating probabilities with respect to one particular clone, as an example, and then to use these results in order to outline several characteristics of the mating system realized in an actual breeding population. Moreover, the gametic contribution of this same clone will be monitored in all-orchard seed probes. Some preliminary results of this study were presented earlier (MÜLLER-STARCK 1979). Enzyme gene marker studies with respect to single clones in pine seed orchards were also presented by RUDIN and LINDGREN 1977, ADAMS and JOLY 1980a, MORAN *et al.* 1980, SHEN *et al.* 1981. More details are given in the results and discussion section.

#### Material and Methods

The clonal seed orchard is situated in a large area with stands of only *Picea abies* and a few deciduous tree species so that it can be expected to be effectively isolated from external pine pollen immigration. It was established in 1959 by the Hessische Forstliche Versuchsanstalt, Hann. Münden, in compartment 384 of the forest district of Reinhardshagen. A block design was applied with two randomly arranged individuals per clone in each block. To the initial number of 32 clones from Poland and Germany, eight clones were supplemented later, but four of these are now represented merely by a few individuals which have not yet flowered. Therefore, in this study 36 clones are included, averaging 25 individuals each, so that the population size amounts to 900.

The parental clones and their offspring were studied by means of *enzyme gene markers*, analysing the system of leucine aminopeptidase (LAP, EC 3.4.1.1). Tissues of endosperm, embryo or needles were homogenized and the enzymes separated by means of starch gel zone-electrophoresis in a modified discontinuous buffer system (for meth-

ods see POULIK 1957, BERGMANN 1973 and RUDIN 1977). To elucidate the genetic control of the LAP-system, seeds originating from controlled crossings were analysed. A portion of this material was provided by the Hessische Forstliche Versuchsanstalt.

The *genotypes at the LAP-B gene locus* of the 36 clones were identified by endosperm analysis, using a sample of nine seeds per clone. One clone was suitable to be chosen as a marker clone, because it carried a unique allele. Additional needle analysis of the same clones proved the LAP-phenotypes to be identical in needles and endosperm mixtures. Thus the genotypes at the LAP-B gene locus of the above-mentioned clones which have not yet flowered could be identified by means of pure needle analysis. None of these clones carried the same unique allele as the marker clone. The results of the needle analyses are not included in this study.

The *probability of self-fertilization* with respect to the marker clone was estimated by means of the detection of descendants from self-fertilization among the different open pollinated offspring from eight individuals. A method was used which already has been described and applied by the author (MÜLLER 1976a and 1977a): In the case of a marker clone with one unique allele at the analysed gene locus, two out of a total of four genotypes originating from self-fertilization can be identified precisely from its seeds. Besides that, the pollen contributions from other clones were detected in the embryos of the marker clone and distinguished according to their different genotypes. Material from the three flowering periods 1974–76 was analysed by using seed probes from the same eight individuals of the marker clone.

The *probabilities of cross-fertilization* were estimated by detecting the seeds with an identifiable male contribution from the marker clone among the open pollinated seed probes of 14 neighboring trees ("sample trees") from other clones. For methods see MÜLLER (1977b). Also, the pollen contribution of clones other than the marker clone are detectable.

The distances between each of the 14 sample trees and the nearest individuals of the marker clone vary from 4.5 m to a maximum distance of 21 m. The sample trees were selected at random from the northern third of the seed orchard where the condition of the trees was better and closer to uniformity than in the remainder of the area. Seed probes from the two flowering periods 1974 and 1976 were analyzed by using material from the same 14 sample trees. The period 1975 had to be omitted, because not enough seeds were available from some of the sample trees. The male gametic contribution of the marker clone as detected in the embryos of a sample tree may originate mainly, but not exclusively, from the nearest individual of the marker clone. This was taken into account separately by application of a model (GREGORIUS and MÜLLER 1975) in which the relative contribution of each marker clone individual to a particular sample tree depends on its distance to this tree, as described by experimentally-obtained exponential functions.

The *gametic contribution* of the marker clone to the open pollinated offspring of the entire seed orchard was studied in detail by identifying whose embryos containing either a male or female gamete from the marker clone or both, that is, seeds originating from self-fertilization. Random probes which originate from the all-orchard seed

production from the two flowering periods 1974 and 1976 were analyzed.

Consequences of the actual mating system on the genotypic structures among the offspring were outlined by computing the average coefficients of inbreeding and kinship. A model presented by GREGORIUS and MÜLLER (1975) was applied. This model is based on the following assumptions, which should be realized approximately: no mutation, immigration or genetic selection and no genetically-caused variation of the amounts of pollen production, of the types of pollen dispersal, or of the probabilities of self-fertilization among the trees; simultaneous flowering and sufficient pollen quantities to fertilize all ovules.

### Results and Discussion

#### Clonal genotypes at the LAP-B gene locus

Analyzing the system of leucine aminopeptidase (LAP) in *Pinus sylvestris*, in accordance with RUDIN (1977) and MEJNARTOWICZ (1979), isozyme variation is obtained in the zymogram in the two distinct regions A and B, each of which is commonly accepted to be expressed by a different gene locus (for other Pine species see for example CONKLE 1971, NICOLIC and BERGMANN 1974, CONKLE and ADAMS 1977, GURIES and LEDIG 1978, O'MALLEY *et al.* 1979, ADAMS and JOLY 1980b, MORAN *et al.* 1980). The variation within the LAP-A region is not included in the results, since an unequivocal identification of the pollen contribution to the embryo is not guaranteed.

Three distinct isozyme bands  $B_1$ ,  $B_2$ ,  $B_3$  (see Figure 2) were identified alternatively in the haploid endosperm tissues. Direct combinations of these bands were equally apparent in endosperm mixtures, pollen probes and needle tissues of the respective clones. This indicates a tissue unspecific enzyme activity and a monomeric structure as far as LAP-B is concerned. The isozyme bands are also obtained in the embryo tissue in the form of direct combinations of the male and female contribution. The marker clone CH 2 is the only one out of the 36 clones, including the four clones which have not yet flowered, to carry the  $B_3$ -band.

