MALE FERTILITY VARIATION AND NON-RANDOM SEGREGATION IN POLLEN MIX CROSSES OF *PICEA ABIES*

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ABSTRACT

Grafts of the same clones of *Picea abies* were pollinated with mixtures of pollen from 2 – 3 clones at a southern and a northern locality in Sweden (lat. 60° and 65°). The pollen parents were both of southern and northern Swedish origin. The contributions of offspring from each of the pollen parents in the mixes were determined by isozyme analyses.

There were large differences in paternal success between the pollen parents, indicating pollen competition. These effects were in most cases consistent across several maternal parents and between the two localities. They were also verified by measurements of frost damage and height growth in comparisons of pollen mix progenies and their constituent full-sib families. Segregation distortions for paternal gametes were observed in more than 50 % of the identified matings from the pollen mixes, but not for maternal gametes. In one mating the distortion was most likely due to selective fertilization or post-zygotic viability selections. The non-random segregations could be one factor contributing to the differences in paternal reproductive success.

Key words: pollen competition, isozymes, segregation distortion, Norway spruce

INTRODUCTION

Variability in male reproductive success between individual coniferous forest trees has been demonstrated both in stands (MÜLLER 1977; XIE and KNOWLES 1992), in seed orchards (SCHOEN and STEWART 1987; ERICKSON and ADAMS 1989) and after controlled crosses with mixtures of pollen from different clones (MORAN and GRIFFIN 1985; CHELIAK *et al.* 1987; APSIT *et al.* 1989). Under natural and seed orchard conditions, parts of these variations can be due to variability the numbers of pollen produced (SKRØPPA and TUTTUREN 1985) or in the timing and duration of the period of pollen release (ERIKSSON *et al.* 1973). However, if differential paternal success occurs after controlled pollinations with mixes of equal numbers of viable pollen from a set of males, then either genetic processes taking place during the sexual reproductive cycle must be involved or environmental factors may condition the pollen from different males differently prior to collection. Possible genetic events are prezygotic pollen competition, which may cause selection at the male gametophytic level, gametophytic mating (fusion) incompatibility (GILLET and GREGORIUS 1992), and postzygotic embryo abortion (MORAN and GRIFFIN 1985; NAKAMURA and WHEELER 1992).

Under the assumption of regular Mendelian segregation, random fertilization and lack of postzygotic viability selection, all gametic types from each specific male are expected to be equally represented among its successful gametes. In addition to non-regular meiosis and post-zygotic viability selection, segregation distortion may occur as a result of differences in viability or fertilizing ability among the gametic types (GILLET and GREGORIUS 1992). Thus, if specific male gametic types have intra- or interclonal selective advantages, segregation distortions may be one factor contributing to variations in male reproductive success (MORAN and GRIFFIN 1985). Segregation distortions have been observed in isozymes studies with conifers, although not frequently (e. g. STRAUSS and CONKLE 1986; MUONA *et al.* 1987; ADAMS *et al.* 1990).

This report presents results of crosses with pollen mixes on Norway spruce (*Picea abies* (L.) KARST.) grafts of the same clones located at different latitudes in Sweden. The objective was to study competition between pollen from males with assumedly different adaptive properties and the effects of performing

crosses at two sites with different climates.

