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Reproductive systems in conifer seed orchards

2. Reproductive selection monitored at an LAP gene locus in *Pinus sylvestris* L.

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Summary. In a Scots pine seed orchard the genetic structures at an enzyme gene locus active in pine seeds were compared among the parental clones and the all-orchard seeds produced over a period of three years. The genotypes of the seeds were identified as ordered pairs consisting of the female and male contribution. The sexual reproductive function monitored at the studied locus differed significantly between the two sexes. This fact proves the necessity of taking sexual asymmetry into account in studies of reproductive selection. This is done by comparing the observed genotypic structures among the offspring with the corresponding multiplicative structure expected under random gametic fusion. Additionally, accounting for partial self-fertilization increased the conformity between the observed and the hypothesized model structures. The differences in female and male gametic contributions to the offspring were used to estimate female and male relative fitness components. Significant deviations between the allelic and/or genotypic structures of orchard clones and their seed, and between seed lots collected in different years, may reduce the efficiency of realizing breeding gains in seed orchards.

Key words: Reproductive selection – Gametic fitness components – Sexual asymmetry – Pine seed orchard

Introduction

The realization of breeding gains primarily depends on how closely the genotypic structure of the offspring resembles that of the parental breeding population. In forest tree breeding, a high degree of conformity between these structures is crucial for the attainment of maximum phenotypic similarity between the selected parent trees and their offspring. The tree breeder may exploit a selection differential according to quantitative-genetic theory only if Hardy-Weinberg equilibrium exists at all gene loci controlling those traits. This requires that the gamete production yield unaltered frequencies of genes controlling the traits under artificial selection and that the gametes carrying such genes combine randomly.

In monoecious conifer species such as Scots pine (*Pinus sylvestris* L.), the following phenomena may exert influence on the genotypic parent-offspring relationship:

Fertility selection: The parent trees may not have the same fertility, and they may vary in their individual production of male and female gametes. We cannot then expect all genes controlling traits under artificial selection to be transmitted with the same probability to the zygotes of the subsequent generation. This may imply that those gametes which are contained in the seeds and thus have become successful carry alleles at the controlling gene loci in frequencies different from those which are encountered in the parent individuals.

Characteristics of the mating system. Phenomena such as genetic incompatibilities between parent trees or genetically determined deviations in flowering time will induce the assortative union of gametes carrying certain genes. This gives rise to deviations from Hardy-Weinberg proportions among the offspring at least at single gene loci and possibly in larger portions of the genome. Deviations will also be caused by non-random union of gametes as a consequence of systems of inbreeding. These are not under genetic control, and they affect the totality of the genome. Systems of this kind are established inevitably when the probabilities of self-fertilization exceed the reciprocal value of the population size, and/or when the effective pollen dispersal is restricted and, thus, preferential mating within limited neighborhoods is favored.

Viability selection. Carriers of different genotypes at loci controlling the traits under artificial selection may not possess equal viability. This means that the genotypic structure of the offspring is affected by the mortality between zygote formation and the age at which breeding merit is evaluated or utilized economically or at which the next generation is produced.

The amounts of the described deviations depend on the properties of the populations studied. Hence, they cannot, in general, be predicted, even though certain phenomena may always prevail. It kan be stated that gametic selection due to meiotic drive or gene conversion (Gutz and Leslie 1975) is not evident, since an earlier study (Müller-Starck 1982a) did not reveal any significant deviations from the 1:1 segregation ratio expected among the endosperms of single clones which contributed the female gamete. Gametic selection due to differential gametic viability cannot be evaluated in this study, and no information on this condition in conifers has so far been presented in the literature. The possible effects of fertility selection due to differential production of female and male gametes under partial self-fertilization have been pointed out by Ziehe (1982a, b); some applications on this are presented by Müller-Starck (1982b) and Müller-Starck et al. (1982).