To elucidate the genetic control of the polymorphism in the LAP-B region, the endosperm and the corresponding embryo of seeds originating from seven controlled crossings including reciprocal crossing and self-fertilization were analysed. As can be seen in Table 1, the detected proportions of the respective embryonic LAP phenotypes segregate without significant statistical deviation according to a simple Mendelian mode of inheritance or stay uniform in the case of uniform parental LAP phenotypes.

For the present, it can be stated that the LAP-B system in endosperm and embryo is controlled genetically by one gene locus with at least three codominant alleles  $B_1$ ,  $B_2$ ,

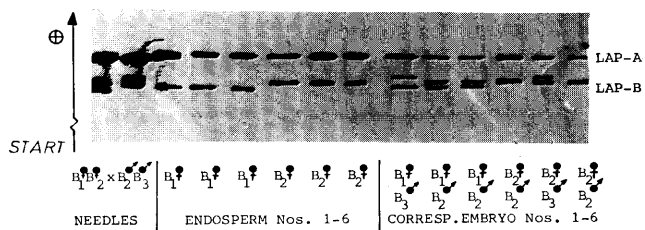


Figure 2: LAP phenotypes of parental trees (needle tissue) and offspring (endosperm and embryo) of 6 seeds from the controlled cross CH 10 ♀ x CH 2 ♂. The designation refers to LAP-B.

Table 1. — Results of analyses of seeds from seven controlled crossings. The given values for the expected phenotypic segregation refer to the Mendelian proportions.

PARENTAL CLONES		Number of seeds	OFFSPRING		$\chi^2$ +)
Controlled crossings ♀ x ♂	LAP-phenotypes		Segregation of LAP-phenotypes Expected	Detected	
CH9 x CH3	$B_2B_2 \times B_2B_2$	54	$B_2B_2$ uniform	$B_2B_2$ uniform	++)
CH2 x CH16	$B_2B_3 \times B_2B_2$	45	$B_2B_2 : B_2B_3 = 22,5$ each	22:23	0.022 n.s.
CH9 x CH2	$B_2B_2 \times B_2B_3$	42	$B_2B_2 : B_2B_3 = 21$ each	21:21	0.000 n.s.
CH2 x CH11	$B_2B_3 \times B_1B_2$	45	$B_1B_1 : B_1B_2 : B_2B_2 : B_2B_3 = 11,25$ each	9:13 11:12	0.778 n.s.
CH11 x CH2	$B_1B_2 \times B_2B_3$	48	$B_1B_2 : B_2B_3 = 12$ each	12:10 13:13	0.500 n.s.
CH10 x CH2	$B_1B_2 \times B_2B_3$	46	$B_1B_2 : B_2B_3 = 11,5$ each	11:16 11:8	2.870 n.s.
CH10 x CH10	$B_1B_2 \times B_1B_2$	19	$B_1B_1 : B_2B_2 = 4,75$ each	4:4 8:3	3.105 n.s.

+)  $\chi^2$ -test "Goodness of fit"  
++) At 5 % level

$B_3$  which are represented phenotypically by distinct single isozyme bands (allozymes). Thus the genotypes at this gene locus can be identified directly by means of the respective allozymes.

#### Probabilities of self-fertilization

The genotypes at the LAP-B gene locus of endosperm and corresponding embryo of open pollinated seeds from each of eight individuals of the marker clone CH 2 are given in Table 2. All results from three flowering periods are included. Since the marker clone carries the frequent allele  $B_2$  and the unique allele  $B_3$ , the embryos of its seeds which contain the pollen contribution  $B_1 \delta$  must originate from cross-fertilization, those with  $B_2 \delta$  from cross- or self-fertilization and those with  $B_3 \delta$  from self-fertilization.

In Table 2 the frequencies of the analysed embryos are listed according to the respective female and male gametic contributions. On the right side of the table, the relative allelic frequencies as detected among the pollen contributions (successful male gametes) are given comprehensively for each flowering period and are contrasted with the relative allelic frequencies of the 36 parental clones. The latter ones represent the expected values according to the panmixia hypothesis. Applying the standard  $\chi^2$ -test "goodness of fit", highly significant deviations are obtained between both allelic frequencies in all three flowering periods. The respective  $\chi^2$ -values for the years 1974 to 1976 range between 179.63\*\*\* and 279.33\*\*\*. These deviations are indicated mainly with respect to the alleles  $B_3$  and  $B_1$ : the marker allele  $B_3$  is obtained about four times as frequently and  $B_1$  only half as frequently as expected. This can be explained by assuming different flowering intensities depending on the respective clonal genotypes (fertility selection) and possibly by gametic or zygotic selection. The degree to which genotypic assortative mating caused by genetic incompatibility is involved can be studied if more marker clones are available.

Only minor differences can be observed between the allelic frequencies among the successful male gametes of the three flowering periods. Applying the  $k \times 2$  table  $\chi^2$ -test according to BRANDT and SNEDECOR (e.g. SACHS 1974) and contrasting the frequencies of one allele in the three periods with those of the remaining two alleles, no significant deviations can be found out. The  $\chi^2$ -values range between 1.02 for  $B_1 \delta$  and 1.73 for  $B_2 \delta$ .

Table 2. — Results of analyses of open-pollinated seeds from eight individuals of the marker clone CH 2 during three flowering periods. The allelic frequencies among the pollen contributions are contrasted with those of the 36 parental clones. The given genotypes refer to the LAP-B gene locus.