MATERIALS AND METHODS

Crosses performed

The same sets of controlled crosses were performed on grafts in 1989 in a Norway spruce seed orchard in northern Sweden (Lillpite, lat. 65.4°) and in a clonal archive in southern Sweden (Röskär, lat. 59.4°). Some of the pollen for the crosses was obtained from another seed orchard in southern Sweden (Saleby, lat. 58.4°). Twelve seed orchard clones of northern (N) Swedish origins grafted both at Lillpite and Röskär were divided into four groups. The members of each group were pollinated with a specific pollen mix which consisted of equal weight units of fresh pollen from two or three clones. In each pollen mix, at least one of the pollen parents was of northern Swedish origin (lat. 64.3 – 66.7°), collected from grafts at Röskär, and at least one was of southern (S) Swedish origin (lat. 58.5 – 59.9°), collected from grafts at Saleby. The compositions of the four pollen mixes are shown in Table 1. All clones involved had known isozyme genotypes at 14 different loci from earlier studies (PAULE *et al.* 1993). However, for some of the clones this knowledge was based on only a single graft, and evident identification errors had to be corrected. The males in each pollen mix were selected so that their gametes could be uniquely identified. However, one gametic type in each of MIX 3 and MIX 4 could be produced by two of the males in the pollen mix. In addition to the pollen mix crosses, full-sib families were produced by pollinations of the same maternal clones with each individual pollen type in the pollen mix. No tests were made of the viability of pollen from the different parents.

Each cross was repeated on two grafts of each maternal clone at both sites. At Röskär, pollinations were performed between May 7 and May 12. Each graft was pollinated on two occasions (days). Due to unusual high temperatures and fast development, some of the female strobili were close to the end of their receptive period at the time of pollination. For this reason no seeds were obtained from some of the crosses at Röskär. At Lillpite, the period of receptivity of the female strobili took place more than two weeks later, and pollinations were performed in the period of May 25 – 27. The isolation bags were removed three weeks after the pollinations, and the cones were harvested in the first week of September.

Table 1 The composition of the four pollen mixes and the number of clones (all northern) that were pollinated by each mix. Origin of male parents S: southern, N: northern

Pollen mix	Pollen parents	# of maternal parents
MIX 1	BD 1012 N, P 3017 S, T 2004 S	4
MIX 2	AC 2003 N, BD 1006 N, T 3018 S	4
MIX 3	AC 1013 N, S 3097 S, S 3105 S	4
MIX 4	BD 200x[1)], R 1003 S	3

[1)] This clone is of northern origin, but its exact identity is not known.

Electrophoretic methods

Electrophoretic analyses were performed for the following loci, with the number of alleles observed per locus in parentheses: *Lap-1* (4), *Lap-2* (4), *Got-2* (2), *Gdh* (3), *Aco* (2), *Pgm-2* (3), *Pgi-2* (5), *Mdh-2* (2), *Idh-1* (2), *F-Est* (2). The procedures of electrophoresis and staining were as described by MUONA *et al.* (1987), who also verified that the banding patterns could be associated to genetic loci for Norway spruce. If all males in a pollen mix were known to be homozygous for the same allele, that enzyme was not stained for in the crosses with this mix. Both embryo and endosperm were analysed from as many as 100 seeds (Tables 2 – 5), if available, from each pollen mix family. The haploid paternal gamete was identified by comparing the diploid embryo genotype and the maternal endosperm. The paternal parentage of each fertilized embryo was identified by multilocus gametes unique to each male. Mix 3 and Mix 4 both contained one gametic type that could be produced by two males. These gametes were assigned to the males according to the ratios of the other gametes. Loci that could not be safely decided upon when evaluating a paternal gamete were excluded.

Calculations

An index of the reproductive success of each male (RS) was calculated by multiplying the average relative paternal contribution at the two sites by the number of males in the pollen mix. The value of this index should equal 1 when the paternal contributions are equally frequent.

Allelic frequencies at individual loci were calculated for successful male gametes from each male in the four pollen mixes. Frequencies were calculated both for each individual cross and for the pooled contribution from each male in all crosses with the same pollen mix. Allelic frequencies were similarly calculated for female gametes in the identified crosses with each male and with the pooled pollen mix.

Calculations of two-way distributions of associations of female and male gametic types (fertilization distributions, GILLET and GREGORIUS 1992) were attempted for loci where both parents were heterozygous.

χ^2 tests were used to test the homogeneity of observed frequencies in male contributions across females and across the two crossing locations. They were also used to test deviations from expected 1:1 segregation ratios and the homogeneity of such deviations.