Several authors have studied the mating system in breeding populations of Scots pine by using enzyme gene markers (Rudin and Lindgren 1977; Müller(-Starck) 1977a, b, 1979, 1982a; Shen et al. 1981). Offspring originating from self-fertilization after open pollination were identified in frequencies far exceeding the reciprocal of the population size. The presence of genotypically identical individuals can increase these frequencies in breeding populations such as clonal seed orchards. It has also been proved that probabilities of cross-fertilization rapidly decrease with increasing distance between individuals, so that preferential mating among neighboring trees is emphasized. The latter effect can be counterbalanced by the arrangement of the individuals of the clones in a seed orchard (Müller-Starck 1979): The random position of single individuals of all clones in many different blocks results in varying neighborhoods in each of these blocks, so that all possible mating constellations may be realized simultaneously. This results in elimination of the effect of distance on the probabilities of cross-fertilization between the clones if minor marginal effects are neglected (Gregorius and Müller 1975).

Other phenomena such as deviations in flowering time, may play a minor role if all representatives of the parent population originate from the same geographic habitat with similar environmental conditions. Furthermore, there is no evidence for prezygotic genetic incompatibilities in coniferous species which could prevent the union of gametes from the same trees or any different trees, although inviabilities after zygote formation due to semilethal or lethal genes are well known (Koski 1971; Hagman 1975; Lindgren 1975). Viability selection in later stages was studied by Lundkvist (1980) in a field experiment with provenances of Norway spruce (*Picea abies* (L.) Karst.), which showed the existence of selective mortality of homozygotes at an esterase gene locus up to the age of 15 years.

The same tendency became manifest in an experiment with beech (*Fagus sylvatica* L.) conducted by Kim (1980). The proportion of homozygotes at an LAP gene-locus observed in the formant nuts was significantly less in the seedling population grown by disseminating the nuts onto the ground in a forest during late winter. Bergmann (1978) detected pronounced differences in the frequencies of certain alleles at an acid phosphatase gene locus among geographic provenances of Norway spruce, which represent the gross effect of past or present viability and/or fertility selection.

The present paper refers to the genotypic frequencies in the endosperm and the corresponding embryo of viable all-orchard seeds, as detected in an earlier study (Müller-Starck 1982a). As outlined before, such material should mainly reflect the effects of fertility selection, self-fertilization and viability selection between zygote formation and the stage of the fully-developed embryo. The influence of viability selection cannot be evaluated separately due to the lack of information about the genotypic structure immediately after zygote formation. It is the intention of this paper to monitor the reproductive function of the LAP-B gene locus by contrasting the genotypic structures of parents and offspring and to test and discuss hypotheses on fertility selection and partial self-fertilization. An additional aim is the estimation of female and male relative fitness components.

Material and methods

Seed orchard population

The studied Scots pine graft seed orchard includes L = 36 clones, each represented by a number of K = 25 genotypically identical individuals, so that the total population size is $n = 900$. It was established in 1959 by the Hessische Forstliche Versuchsanstalt, Hann.-Münden, applying a block design with 2 randomly arranged individuals per clone in each block. The seed orchard can be expected to be almost completely isolated from foreign pine pollen influx as it is located in a large block of stands of other species.

Seed samples

Seeds originate from routine all-orchard collections made in the years 1975, 1976 and 1977 and thus refer to the open pollination of the flowering periods 1974, 1975 and 1976, respectively. The material was supplied by the Hessische Forstliche Versuchsanstalt, Hann.-Münden. The samples were drawn from the all-orchard seed in cold-storage. Hence, they are to be viewed as being representative of orchard-produced seed material used in silviculture. The size of the studied random sample was 640 for each flowering period.

Identification of genotypes

Genotypes of clones and their offspring from the flowering periods 1974 and 1976 have already been identified in an earlier study (Müller-Starck 1982a) by enzyme gene markers in viable seeds: the system of leucine aminopeptidase (LAP, EC 3.4.1.1) was analyzed by means of starch gel zone-electrophoresis in a modified discontinuous buffer system (Bergmann 1973; Rudin 1977). The genetic control of the LAP-system was eludicated by analyzing seeds from controlled crosses with clones from the same seed orchard. Clonal genotypes at the LAP-B gene locus were studied by endosperm analysis, the genotypes of the offspring by analysis of endosperm and corresponding embryos of individual seeds. The haploid endosperm represents the female gamete, since it has the same origin (i.e. the megaspore) and the same genetic information. By comparing the observed genotype in the diploid embryo of an individual seed with that of the corresponding endosperm, the male and female gametic contribution to the embryo can be identified as an ordered pair (Müller 1976).