Individuals of the marker clone CH 2	CH2/28	CH2/73	CH2/119	CH2/175	CH2/236	CH2/302	CH2/339	CH2/417	Σ	Allelic frequencies of OFFSPRING (σ <sup>2</sup> contrib.) PARENTAL CLONES	
<b>FLOWERING PERIOD 1974</b>											
Total number of seeds per individual	120	120	120	120	120	120	120	120	960		
Number of seeds with endosperm genotype											
$B_2 \sigma$ $B_3 \sigma$	65 55	63 57	61 59	58 62	58 62	60 60	52 68	75 45	960		
Number of corresponding embryos according to pollen contributions											
$B_2 \sigma B_1 \sigma$ $B_3 \sigma B_1 \sigma$	3 3	4 -	2 6	2 3	6 1	3 4	1 6	2 1	23 24	0.049	0.111
" $B_2 \sigma$ " $B_2 \sigma$	59 49	56 54	52 49	54 54	50 59	54 52	49 57	67 43	441 417	0.894	0.875
" $B_3 \sigma$ " $B_3 \sigma$	3 3	3 3	7 4	2 5	2 2	3 4	2 5	6 1	28 27	0.057	0.014
<b>FLOWERING PERIOD 1975</b>											
Total number of seeds per individual	120	120	120	120	120	120	120	120	960		
Number of seeds with endosperm genotype											
$B_2 \sigma$ $B_3 \sigma$	63 57	58 62	53 67	67 53	60 60	55 65	57 63	65 55	960		
Number of corresponding embryos according to pollen contributions											
$B_2 \sigma B_1 \sigma$ $B_3 \sigma B_1 \sigma$	2 3	1 1	1 6	3 6	4 2	3 1	5 6	4 9	23 34	0.059	0.111
" $B_2 \sigma$ " $B_2 \sigma$	56 54	53 58	47 57	60 45	53 56	49 64	49 54	53 40	420 428	0.883	0.875
" $B_3 \sigma$ " $B_3 \sigma$	5 -	4 3	5 4	4 2	3 2	3 -	3 3	8 6	35 20	0.057	0.014
<b>FLOWERING PERIOD 1976</b>											
Total number of seeds per individual	120	120	120	120	120	120	120	120	960		
Number of seeds with endosperm genotype											
$B_2 \sigma$ $B_3 \sigma$	68 52	57 63	65 55	61 59	60 60	62 58	62 58	69 51	960		
Number of corresponding embryos according to pollen contributions											
$B_2 \sigma B_1 \sigma$ $B_3 \sigma B_1 \sigma$	1 2	3 2	3 2	5 7	5 3	3 5	4 4	2 2	26 27	0.055	0.111
" $B_2 \sigma$ " $B_2 \sigma$	63 48	51 56	58 46	48 44	50 54	55 52	55 51	65 44	445 395	0.875	0.875
" $B_3 \sigma$ " $B_3 \sigma$	4 2	3 5	4 7	8 8	5 3	4 1	3 3	2 5	33 34	0.070	0.014

The probabilities of self-fertilization are estimated by means of the frequencies of the embryo genotypes which contain the marker allele as male contribution ( $B_2 \sigma B_3 \sigma$  and  $B_3 B_3$  — see Table 2, bottom line of the respective flowering period data). These represent two out of a total of four genotypes originating from self-fertilization ( $B_2 B_2$ ,  $B_3 \sigma B_2 \sigma$ ,  $B_2 \sigma B_3 \sigma$ ,  $B_3 B_3$ ). According to the studied genetic control of the LAP-B system, these four genotypes are to be expected with the same frequencies. This is valid for the identified genotypes  $B_2 \sigma B_3 \sigma$  and  $B_3 B_3$  in the periods 1974 and 1976, but not in 1975. The detected frequency distortion of 35:20 (see also Table 3) no longer agrees unequivocally with the 1:1 hypothesis, since the probability function for this explicit case

$P(x) = \binom{n}{x} \cdot p^x \cdot (1-p)^{n-x}$  with  $p = (1-p) = 0.5$  and  $n = 55$  results in a value of 94,2% for the interval  $20 < x < 35$ . This indicates that a segregation such as the one detected has only the probability of 5,8% of occurring as a random event, so that systematic causes for this distortion seem to be more realistic. It cannot be induced by gametic selection, as the gametic segregation is close to the expected 1:1 proportion, and selection with respect to  $B_3$  is not evident

because it is contained in both identified genotypes. To take into account the possible effects of genotypic selection, as for example selection resulting from archegonical polyembryony (for details see MÜLLER 1977a), two methods are applied in Table 3 to estimate the expected probabilities of self-fertilization. Method (1) is based on the expectation that the frequencies of the two identified genotypes and the two remaining genotypes are identical, and method (2), that all genotypes except the identified homozygote  $B_3 B_3$  have the same frequencies.

The estimated probabilities of self-fertilization show only small variation: the average period values vary between 0.115 (11.5 %) and 0.139 (13.9 %). The detailed results concerning the individuals of the marker clone can be derived analogously from the data given in Table 2. These individual values vary between 0.063 and 0.267. The average probability of self-fertilization of the three flowering periods is 0.126. Values for individual trees in Scots pine stands instead of a clone in a seed orchard were estimated by the author (MÜLLER 1977a) by applying the same methods to average 0.062.

Table 3. — Probabilities of self-fertilization of the marker clone CH 2 for three flowering periods (data taken from table 2).

Flowering period	Number of seeds	Frequencies of identifiable LAP-genotypes from self-fertilization		Probabilities of self-fertilization estimated by 2 methods	
		$B_2 \sigma B_3 \sigma$	$B_3 B_3$	(1)	(2)
1974	960	28	27	0.115	0.116
1975	960	35	20	0.115	0.130
1976	960	33	34	0.140	0.139

Additional marker clones will be studied to determine whether or not the observed presence of twice as many descendants from self-fertilization in the seed orchard as compared to natural stands is representative. Previous enzyme gene marker studies on self-fertilization with respect to single clones in pine seed orchards resulted in the following values: 0.060 (6.0%) for *Pinus sylvestris* in Sweden (SHEN *et al.* 1981), 0.012 for *Pinus taeda* in USA (ADAMS and JOLY 1980a) and 0.140 for *Pinus radiata* in Australia (MORAN *et al.* 1980).

Probabilities of cross-fertilization

Seed probes from each of 14 trees from clones other than the marker clone ("sample trees") were studied. In Table 4 the frequencies of the analysed embryos from each of the sample trees are listed according to pollen contribution and flowering period. The ordering of the sample trees is according to the respective distance to the closest individual of the marker clone. These distances vary between 4.5 and 21 m. On the right side of the table, the relative allelic frequencies among the pollen contributions in the embryos are given comprehensively for each flowering period and contrasted with the allelic frequencies of the 36 parental clones, which fulfill the panmixia expectation. Applying the standard chi<sup>2</sup>-test "goodness of fit", the parental values can be proven to deviate significantly from the frequencies among the successful male gametes. The chi<sup>2</sup>-value is 1804.96\*\*\* for 1974 and 1008.30\*\*\* for 1976. These deviations are mainly reflected in the frequencies of the alleles B<sub>3</sub> and B<sub>1</sub>: the first is obtained on the average 6.9 times, the latter only 0.41 times as frequently as expected. These results confirm the frequencies which were determined in the offspring from the marker clone.

