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Reproductive systems in conifer seed orchards

3. Female and male fitnesses of individual clones realized in seeds of *Pinus sylvestris* L.

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Summary. In order to quantify female and male fitness values of clones in a *Pinus sylvestris* L. seed orchard, multilocus-genotypes of parental clones were compared with those of open pollinated seeds in the bulked orchard crop. Female and male contributions to individual seeds were distinguished by observing enzyme gene loci active in both endosperm and embryo tissue. Seed probes from two successive flowering periods were surveyed. The female and male fitnesses of five parental clones measured relative to the population mean were derived. The contributions of four clones were found to be sexually asymmetric. One clone, for instance, made exclusively female contributions in one flowering period. Variations existed in fitness values between clones. Deviations in sex specificity occurred between flowering periods: one clone contributed asymmetrically in both periods, but in sexually reversed proportions. A method to comprehensively quantify and illustrate the observed phenomena is proposed.

Key words: Reproductive selection – Clonal fitness values – Sexual asymmetry – Enzyme gene loci – Pine seed orchard

Introduction

During reproduction of Scots pine (*Pinus sylvestris* L.), three prevailing phenomena are expected to affect the parent-offspring relationship: differential production of female and male gametes (fertility selection), mating systems with non-random fusion of gametes (assortative mating, inbreeding), and differential viabilities of the zygotes (viability selection) (Müller-Starck et al. 1983). Prezygotic incompatibilities have not been observed in pine species.

Previous gene marker studies in pine species deal mainly with components of the mating system (Rudin and Lindgren 1977; Adams and Joly 1980; Moran et al. 1980; Shen et al. 1981; Chung 1981; Friedman and Adams 1982; Squillace and Goddard 1982; Müller-Starck 1979, 1982a, b). The existence of fertility selection and partial self-fertilization has been demonstrated by Müller-Starck (1982a) and Müller-Starck et al. (1983). Associations between differing fertilities and specific enzyme genotypes have been revealed by Linhart et al. (1979).

In the present paper, a method of characterizing reproduction is proposed and examined which consists of estimating the reproductive efficiency (fitness values) of individual genotypes. By comparing the genotypic structure of a well-defined parental population with those of their bulked offspring at the stage of fully-developed seeds, the object of this study is to evaluate female and male fitness components of parental genotypes realized in annual reproduction periods. Consequently, all fitnesses refer to the successful gametes, i.e. gametes which are incorporated into zygotes. The parental fitnesses in earlier reproduction periods or non-reproductive stages are not taken into account. All fitness values included in this study are estimated and illustrated relative to the population mean.

We chose an experimental situation in which the environment is more uniform than that found in natural forest tree populations and one in which the reproductive potential of the parental population can be intensively used to establish future tree generations. This is the case in breeding populations, such as clonal seed orchards. These populations consist of a limited number of selected parental trees (clones) which are represented in the orchard by numerous genotypically identical replicates. In many pine species, including Scots pine, the reproductive potential will increasingly become focussed on seed orchards because their bulked crop usually is favoured as compared to the crop from natural forest populations.

Materials and methods

Studied population

Scots pine clonal seed orchard at the ages of 16 (1975) and 17 (1976) years; population size $N = 900$, number of clones $L = 36$ with an average number of 25 individuals per clone (ranging in the case of the studied clones between 24 and 26); isolated location from other pines, in a large area with spruce and deciduous leaf tree stands. For further details see Müller-Starck (1982b).

Seed samples

Random samples of 640 seeds each from the harvested bulked orchard crop consisting of cones from all clones of two succeeding flowering periods.

Identification of genotypes

Multilocus-genotypes of parental clones and the seeds in the crop sample have been identified in previous studies (Müller-Starck 1982a, b) by electrophoretically assaying the enzyme systems leucine aminopeptidase and glutamate oxal acetate transaminase (LAP, EC 3.4.11.1 and GOT, EC 2.6.1.1). The genetic control of both systems was verified by analyses of seeds from controlled crossings. For a more detailed characterization of the LAP-system, see Müller-Starck and Hüttermann (1981). Four gene loci, LAP-B, GOT-A, -B, and -C, were scored simultaneously. The genotypes of the offspring were identified by the analysis of endosperm (♀) and corresponding embryos (♀♂) of each individual seed, so that the female and male contributions could be detected and determined unequivocally as an ordered pair (Müller 1976). Clones which carry unique alleles or unique multilocus-combinations can then be identified as female and/or male parents in orchard offspring.