Freezing tests

Seeds from families that had more than 40 filled seeds were germinated, and seedlings were cultivated during the summer of 1990. Details of plant cultivation and freezing tests that were performed during the period of hardiness development in that autumn are described by SKRØPPA *et al.* (in prep.). Six weeks after the freezing test, the damage of each individual seedling was classified according to the scale 0 = no visible damage, 1 – 10 = brown or discoloured needles in ten percent classes, 11 = all needles completely brown, seedling dead. Mean damage values of families are reported here. Before testing differences between families by analyses of variance, arcsine transformations were made of plot mean values (SKRØPPA *et al.* in prep).

RESULTS

Paternity analyses

Paternal success in polycrosses

The male parents contributed their genes very differently to the seeds in the families from the pollen mix crosses, as shown by the number of seeds sired by each male, presented in Tables 2 – 5. The χ^2 test of the hypothesis of equal male contributions showed significant deviations at the 0.1 % level in all maternal families from crosses with MIX 1, MIX 2 and MIX 4 at both sites. In MIX 3, significant differences were found in two of the six maternal families (p = 0.02). When all families were pooled, however, the contributions from the three male parents differed significantly (p = 0.001) also in this pollen mix.

Considerable variation was present between the reproductive success indices of the males in MIX 1, MIX 2 and MIX 4. Four of the southern males had an index above 1 and two below 1. For the northern males, one had an index above 1 and four below 1. Thus, there is an indication that southern males may be more reproductively successful.

Homogeneity among maternal parents

The χ^2 tests of homogeneity of the paternal contributions among the maternal parents showed no significance in MIX 1, MIX 2 and MIX 3 at any of the two sites (p varies between 0.10 and 0.72). In MIX 4, with only two paternal parents, one significant heterogeneity was found for mother BD 2009 (p = 0.0004), although father R 1003 had a consistent higher RS for all female clones.

Homogeneity between crossing sites

A test for consistency of the paternal contribution across the two crossing sites could be tested in MIX 1 with 4 and in MIX 2 and MIX 3 with 2 common females, after pooling the number of seeds sired by each male at each site. No heterogeneity was present in MIX 1 and MIX 3 (p = 0.09 and p = 0.73). In MIX 2, some discrepancies between the two sites were found (p = 0.001). It is due to a considerably lower paternal contribution from the clone T 3018 at Lillpite than at Röskär.

Homogenity within clones

No differences were found in paternal contributions between identical crosses made on two different grafts of the clones BD 1004 (MIX 1) and AC 1008 (MIX 3) at Lillpite, as verified by the χ^2 tests (p = 0.09 and p = 0.78, respectively).

Segregation of isozymes

No significant deviations from the expected 1:1 ratio were found for female gametes in any of the 21 polycross families, based on the total pollen pool in each pollen mix.

Table 2 Number of seeds sired by each of the three male parents in MIX 1 and their reproductive success indices (RS). Origin of male parents S: southern, N: northern

♂ / ♀	Site	BD 1012 N	P 3017 S	T 2004 S	Sum
AC 2003	Röskär	8	63	29	100
AC 2003	Lillpite	6	66	28	100
AC 1001	Röskär	1	5	6	12
AC 1001	Lillpite	3	30	11	44
AC 1004	Röskär	3	25	30	58
AC 1004	Lillpite	5	61	40	106
AC 1011	Röskär	9	50	40	99
AC 1011	Lillpite	8	61	28	97
Sum		43	361	212	616
Percentage		7.0	58.6	34.5	100
RS		0.21	1.76	1.03	

Table 3 Number of seeds sired by each of the three male parents in MIX 2 and their reproductive success indices (RS). Origin of male parents S: southern, N: northern