Comparing the frequencies of the pollen contributions B<sub>1</sub>♂, B<sub>2</sub>♂ and B<sub>3</sub>♂ among the offspring of the 14 sample trees, deviations are observed which concern all three alleles to a similar extent. For instance, the frequencies of B<sub>1</sub>♂ resp. B<sub>3</sub>♂ vary in 1974 between 1 and 16 resp. 12 and 33, in 1976 between 1 and 19 resp. 3 and 28. As can be seen in Table 4, such minimal and maximal values in the two flowering periods are always obtained from different trees. This fact can be interpreted as being a result of interferences caused by environmental conditions. Such a conclusion also seems justified in the case of a comparison between the average frequencies of the three alleles in the two flowering periods: Applying the k × 2 table chi<sup>2</sup>-test according to BRANDT and SNEDECOR, significant deviations between 1974 and 1976 are obtained for the alleles B<sub>2</sub>♂ and B<sub>3</sub>♂. The chi<sup>2</sup>-values are 13.54\*\*\* and 10.82\*\*, respectively. The value of 2.26 for the allele B<sub>1</sub>♂ implies an insignificant deviation. These results indicate a more intensive male flowering of clones which carry the allele B<sub>2</sub> in the period 1976 and of the marker clone in 1974.

Considering the frequencies of only those embryos which contain the B<sub>3</sub>♂ pollen contribution from the marker cone, no correlation is evident in Table 4 between these values and the distance values of the respective trees to the nearest individual of the marker clone. In order to describe the probabilities of cross-fertilization with respect to the marker clone (in spite of the obvious frequency fluctuations), exponential distribution functions were tentatively fitted to the data by means of a least squares estimation: the functions are f(x) = 19.39 · e<sup>0.001 x</sup> for the flowering period 1974 and f(x) = 19.01 · e<sup>-0.025 x</sup> for 1976 (x = distance). As can be seen in Figure 3, with increasing distances the functional values are either very slightly increasing (1974) or decreasing (1976). Both functions together confirm that the frequency of the male gametic contribution of the marker clone cannot be related unequivocally to the spatial arrangement of the respective trees. Results of a study of RUDIN and LINDGREN (1977, 4.2.3) in Swedish Scots pine seed orchards allow the same conclusion, while in a previous study (SHEN *et al.* 1981) preferential mating within small neighborhoods is evident.

The obtained results illustrate a mating situation which is expected to be typical for seed orchards. Contrary to the results of analogous studies in Scots pine stands by the author (MÜLLER 1977b), the probabilities of cross-fertilization no longer depend clearly on the distance of the respective trees. This can be explained as a consequence of overlay effects due to the presence of many genotypically identical individuals within a small area: Individuals of each clone are arranged randomly in many small blocks, so that varying neighborhoods are given and nearly all mating constellations can be realized at the same time.

Comparing the average frequencies of the pollen contribution B<sub>3</sub>♂ in tables 2 and 4, a much lower representation of B<sub>3</sub>♂ is evident among the offspring from the marker clone (descendants from self-fertilization) than from the offspring of the sample trees: the average relative values for the flowering periods 1974 and 1976 are 0.064 for Table 2 and 0.096 for Table 4, which in this case means a reduction of the B<sub>3</sub>♂ frequency after self-fertilization as compared to cross-fertilization by one-third. This difference cannot be explained by gametic selection because the results always refer to the same B<sub>3</sub>♂ gametes.

Table 4. — Results of analyses of open-pollinated seeds from 14 individual trees of other clones than the marker clone during three flowering periods. The respective distances to the nearest individual of the marker clone are given in parentheses. The allelic frequencies among the pollen contributions are contrasted with those of the 36 parental clones. The genotypes refer to the LAP-B gene locus.

Tree-No. (distance)	88 (4.5m)	174 (5.0m)	185 (7.5m)	177 (10.0m)	229 (10.5m)	69 (11.0m)	34 (11.5m)	134 (12.0m)	157 (13.5m)	76 (15.0)	149 (16.0m)	126 (18.5m)	179 (20.0m)	220 (21.0m)	Σ	Allelic frequencies of OFFSPRING (#contrib.)		PARENTAL CLONES
<b>Flowering Period 1974</b>																		
Total number of seeds per individual	192	192	192	192	192	192	192	192	192	192	192	192	192	192	2688			
Number of embryos with pollen contribution																		
B <sub>1</sub> ♂	1	10	16	11	5	6	7	16	13	10	15	12	11	1	134	0.050	0.111	
B <sub>2</sub> ♂	176	170	149	148	162	168	165	154	157	164	148	165	166	169	2261	0.841	0.875	
B <sub>3</sub> ♂	15	12	27	33	25	18	20	22	22	18	29	15	15	22	293	0.109	0.014	
<b>Flowering Period 1976</b>																		
Total number of seeds per individual	192	192	192	192	192	192	192	192	192	192	192	192	192	192	2688			
Number of embryos with pollen contribution																		
B <sub>1</sub> ♂	6	4	19	9	9	2	1	5	13	3	17	16	8	3	111	0.041	0.11	
B <sub>2</sub> ♂	164	169	145	158	180	183	173	175	160	175	162	164	169	178	2355	0.876	0.875	
B <sub>3</sub> ♂	22	19	28	25	3	7	18	12	19	14	17	12	15	11	222	0.083	0.014	

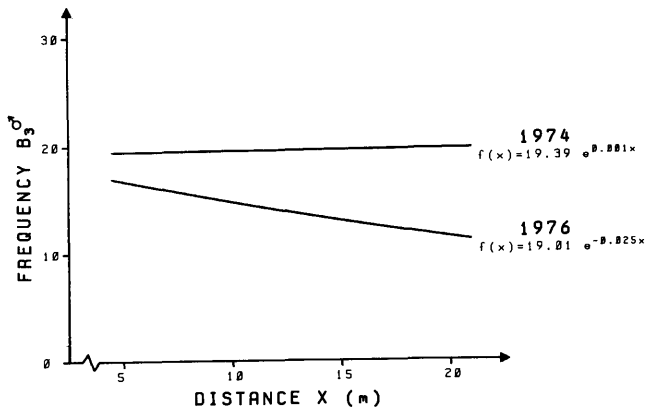


Figure 3. — Absolute frequencies of embryos containing the pollen contribution  $B_3\delta$  of the marker clone as a function of the distance  $X$  between 14 sample trees and the respective nearest individual of the marker clone. Both functions are derived from data in table 4.