Estimation of fitness values

The term "fitness" as used here corresponds to the Darwinian concept of fitness and is justified and discussed in detail by Gregorius (1982): fitness of an individual organism is the number of successful gametes this individual produces during its life time. Fitness of clones or genotypes is defined as the average fitness of a corresponding cohort. In the present paper, those fitness values are investigated which refer to single annual reproductive periods (annual realized generative fitness values).

Fitness values

For a clone of type i, the clonal values of female fitness $w_i^{♀}$, and male fitness, $w_i^{♂}$, designate the average number of successful female and male gametes which are produced during a certain reproductive period by each individual of this clone, i.e. incorporated into zygotes (Gregorius and Ross 1981). Since the total amount of zygotic production is unknown, only relative fitness values $w_i^{♀}/\bar{w}^{♀}$ and $w_i^{♂}/\bar{w}^{♂}$ are used, where $\bar{w}^{♀}$ and $\bar{w}^{♂}$ denote population means (compare Müller-Starck et al. 1983). Assuming that pollen emigration and immigration is negligible, clearly $\bar{w}^{♀} = \bar{w}^{♂} = w_\Sigma/N$, where w_Σ is the total number of zygotes produced by the seed orchard and N is the number of individuals in the population. Functional sexes F_i (Ross and Gregorius 1983) of single clones measure the relative degree of female function and are defined according to $F_i = w_i^{♀}/[w_i^{♀} + w_i^{♂}]$. Thus, F_i varies between 0 and 1, where $F_i = 0$ holds for a clonal type with reproduction effective exclusively via ovule contribution, and $F_i = 1$ holds for those which contribute to the offspring solely via pollen.

Assumptions required

Firstly, since fitness becomes manifest in the zygotes, the genotypic survival rates between the zygote stage and that of fully developed seeds is assumed to be approximately identical for all zygotes. That is, it is assumed that each zygote has the same probability, say s $(0 < s \leqq 1)$, to reach the census state of the seed. Accordingly, the average female or male contribution of individuals of a particular clone to the seed is identical to the clonal female or male fitness reduced by the factor s, and thus the corresponding relative contributions and the relative fitnesses coincide. The assumption of a genotypically ineffective viability selection is supported by previous studies (Müller-Starck 1982a, b; Müller-Starck et al. 1983).

The second assumption is that alleles of a particular heterozygous clone should not show deviations from a Mendelian 1 : 1 segregation within zygote contributions. This excludes reproductive effects such as gametic incompatibilities.

The model

Consider an allele which is present in heterozygous form in a single clone only, and denote by $p_i^{♀}$ the relative frequency of this allele among the female contributions to the seed crop. Assuming undistorted (Mendelian) gametic segregation with respect to zygotic contributions, the estimated total number of female contributions of this clone to zygotic seeds is $(2p_i^{♀})w_\Sigma$, whereas the contributions to seeds at census must be reduced to $(2p_i^{♀})w_\Sigma \cdot s$. Letting N_i be the number of individuals of clonal type i and $w_i^{♀}$ be the clonal female fitness, then the mean number of female contributions descending from i-type individuals to the seeds at census is $N_i w_i^{♀} \cdot s$, so that $2p_i^{♀} w_\Sigma \cdot s = w_i^{♀} N_i \cdot s$ is expected to hold. If all clones consist of the same number of individuals (so that $N_i = N/L$ with L = number of clones), then since $w_\Sigma/N = \bar{w}^{♀}$, the above equation yields for the estimated relative female fitness of the corresponding clones $w_i^{♀}/\bar{w}^{♀} = 2\,L\,p_i^{♀}$. The equivalent male fitness with corresponding definition of $p_i^{♂}$ is: $w_i^{♂}/\bar{w}^{♂} = 2\,L\,p_i^{♂}$.

Results and discussion

Gametic contributions in seeds

Gametes which can be assigned uniquely to certain clones are surveyed in Table 1.

In each of the two flowering periods, the observed female frequencies differed substantially from the respective male ones: applying a 2×2 homogeneity test, 6 out of 10 of these deviations are statistically significant (Table 1). Considering clone RD 1 in flowering period 1976, seeds with its female contribution unique to RD 1 are frequently represented, whereas those with the corresponding male contribution are not present in the studied seed probe. In addition, the female contribution descending from CH 4 in 1975 is 5.3 times as frequent as its male contribution. The opposite relationship holds for CH 14 in the period 1976, in that the frequency of

Table 1. Genotypes and observed numbers of gametes in the offspring which can be assigned to certain clones within two flowering periods