♂ / ♀	Site	AC 2003 N	BD1006 N	T 3018 S	Sum
AC 1008	Röskär	67	7	11	85
AC 1008	Lillpite	75	0	5	80
AC 1013	Röskär	41	3	11	55
BD 1001	Röskär	4	2	1	7
BD 1001	Lillpite	77	6	1	84
BD 1012	Lillpite	75	4	8	87
Sum		339	22	37	398
Percentage		85.2	5.5	9.3	100
RS		2.56	0.16	0.28	

Table 4 Number of seeds sired by each of three male parents in MIX 3 and their reproductive success indices (RS). Origin of male parents S: southern, N: northern

♂ / ♀	Site	AC 1013 N	S 3097 S	S 3105 S	Sum
AC 1005	Röskär	10	14	9	33
AC 1005	Lillpite	27	21	42	90
AC 1008	Röskär	24	22	43	99
AC 1008	Lillpite	33	23	35	91
BD 2001	Lillpite	21	35	33	89
BD 2006	Lillpite	31	22	45	98
Sum		146	147	207	500
Percentage		29.2	29.4	41.4	100
RS		0.88	0.88	1.24	

The observed segregations of male gametes in the identified matings in the polycross families, pooled acrossthe female parents and crossing locations, differed significantly from the 1:1 ratio in 17 of the 31 cases of heterozygote males (Table 6). The number of loci with segregation distortion varied among the males, but for only two males did the segregation follow the 1:1 ratio at all of their heterozygous loci (two and three loci, respectively). All three males that were heterozygous at *Pgm-2* produced significant segregation distortions. For the *Pgi-2* locus significant segregation distortion was present in all but one of the possible seven cases (Table 6).

No relationship was found between the origin of the parental clone (N or S) and the frequency of non-random segregations of its male gametes (Table 6).

Homogeneity of segregation distortions

Tests were made of the homogeneity of the observed male segregations across maternal parents and between the two crossing locations whenever possible. Significant heterogeneity was found in two cases only, both of which involved the locus *Lap-2*. The paternal parent T 2004 in MIX 1 segregated close to the 1:1 ratio in crosses with two of the female parents, but not with the other two. Similarly, BD 200x in MIX 4 produced a deficit of allele 2 in the cross with BD 2007 at Lillpite, whilst the opposite was the case in the crosses with BD 2001 and BD 2009. The observed male segregation distortions were, apart from these two cases, consistent across the female parents and between the two crossing locations.

The small numbers of gametes in each class and/or homozygosity of one of the parents limited the information from fertilization distributions, which for each locus describes the two-way frequency distribution of associated gametic types in the fertilized seeds of each cross (GILLET and GREGORIUS 1992). One exception was the segregation at the locus *Pgi-2* in matings with the clone AC 2003, used both as a maternal parent with MIX 1 and as a paternal parent in MIX 2 (Table 7).

Consistent and significant deviations from regular segregation were present at the *Pgi-2* locus among AC 2003's male gametes at both sites. Its female gametes, however, showed segregation distortion at neither site. The male gametes of P 3017 showed significant segregation distortion at Röskär, but not at Lillpite, while AC 1008's female gametes showed no distortions. The allele 2 was favoured in both sets of crosses, but to a much larger extent when AC 2003 acted as the paternal parent. In that cross, the genotype 31 was at a strong disadvantage at both sites. The distributions of the male gametes for the two female alleles (female fertility distributions) differed significantly ($p < 0.001$), for both sites pooled they were 21:45 and 6:70 for the alleles 2 and 3, respectively (Table 7). In the cross where AC 2003 acted as the female parent, the segregation distributions also differed significantly ($p = 0.015$). However, this was due to the non-random segregation of the male parent P 3017.