Furthermore, there is no evidence for selection against  $B_3$  gametes, since gametic segregation as detected in the seeds of the marker clone is still congruous with the 1:1 hypothesis. Therefore the obtained differences between the  $B_3\delta$  frequencies clearly indicate the effects of genotypic selection against all or at least the identifiable descendants from self-fertilization.

#### Illustration of mating probabilities

Mating probabilities of the marker clone are presented in the form of the frequencies of viable seeds containing the  $B_3\delta$  gametic contribution of the marker clone which are expected for each tree in the seed orchard, including the sample trees and the individuals of the marker clone. The model described in GREGORIUS and MÜLLER 1975 is applied to calculate the expected  $B_3\delta$  frequencies (Figure 4) and to contrast these results with an approximation to the

experimentally evaluated data (Figure 5). In both cases, the given results refer to the actual population size and position of the trees in the seed orchard. Because the genotypic identity of the individuals of the same clone does not allow the evaluation of the probabilities of self- and cross-fertilization for each individual separately, results from analogous experiments were applied which refer to individual trees in Scots pine stands (MÜLLER 1977a, b and unpublished data). The value for the average individual probability of self-fertilization is  $S_i = 0.06$  for Figure 4, the function which represents the probability of cross-fertilization is  $f(x) = 0.052 \cdot e^{-0.038 \cdot x}$  with  $x =$  distance. In the pictured plot diagrams there is an exact correspondence between the columns and the actual positions of the individual trees in the seed orchard. In Figure 4 the positions of the individuals of the marker clone are identical with the protruding columns. The positions of the sample trees are represented by dark squares.

The values obtained from the model deviate considerably from the experimentally detected ones. Whereas the observed frequency of the  $B_3\delta$  contribution in the seeds of individuals from the marker clone was on the average only one-third that of seeds from other individuals (see Tables 2 and 4), the frequency to be expected from the model in the marker clone seeds is twice that of the other seeds. Such overestimation of the presence of descendants from self-fertilization can only be due to differences in the actual conditions from those assumed in the model. Two variables are concerned more than the others:

Firstly, the assumed range of pollen dispersal may differ from the actual situation in the seed orchard. However, additional computations with different values for this variable resulted only in deviations of less than 10%, even in the case of extreme pollen dispersal hypotheses.

Secondly, the calculated probabilities of the formation of descendants from self-fertilization may not be realized

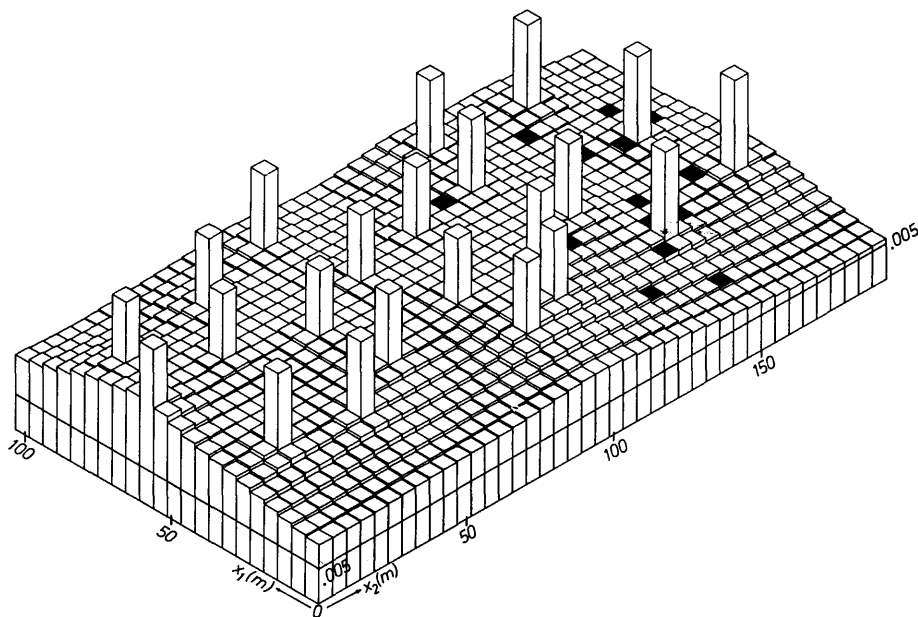


Figure 4. — 3-dimensional presentation of relative frequencies of embryos containing the male gametic contribution  $B_3\delta$  of the marker clone as estimated for the individual tree at each position  $(x_1, x_2)$ . The protruding columns represent the individuals of the marker clone; the dark squares are the sample trees. Selection against descendants from self-fertilization is excluded.

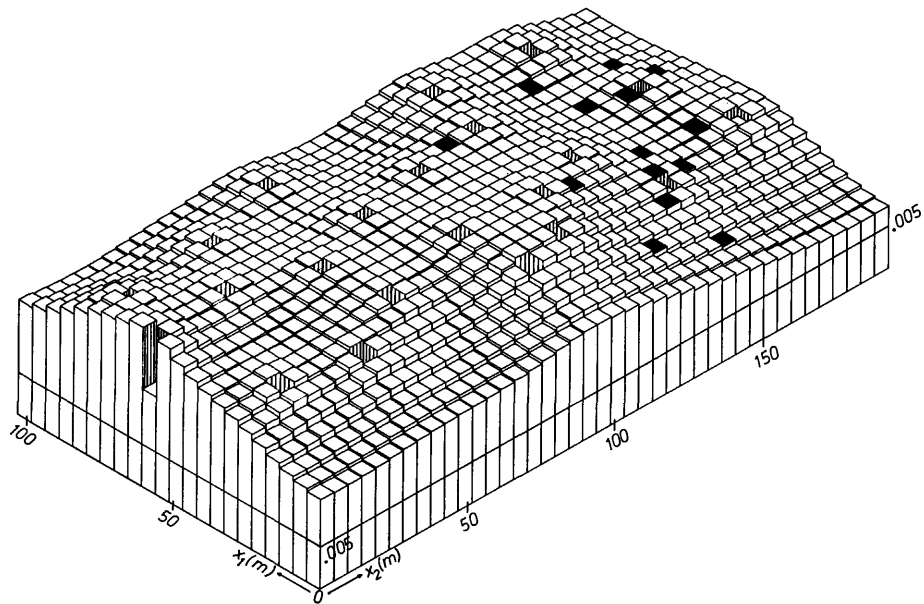


Figure 5. — Approximation to experimental data by assuming selection against descendants from self-fertilization. For legend see figure 4, for further explanation see text.

in the seed orchard. The presence of such descendants may be much lower than can be expected under conditions which exclude genetic selection.