Clone					Unique gametic genotypes				Observed nos. in seed samples of size 640 from two flowering periods and respective χ^2-values for homogeneity tests					
Designation	Genotypes (unordered)								1975			1976		
	>LAP-B	>GOT-A	>GOT-B	>GOT-C	LAP-B	GOT-A	GOT-B	GOT-C	♀	♂	χ^2	♀	♂	χ^2
CH 4	2 2	1 2	3 5	3 3	2 2	1 1	3 5	3 3	8 8 } 16	1 2 } 3	9.03**	8 8 } 16	8 5 } 13	0.32 n.s.
CH 9	2 2	2 2	1 3	3 3	2	2	1	3	6	20	7.69**	17	10	1.85 n.s.
CH 14	2 2	2 2	3 7	3 3	2	2	7	3	5	15	5.08*	5	22	10.93***
RD 1	2 2	2 2	5 5	1 2	2	2	5	2	15	3	8.11**	15	0	15.18***
RD 4	2 2	2 2	2 5	3 3	2	2	2	3	7	11	0.90 n.s.	5	13	3.61 n.s

* $0.05 > P \geqq 0.01$; ** $0.01 > P \geqq 0.001$; *** $P < 0.001$;
n.s. = not significant

Table 2. Relative fitness values and functional sexes (F_i) of particular clones estimated for two flowering periods

Designation of clone	Flowering period 1975				Flowering period 1976			
	Relative fitness values				Relative fitness values			
	♀	♂	♀♂	F_i	♀	♂	♀♂	F_i
CH 4	1.80	0.34	1.07	0.84	1.80	1.46	1.63	0.55
CH 9	0.68	2.25	1.46	0.30	1.91	1.13	1.52	0.63
CH 14	0.56	1.69	1.13	0.25	0.56	2.48	1.52	0.19
RD 1	1.69	0.34	1.01	0.83	1.69	0	0.84	1
RD 4	0.79	1.24	1.01	0.39	0.56	1.46	1.01	0.28

seeds with its male contribution is 4.4 times its corresponding female contribution.

Male and female contributions of clones also vary between seed crops. Deviations are pronounced for the gametic type descending from CH 4 with respect to its male gametic representation; a change in sexually opposite directions is clearly indicated for the gametes descending from clone CH 9.

Relative female and male fitnesses

The estimated fitness values of the five clones are summarized in Table 2. They are calculated according to the above formula by obtaining the relative frequencies $p_i^{♀}$ and $p_i^{♂}$ from the female and male gametic observations of Table 1 divided by the actual sample size. Table 2 also contains estimates of the relative fitness (compare Müller-Starck et al. 1983) which takes into account the sum of the absolute female and male fitness relative to the corresponding population mean. Since the total fitness w_i is defined as the number of *all* successful gametes, i.e. $w_i = w_i^{♀} + w_i^{♂}$, and since $\bar{w}^{♀} = \bar{w}^{♂} = \bar{w}$, the relative fitness is given by $w_i/(2\bar{w})$ and is thus identical to the arithmetic mean of relative female and relative male fitness. As an additional parameter to reflect the degree of female functioning and thus the sexual asymmetry in fitness values, functional sexes $F_i = w_i^{♀}/(w_i^{♀} + w_i^{♂})$ are calculated for each clone.

The given fitness values reveal considerable inferiority and superiority among the clones: over the two reproduction periods the female fitnesses of the clones range between 0.56 and 1.91, the male between 0 and 2.48. Environmental and/or ontogenetic effects are indicated by large differences between the two flowering periods. Superiority in the total fitness of clones is noticeable for CH 9 in both periods and also for CH 4 and CH 14 in 1976. Inferiority is indicated in the case of the clone RD 1 in 1976.

The functional sex of a clone, which denotes the proportion of total fitness due to female contributions, might be expected to be approximately 0.5 for each clone of a monoecious species such as *Pinus sylvestris*. However, estimates of functional sex values deviated clearly from this expectation, ranging between 0.19 and 1. As can be seen, for instance in the case of the clones CH 9 and CH 4, the sex function of clones can change substantially from one year to the next. As regards changes from 1975 to 1976, clones RD 1 and CH 9 became less effective as male parents (i.e. F_i increased), while the remaining clones became more effective. All clones except CH 9 maintain their predominant determination as males or females over both reproduction periods. The question remains open as to how much total fitness and sex function are under genetic control.

Illustration of clonal fitness values

The results of the investigation of female and male clonal fitnesses are illustrated graphically in Fig. 1. The coordinate axes are scaled with respect to the population means for each of the reproductive periods. Arrows indicate the changes in fitness components between the flowering periods 1975 and 1976. The purpose of the diagram is a comprehensive demonstration of reproductive characteristics.