Results and discussion

1 Evidence for an adaptive function of the LAP-B gene locus in reproduction

The identified genotypes at the LAP-B gene locus of the all-orchard seeds originating from open pollination during 3 different flowering periods are listed in Table 1. In this table and in the following sections, B_iB_j denotes an ordered genotype which received the first allele, B_i, from the female and the second, B_j, from the male parental gamete.

An elementary hypothesis about sexual reproduction, reflecting the absence of any selective effects, is that of panmictic reproduction. With respect to the organism considered, this means regular Mendelian segregation, no viability selection, no differences within female and male gamete production, and random fusion of gametes to zygotic offspring. This hypothesis is contrasted with the obtained genotypic structures among the offspring as follows:

We denote the relative frequency of an ordered genotype B_iB_j by P_{ij}, the relative frequency of an allele B_i by p_i, and indicate the corresponding offspring frequencies by primes. Then, the hypothesis of panmictic reproduction, in particular, requires $P'_{ij}=p_ip_j$ for all ordered offspring genotypes. An expected offspring structure based on the seed orchard structure is already presented in Table 1. The application of the standard χ^2-test of goodness of fit to each of the different offspring generations (B_1B_3, B_3B_1, B_3B_3 pooled) with the hypothesis of panmictic reproduction results in $P<0.001$ (***) for each of the flowering periods 1974, 1975 and 1976. These values imply rejection of the hypothesis of panmictic reproduction for each of the 3 flowering periods.

Table 1. Genotype frequencies at the LAP-B gene locus among parental clones and offspring from three flowering periods, and the expected frequencies under panmixia

LAP-genotype	Frequencies among parental clones	Expected offspring frequencies under panmictic reproduction	Offspring frequencies from flowering period		
			1974	1975	1976
B_1B_1	0	7.9	9	9	1
B_2B_2	27	490.0	444	528	522
B_3B_3	0	0.1	0	1	1
B_1B_2	8	62.2	96	41	30
B_2B_1		62.2	44	22	36
B_1B_3	0	1.0	8	3	5
B_3B_1		1.0	0	1	2
B_2B_3	1	7.8	30	23	26
B_3B_2		7.8	9	12	17
Total	36	640	640	640	640

Table 2. Allelic frequencies at the LAP-B gene locus among parental clones and offspring from three flowering periods, and the expected frequencies under panmixia

LAP-allele	Frequencies among parental clones	Expected offspring frequencies under panmictic reproduction	Offspring frequencies from flowering period		
			1974	1975	1976
B_1	8	142.2	166	85	75
B_2	63	1120.0	1067	1154	1153
B_3	1	17.8	47	41	52
Total	72	1280	1280	1280	1280

The next question is whether the significant deviations from the genotypic structure expected under panmictic reproduction reflect the same effect and differ only randomly between the observed flowering periods. Application of a homogeneity test for the 3 genotypic offspring structures leads to $P<0.001$ (***) and thus to rejection of the hypothesis of a homogeneous environmental influence on reproduction in various flowering periods.

A change in the genotypic structures from the adult generation to their offspring does not necessarily lead to a change in allelic frequencies. Thus, allelic frequencies should be investigated additionally. Under the hypothesis of panmictic reproduction, $p'_i=p_i$ with no allelic change must be expected for all alleles. Table 2 contains the pertinent observations and expectations.

χ^2-tests of goodness of fit for the hypothesis of panmictic reproduction yield $P<0.001$ (***) for each of the flowering periods separately and thus rejection of the hypothesis of no allelic change. A homogeneity test also delivers $P<0.001$ (***) and rejection of a temporarily homogeneous deviation from allelic constancy.

All presented tests confirm the occurrence of significant nonrandom deviations of genotypic and allelic structures at the LAP-B gene locus from those expected under panmictic reproduction. Furthermore, the deviations show varying directions and quantities for different flowering periods. These results indicate at least one of the following functions of LAP-B:

i) The LAP-B gene locus participates directly in an adaptive reproductive function within sexual reproduction.
ii) The LAP-B gene locus contributes to differential survival abilities between zygotic formation and census of the seed.
iii) The LAP-B gene locus is linked to at least one gene locus with a function according to i) or ii).