Only the latter assumption can sufficiently explain the deviation between the results of the applied model and the experimental data. To prove this, calculations with reduced probabilities of individual self-fertilization  $S_i$  were performed. A good approximation to the experimental data was obtained with  $S_i = 0$ . These results are presented in Figure 5. The positions of the individuals of the marker clone are identical with the low columns, the neighboring columns of which are shaded.

The fact that with  $S_i = 0$  the approximation conditions are met with respect to the frequency proportion of  $B_3 \sigma$  between the descendants of the marker clone and other clones indicates a strong genetic selection against individuals originating from self-fertilization. The expected proportion of all individuals which can originate either from individual self-fertilization or from cross-fertilization between the individuals of the same clone is reduced un-

der the given experimental conditions to an extent which approximately equals the frequency of the first category.

In spite of this obvious reduction, the remaining descendants from self-fertilization of all individuals of the marker clone are still over-represented when compared with a population where all individuals have a different genotype. Some population genetic consequences of this constellation are outlined under the topic "inbreeding and kinship".

The graduation in Figure 5 makes it obvious that the obtained values according to the model assumptions are on the average much lower than the experimentally detected ones (see Tables 2 and 4). This is a clear indication of an above-average male flowering intensity of the marker clone. The extent to which this affects the transferability of the estimated probabilities of self-fertilization to other clones is not predictable, because many clone specific variables are involved due to genotypic and/or genotype-environment interactional characteristics. It may imply the tendency to slightly overestimate the probabilities of

Table 5. — Results of analyses of open pollinated all-orchard seed probes originating from two flowering periods. Allelic frequencies among the offspring are contrasted with those of the 36 parental clones.

	Number of corresponding embryos with the pollen contribution			Allelic frequencies of OFFSPRING (#contrib.)	
	$B_1 \sigma$	$B_2 \sigma$	$B_3 \sigma$	PARENTAL CLONES	
Flowering period 1974					
Number of seeds with endosperm genotype					
$B_1 \sigma$	9	96	8	0.177	0.111
$B_2 \sigma$	44	444	30	0.809	0.875
$B_3 \sigma$	-	9	-	0.014	0.014
Allelic frequencies of OFFSPRING (#contrib.)	0.083	0.858	0.059		
Flowering period 1976					
Number of seeds with endosperm genotype					
$B_1 \sigma$	1	30	5	0.056	0.111
$B_2 \sigma$	36	522	26	0.913	0.875
$B_3 \sigma$	2	17	1	0.031	0.014
Allelic frequencies of OFFSPRING (#contrib.)	0.061	0.889	0.050		

self-fertilization with respect to the other clones. Possible consequences are discussed in the section "Inbreeding and kinship".

In both figures considerable fluctuations of the frequencies of descendants with the  $B_3 \sigma$  contribution are obtained between individuals of the non-marker clones. The largest values are more than 2.5 times the smallest. This is due to the spatial distances of the trees to the nearest individuals of the marker clone. The fluctuations are less pronounced than the experimentally detected ones, but still clearly indicate deviations from the panmixia hypothesis.

The given results are a first step towards the presentation of mating probabilities. Further studies are required for an optimal approximation to the obtained experimental data.

#### *Gametic contribution in all-orchard seeds*

Probes of all-orchard harvests of seeds originating from the flowering periods 1974 and 1976 were studied using a sample of 640 seeds per period. Genotypes of the endosperm and the corresponding embryo of each seed are presented in *Table 5*. The detected relative allelic frequencies among the offspring are given separately for the male and the female gametic contributions and contrasted with the values of the parental clones. Comparing the latter frequencies, which represent the panmixia expectation, with the pooled male and female allelic frequencies among the offspring, highly significant deviations are obtained in both flowering periods: The  $\chi^2$ -values according to the standard test "goodness of fit" are 54.39\*\*\* for 1974 and 98.44\*\*\* for 1976. Such deviations are also revealed when the male resp. female frequencies alone are compared with the parental ones: The  $\chi^2$ -values range between 27.84\*\*\* (female contribution 1974) and 99.97\*\*\* (male contribution 1974). As is also the case for the offspring of the marker clone and the sample trees,  $B_3 \sigma$  is overrepresented and  $B_1 \sigma$  underrepresented in the all orchard offspring in both flowering periods. It can be seen in *Table 5*, that these tendencies are not evident among the female allelic frequencies:  $B_3 \sigma$  is obtained too frequently only in 1976;  $B_1 \sigma$  is overrepresented in 1974 and underrepresented in 1976 and  $B_2 \sigma$  vice versa.

The pooled male and female allelic frequencies of the two flowering periods were compared by applying the  $k \times 2$  table  $\chi^2$ -test according to BRANDT and SNEDECOR. The value of 37.94\*\*\* proves the deviations between both offspring populations to be highly significant. This test was also applied to compare the male allelic frequencies in the two periods. The same was done for the females. The frequency of one allele and the frequencies of the remaining two alleles were compared between the periods. Significant deviations are indicated with respect to the frequencies of  $B_1$ ,  $B_2$  and  $B_3$  in the female but not in the male contributions. The  $\chi^2$  values for the female contributions range between 4.27\* and 45.03\*\*\*, for the male between 0.54 and 2.30 only. The fact that outstanding deviations between 1974 and 1976 are expressed only in the female contributions to the offspring indicates that the female flowering of the genotypes may be influenced more by the particular environmental conditions than the male flowering. The obvious deviations between the male and the female allelic frequencies within each flowering period reflect the effects of sexually asymmetric fertility selection. This phenomenon is studied in more detail (MÜLLER-STARCK *et al.*, submitted for publication).