Fitness is defined as the sum of female and male gametic fitnesses. Thus, the straight line connecting $(0; 2\bar{w}^{♂})$ via $(\bar{w}^{♀}, \bar{w}^{♂})$ with $(2\bar{w}^{♀}; 0)$ represents points with mean population fitness. With the exception of RD 1 for flowering period 1976, the sampled clones possess above-average female and male fitness values. In the case of the clones CH 4, CH 9, and CH 14, the gametic fitnesses exceed the mean population fitness by more than 50 percent of the population mean.

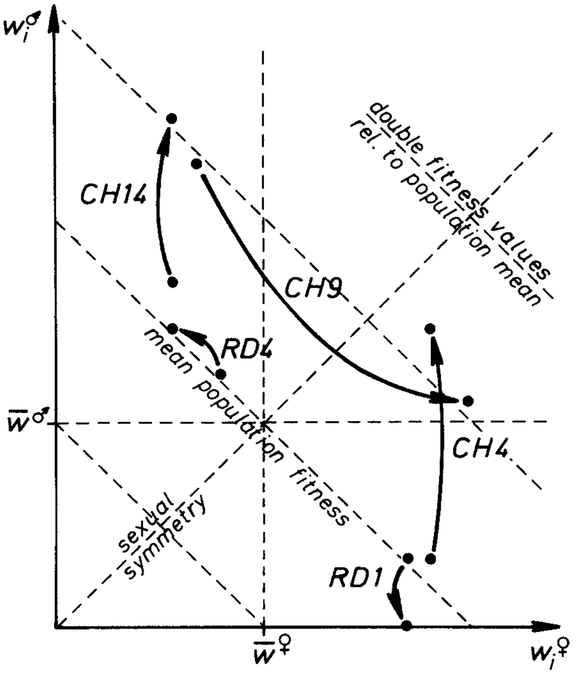

Fig. 1. Female and male gametic fitnesses of particular clones in two subsequent flowering periods (1975 → 1976)

Symmetry in female and male reproductive efficiency is reflected by the identity line, i.e. $w^{♀} = w^{♂}$. All clones deviate for both reproduction periods substantially from sexual symmetry as indicated also by the clonal functional sexes (Table 2). As pointed out before, changes occur towards both female and male fitnesses and are extreme in the case of the clone CH 9.

Concluding remarks

The obtained results indicate that the occurrence of selection during reproduction acts asymmetrically in the female and male sex function of single clones. Such selection was previously confirmed by statistical tests in the case of genotypes at the LAP-B gene locus by comparing allelic structures among parents and offspring (Müller-Starck et al. 1983). One consequence of the observed asymmetries concerns application of standard tests for revealing inbreeding: sexually asymmetrical selection can reduce the proportion of homozygotes in a population relative to the Hardy-Weinberg proportions and thus potentially mask inbreeding effects (Ziehe 1982 a). Those classical standard tests which are based on inbreeding as the unique non-random factor influencing offspring genotypic structures cannot, therefore, be applied. For examples of sexually asymmetrical selection counterbalancing inbreeding effects see Ziehe (1982 a, b) and Müller-Starck (1982 a).

A consequence which may also be valid for natural populations is that selection in reproduction acts intensively, even in the case of a population where all genotypes are flowering.

In the above-mentioned examples, the female and male fitness values of single clones deviate from their population mean by a factor between zero and 2.48. Also, the total fitnesses relative to the corresponding population mean vary within and between the annual reproduction periods. Annual effects are more pronounced in male than in female fitnesses. Ontogenetic influences may be involved in such changes although rapid effects between two years are rather improbable for long-lived species. More likely, environmentally dependent genetic selection with respect to sex-specific fitness components of single clones can be assumed. It is quite probable that selection during reproduction also affects ecologically and economically relevant characters of the offspring population, for example via linkage and epistatic effects. This concerns breeding populations such as clonal seed orchards in particular since the purpose is to achieve maximal phenotypic similarity between the original parental population and their offspring in corresponding age classes.

The obtained fitnesses suggest a certain form of presentation which comprehensively illustrates the detected phenomena. The proposed diagram can also serve as a criterion for the decision whether the bulked orchard harvests from different flowering periods should be handled separately or mixed in order to increase the average gametic contribution of single clones to the offspring. It may additionally yield criteria for the decision as to whether clones should stay in the breeding population or be removed or replaced because of too small reproductive efficiency. Further studies are required to decide to what degree the reproductive system in a seed orchard can be expected to maintain genetic characteristics of the parental population.

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