Reconsidering Table 1, an asymmetry within female and male allelic contributions to genotypes of the offspring generations is already obvious. (Compare for example the frequencies of B_1B_2 and B_2B_1 from flowering period 1974 and 1975 or B_2B_3 and B_3B_2 from 1976). Table 3 contains the total number of allelic contributions to the observed offspring via female and male gametes, respectively. These data were computed by counting all observed offspring which received an allele B_i from a female or, in the other case, from a male gamete.

Each structure of female and male allelic contributions is compared with the expected structure under panmictic reproduction using the χ^2-test of goodness of fit. The results are:

$P < 0.001$ (***) for the female contribution from each of the flowering periods 1974 and 1976, $P < 0.05$ (*) for the female contributions from flowering period 1975, and $P < 0.001$ (***) for the male contributions of each of the flowering periods 1974, 1975 and 1976. Thus deviations of the sex-specific contributions from panmictic expectations are significant.

A comparison between female and male contributions within each flowering period results in $P < 0.001$ (***) for flowering period 1974, $P < 0.01$ (**) for 1975, and $P > 0.05$ (n.s.) for flowering period 1976.

The differences between female and male contributions are significant except among offspring from flowering period 1976. These results clearly indicate the occurrence of sexual asymmetry, so that hypotheses about a selective function of LAP-B must take sex-specific components into account.

2 Testing the hypothesis of sexually asymmetric fertility selection and partial self-fertilization

The reproductive phase from the flowering of the seed orchard until census of the seed can be divided into three main reproductive parts, each underlying specific selectionary effects:

i) Differential production of ovules and pollen (sexually asymmetric fertility selection).
ii) Selective fusion of the produced female and male gametes including partial self-fertilization and prezygotic incompatibilities (mating system components).
iii) Postzygotic viability selection until census takes place.

Table 3. Female and male allelic contributions to offspring from three flowering periods and the expected frequencies under panmixia

LAP-allele	Expectation under panmictic reproduction	Offspring frequencies from flowering period					
		1974		1975		1976	
		♀	♂	♀	♂	♀	♂
B_1	71.1	113	53	53	32	36	39
B_2	560.0	518	549	573	581	584	569
B_3	8.9	9	38	14	27	20	32
Total	640	640	640	640	640	640	640

First we hypothesize (i) as the main selective component, assuming Mendelian segregation, random gametic fusion and equal zygotic viabilities. Sexually asymmetric gametic contributions to the offspring generations are already shown in Section 1 of this chapter. Thus, fertility selection is assumed to act asymmetrically in the gametic sexes. Nevertheless, random gametic fusion to zygotes without any restrictions on fertilities always results in the following sexually asymmetric multiplicative structure (indicated by (m)) immediately after zygote formation:

$$P_{kl}^{(m)'} = p_k^{♀'} \cdot p_l^{♂'} \text{ for all k, l} \qquad (*)$$

For any genotypic structure, the corresponding multiplicative structure can be determined by (*) with $p_k^{♀'} = \sum_j P'_{kj}$ and $p_l^{♂'} = \sum_i P'_{il}$.

If compared with corresponding Hardy-Weinberg proportions, this asymmetric multiplicative structure generally shows a homozygote deficiency and an excess in the sum of all heterozygotes. These deviations vanish only if female and male gametic contributions to zygotes are equal e.g. as under sexually symmetrical fertilities. Ziehe and Gregorius (1981) discussed the range of possibly occurring deviations from Hardy-Weinberg proportions in more detail.

Agreement with structure (*) can be used as an indicator of random gametic association to zygotes. Table 4 contains the observed genotypic structures among the offspring and the corresponding multiplicative structure (*) for each flowering period. A χ^2-test of goodness of fit (B_1B_3, B_3B_1 and B_3B_3 pooled) for each flowering period leads to $P > 0.05$ (n.s.) for flowering period 1974, $P < 0.001$ (***) for 1975, and $P > 0.05$ (n.s.) for flowering period 1976.