Only those descendants of the marker clone can be identified which contain the marker allele  $B_3$  as either the female or the male contribution or as both ( $B_3 \sigma B_1 \sigma$ ,  $B_3 \sigma B_2 \sigma$ ,  $B_1 \sigma B_3 \sigma$ ,  $B_2 \sigma B_3 \sigma$ ,  $B_3 B_3$ ).

The latter originates from self-fertilization and is the only identifiable genotype of the four genotypes from self-fertilization. All seeds with the allele  $B_2$  from the marker clone cannot be distinguished from the others because  $B_2$  is also present in the gametes of other clones. Therefore in *Table 6* the estimated values for the gametic contribution of the marker clone were derived by multiplication by the factor 2 in the case of seeds with  $B_3 \sigma$  or  $B_3 \sigma$  contribution and by the factor 4 in case of the descendant from self-fertilization ( $B_3 B_3$ ). Comparing these values with the expected values according to the panmixia hypothesis (the value 0.027 is derived by  $\frac{L-1}{L^2}$  and 0.001 by  $\frac{1}{L^2}$  where  $L$  = number of clones), it can be stated that in both flowering periods the marker clone is substantially overrepresented in the all-orchard offspring. This is valid especially with respect to the male contribution, but also with respect to the descendants from self-fertilization. The contribution as female parent exceeds the panmixia expectation by the factor 1.6, as male parent by the factor 4, and as both by the factor 3. The total gametic contribution, i.e., the contribution as female or male parent or both is on the average 2.8 times as frequent as expected. The deviations between the flowering periods indicate that the flowering intensity in both sexes must not necessarily respond to the specific environmental conditions in the same way: The results in *Table 6* can be interpreted in the way that the increase in the female flowering of the marker clone in 1976 as compared to 1974 coincides with a decrease in the male flowering of the same clone in the same period.

#### *Inbreeding and kinship*

Descendants from self-fertilization of the marker clone are overrepresented as compared to the panmixia expectation but also with respect to the estimations for individual trees in forest stands. The frequencies of these descendants exceed the panmixia values on the average by the factor 4.5 in the case of the marker clone offspring and by the factor 3 in the all-orchard offspring. Any increase in descendants from self-fertilization induces an increase in the average coefficients of inbreeding and kinship among the offspring populations, although this is not necessarily the only cause. This implies an increase in the portion of homozygote genotypes as compared to the corresponding Hardy-Weinberg proportions. A well-known consequence of this phenomenon is the increasing probability for a phenotypic expression of disadvantageous traits in a population (e.g. ERIKSSON *et al.* 1973) which is evident as long as these effects cannot be counterbalanced by selection.

The given experimental results are used to compute the expected average coefficients of inbreeding ( $f$ ) and kinship ( $\varphi$ ) based on data of the actual seed orchard as well as on varying clone numbers and individuals per clone assuming constant population size  $N = 900$ . A model is applied which is described in detail by GREGORIUS and MÜLLER (1975). The obtained results as presented in *Figure 6* can reflect the actual mating situation only approximately. Variability in the gamete production generally can be expected to reduce the inbreeding effective population size, which in turn increase the average coefficients of inbreeding and kinship



Table 6. — Gametic contribution of the marker clone to the all-orchard offspring from two flowering periods. Expected values are based on the panmixia hypothesis, estimated values refer to Table 5.

Gametic contribution of the marker clone	Rel. frequency of EXP. under PANMIXIA	seeds from the marker clone ESTIMATED for flower periods	
		1974	1976
♀ contribution	0.027	0.028	0.059
♂ contribution	0.027	0.119	0.097
♀ and ♂ contribution (descendants from self-fertilization)	0.001	-	0.006

as compared to homogeneous gamete production. This implies that the derived values are likely to represent a minimum, so that the actual values should exceed the ones in Figure 6.

The following data of the actual seed orchard are included in the calculations: population size  $N = 900$ , probability of self-fertilization  $S = 0.126$  for the number of clones  $L = 36$ , probabilities of cross-fertilization as presented in Figure 3 (averaged values of the two given functions), and the average grid distance between the trees of 4.7 m. For number of clones other than 36, the probabilities of self-fertilization were transformed continuously according to the applied model:  $0.191 \geq S \geq 0.102$  for  $10 \leq L \leq 900$ . The minimum value of  $S = 0.102$  was calculated as an approximation for a hypothetical seed orchard with  $N = L = 900$  which implies that each clone is represented by only one individual. This value for individual self-fertilization exceeds the value of  $S = 0.062$  which refers to individual trees in Scots pine stands (MÜLLER 1977a). Such a deviation may be explained by assuming varying self-fertilities and/or as an effect due to different population characteristics such as population size which may affect the extend of individual self-fertilization.

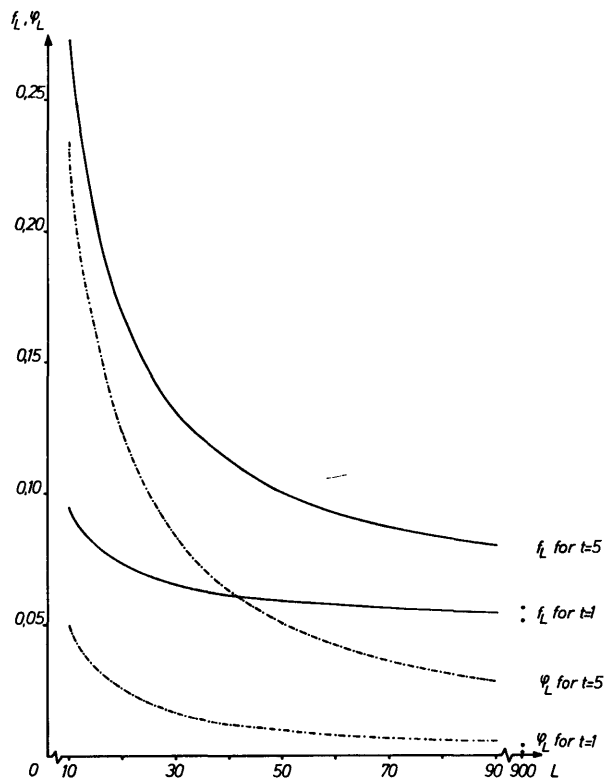


Figure 6. — The expected average coefficients of inbreeding ( $f$ ) and of kinship ( $\varphi$ ) as a function of the number of clones per seed orchard ( $L$ ) for the generations  $t = 1$  and  $t = 5$ .

Population specific aspects imply that model assumption cannot be generalized for all population types.

Because the level of inbreeding and kinship can vary substantially among the respective basic populations from which the parental clones were selected, the coefficients of inbreeding and of kinship among these clones were taken for the present as  $f_0 = \varphi_0 = 0$ . In this way the particular dynamics in seed orchard populations can be evaluated independently from other effects. Results of two different generations  $t$  are presented in Figure 6:  $t = 1$  is equivalent to the offspring of the actual seed orchard,  $t = 5$  to the fifth generation. Conditions for a new generation are met in the case of the selection of seed orchard clones from stands which were established by seeds originating from the previous orchard generation.

Under the given assumptions the calculations for the actual seed orchard result in average coefficients of inbreeding of  $f_1 = 0.063$  and  $f_5 = 0.119$  and in average coefficients of kinship of  $\varphi_0 = 0.014$  and  $\varphi_5 = 0.070$ . In the first few generations the values of  $f$  exceed the ones of  $\varphi$  by far because the initial condition  $f_0 = \varphi_0 = 0$  implies that  $f$  is then determined primarily by the extend of self-fertilization while  $\varphi$  always depends also on other parameters such as cross-fertilization. The differences between both should be reduced with increasing probabilities of self-fertilization. These tendencies are evident if varying number of clones are included in the calculations (see Figure 6). The obtained results demonstrate clearly that the expected average coefficients of inbreeding and of kinship among the offspring increase generally with decreasing numbers of parental clones and increasing numbers of individuals per clone, respectively. This tendency is pronounced with increasing numbers of generations. Under the given conditions, in the first generation the maximum average coefficient of inbreeding is 0.0955 ( $L = 10$  clones) and the minimum one 0.051 ( $L = N = 900$  clones). If for instance the number of clones is increased from 10 to 20, a reduction of the coefficient of inbreeding is obtained totalling 51% of the difference between the maximum and the minimum value. In the case of the actual seed orchard the reduction is then 73%; if the number of clones is further increased, the reduction is 81% for 50 clones, and 90% for 90 clones. With growing numbers of clones and decreasing numbers of individuals per clone, respectively, the reduction rate becomes effectively smaller.

As pointed out before, the given results represent in general minimum values because of the simplifying model assumptions with respect of fertility and mating system parameters. In addition the values for  $f_L$  and  $\varphi_L$  altogether will exceed or can remain under the given ones in the case of higher or lower probabilities of clonal self-fertilization than  $S = 0.126$ . Specific deviations from the obtained values can be expected as follows:

— If values for self-fertilization are used which are valid for individual trees in stands, instead of the transformed values for  $L = N = 900$ , the coefficients of inbreeding and of kinship should then exceed the given values in the case of numbers of clones below 50. For greater clone numbers per seed orchard reduced values are obtained.

If the initial conditions are  $f_0 \neq 0$  and  $\varphi_0 \neq 0$  for the parental clones, the development of inbreeding and kinship values among the orchard offspring depends primarily on  $\varphi_0$ : Assuming the parental clones to be selected from large populations with random arrangement of individuals,

such as artificially regenerated continuous forest stands,  $\varphi_0$  can be expected to be very close to zero (MÜLLER 1976b). This does not necessarily imply the same for  $f_0$ . In any case, the values for  $f_L$  and  $\varphi_L$  then will not deviate clearly from the presented results. If greater values for  $\varphi_0$  can be expected, as for instance in the case of a selection of neighboring trees from small indigenous populations as parental clones, the resulting values for  $f_L$  and  $\varphi_L$  can exceed the given ones substantially.

#### Concluding Remarks

As a first step in the evaluation of characteristics of the mating system realized in an actual breeding population, mating types were studied exemplarily in relation to one marker clone. Important preconditions were the following: The marker clone carried a unique allele, the seed orchard could be expected to be isolated effectively from external pine pollen and the seeds were harvested representatively from different parts of the crowns. The studied mating types refer to viable seeds which originate from open pollination.

Under the given conditions the allelic frequencies among the offspring from the marker clone, the sample trees, and the entire seed orchard are proven to deviate highly significantly from the parental one. These facts clearly indicate that important preconditions for conformity between parental and offspring populations are not met.

Descendants from self-fertilization of the marker clone are generally overrepresented as compared to the panmixia expectation: They are found among the offspring from the individuals of the marker clone 4.5 times as frequently as expected, and 3.0 times as frequently among the all-orchard offspring. This is valid in spite of the illustrated selection against descendants from self-fertilization. The probabilities of cross-fertilization do no longer seem to depend clearly on the distance between the respective trees: As a consequence of the particular arrangement of genotypically identical individuals in seed orchards, nearly all mating constellations can be realized at the same time. Based on the given mating probabilities, coefficients of inbreeding and kinship are obtained, which depend unequivocally on the number of clones per seed orchard.

The total gametic contribution of the marker clone to the all-orchard seed probes exceeds the expected value by the factor 2.8 which indicates substantial deviations with respect to the clonal representation even in a seed orchard where all clones can be expected to flower. The obtained results also demonstrate that the allelic frequencies among the offspring populations can vary between different flowering periods.

Obviously main emphasis has to be put on the degree of clonal self-fertilization. There is no evidence for genetic incompatibilities in Scots pine populations which can prevent self-fertilization. Only post zygotic inviabilitys due to lethal or semilethal alleles can have a reducing effect. Thus it can be concluded that the most effective way to reduce the presence of offspring from self-fertilization is to increase the number of clones and to decrease the number of individuals per clone in the future. The given coefficients of inbreeding and of kinship may be inter-

preted as indicators of an economic risk and function as preliminary criteria for an acceptable number of clones per seed orchard.

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