Table 4. Comparison between the observed offspring structures at the LAP-B gene locus and the corresponding sexually asymmetrical multiplicative structure expected under random gametic fusion

LAP-genotype	Offspring frequencies from flowering period					
	1974		1975		1976	
	Observed	Expected multiplicative structure	Observed	Expected multiplicative structure	Observed	Expected multiplicative structure
B_1B_1	9	9.4	9	2.7	1	2.2
B_2B_2	444	444.3	528	520.2	522	519.2
B_3B_3	–	0.5	1	0.6	1	1.0
B_1B_2	96	96.9	41	48.1	30	32.0
B_2B_1	44	42.9	22	28.7	36	35.6
B_1B_3	8	6.7	3	2.2	5	1.8
B_3B_1	–	0.7	1	0.7	2	1.2
B_2B_3	30	30.8	23	24.2	26	29.2
B_3B_2	9	7.7	12	12.7	17	17.8

Acceptance of the hypothesis of a multiplicative structure within the offspring generation holds for the flowering periods 1974 and 1976; significant deviations occur for 1975; they will be discussed later on.

Thus for flowering periods 1974 and 1976, the deviation of the genotypic structures from those expected under panmictic reproduction can be well explained by differential ovule and pollen production. Moreover, both selective fusion of female and male gametes and viability selection among zygotes generally tend to disturb multiplicative structures, with the exception of some specialized selection models, e.g. in which viability coefficients are factorizable into multiplicative allelic components. These facts suggest that the hypothesis of sexually asymmetric fertility selection can be used as a preliminary hypothesis for the flowering periods 1974 and 1976. Further consequences for these flowering periods will be drawn in Section 3.

For monitoring partial self-fertilization as an additional effect the following definitions are required: for an adult genotype B_kB_l and a given flowering period, let φ_{kl} be the average number of produced ovules which are actually fertilized during this flowering period. It is assumed that φ_{kl} does not depend on whether the corresponding genotype once received the aleles B_k and B_l via a female or a male gamete, so that $\varphi_{kl}=\varphi_{lk}$. Let σ denote the proportion of ovules which are self-fertilized, whereas the remaining portion $(1-\sigma)$ is fertilized at random by pollen from the pollen cloud to which all genotypes contribute all pollen not reserved for self-fertilization. Then, as described in Ziehe (1982a), deviations from a multiplicative offspring structure due to the additional effect of self-fertilization occur and may be written in the following way:

$$P_{kk}^{(m,\sigma)\prime}-P_{kk}^{(m)\prime}=\sigma\left[\tfrac{1}{2}\,p_k^{♀\prime}-p_k^{♀\prime 2}+\tfrac{1}{2}\,P_{kk}\,\varphi_{kk}/\bar{\varphi}\right]$$

and

$$P_{kl}^{(m,\sigma)\prime}-P_{kl}^{(m)\prime}=-\sigma\left[p_k^{♀\prime}\,p_l^{♀\prime}-\tfrac{1}{4}(P_{kl}+P_{lk})\,\varphi_{kl}/\bar{\varphi}\right]$$

for $k\neq l$,

where $P_{kl}^{(m,\sigma)}$ denotes genotypic frequencies expected under additional self-fertilization σ and $\bar{\varphi}$ is the mean genotypic ovule production.

The given equations imply the following:

1) If $\sigma=0$, self-fertilization occurs only randomly. The deviations clearly vanish.

2) If $\sigma>0$, the above deviations reflecting the additional self-fertilization effect are independent of genotypic contributions to the pollen cloud. Thus the genotypic pollen production exceeding that needed for reserved self-fertilization has no influence on the deviation from a multiplicative structure due to partial self-fertilization.

3) The heterozygotic offspring deviations require the following symmetry:

$$P_{kl}^{(m,\sigma)\prime}-P_{kl}^{(m)\prime}=P_{lk}^{(m,\sigma)\prime}-P_{lk}^{(m)\prime}.$$

A significant difference between these deviations leads to rejection of the hypothesis of a combined fertility and self-fertilization influence.

For equal ovule production, it can be shown that a homozygote excess and a deficiency in the sum of all heterozygotes relative to the multiplicative structure occur. Nevertheless, even under high self-fertilization probabilities, even insignificant deviations from the multiplicative structure can occur if the ovule production of single heterozygote genotypes notably exceeds the mean or if the ovule production of homozygote genotypes notably falls short of the mean.

Comparing now the observed offspring numbers from flowering period 1975 with their corresponding multiplicative structure, an homozygote excess for B_1B_1

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