Table 5 Number of seeds sired by each of the two male parents in MIX 4 and their reproductive success indices (RS). Origin of male parents S: southern, N: northern

♂ / ♀	Site	BD 200x N	R 1003 S	Sum
BD2001	Lillpite	20	86	106
BD2007	Lillpite	32	88	120
BD2009	Röskär	20	27	47
BD2009	Lillpite	24	76	100
Sum		96	277	373
Percent.		25.7	74.3	100
RS		0.51	1.48	

Success of full-sib family crosses

The pollen mix crosses generally produced a far larger amount of filled seeds and viable seedlings than did the individual full-sib family crosses. X-ray tests of samples of both types of families at both sites showed that the seed quality (filled seeds or seeds without damage) was generally lower at the southern site Röskär than at Lillpite. In some of the full-sib families only a few seeds germinated, and these families could consequently not be included in the freezing tests. However, several of the pollen parents that contributed poorly to the offspring generation in the pollen mix crosses succeeded in producing offspring in the full-sib family crosses with the same mothers. Some of the paternal parents, on the other hand, functioned poorly in both types of crosses.

In the MIX 1 crosses, sufficient numbers of seedlings were obtained from all three maternal parents in the full-sib family crosses. Filled seeds were produced in the individual crosses with the three paternal parents in MIX 2. However, only a few of the seeds from the crosses with pollen from BD 1006 germinated, and these families could not be included in the freezing tests. T 3018, which was verified by the isozymes to have low paternal success in the pollen mix, produced a sufficient number of seedlings

in two of the three full-sib family crosses at Lillpite. Similarly, in the MIX 3 crosses, AC 1013 did not produce a sufficient number of seedlings to be included in the freezing tests in any of its three full-sib family crosses. S 3105 produced a sufficient number of seedlings only in one of its three full-sib family crosses. In the MIX 4 crosses, viable seedlings were produced in the full-sib families involving the maternal parents BD 2009 and BD 2007 and the two pollen parents in the pollen mix.

The low paternal efficiency of some of the males could not be due to non-functioning pollen in the cases where the full-sib family crosses with the same pollen produced viable seedlings.

Table 6 Observed male gametic segregations at single loci. For each male and heterozygous locus two alleles and segregation ratio are shown. Significant deviations at the 5 % level from the expected 1:1 ratio are shown by an asterisk

Pollen mix Paternal parent	Sample size	*Pgi-2*	*Pgm-2*	*Lap-1*	*Lap-2*	*Gdh*	*Idh-1*	# of signif. distort.	# of heteroz. loci
MIX 1									
BD1012N	39	2:0.18* 3:0.82	2:1	3:0.46 4:0.54	2:1	3:1	2:1	1	2
P 3017S	341	4:0.60* 5:0.40	2:1	1:0.47 5:0.53	1:1	3:1	2:1	1	2
T 2004S	210	2:1	1:0.24* 2:0.76	3:1	1:0.298 2:0.71	1:1	2:1	2	3
MIX 2									
AC2003N	339	1:0.27* 2:0.73	2:1	2:1	2:0.44* 3:0.56	1:1	2:1	2	3
BD1006N	22	2:0.45 4:0.55	2:1	3:1	1:1	1:0.69 2:0.31	2:1	0	3
T 3018S	37	3:1	2:0.33* 3:0.67	3:1	3:0.74* 8:0.26	1:1	2:1	2	2
MIX 3									
AC1013N	145	3:0.40* 4:0.60	2:1	1:0.67* 5:0.33	3:1	1:1	1:0.27* 2:0.73	3	4
S 3097S	141	3:1	1:0.15* 2:0.85	3:0.67 5:0.33	1:0.21* 3:0.79	1:0.78* 2:0.22	2:1	4	5
S 3105S	207	2:1	3:1	1:0.47 3:0.53	2:1	1:1	2:1	0	2
MIX 4									
BD200xN	96	3:0.19* 4:0.81	2:1	1:0.54 3:0.46	2:0.44 3:0.56	1:1	2:1	1	3
R 1003S	277	2:0.39* 4:0.61	2:1	3:0.48 5:0.52	3:1	1:1	2:1	1	2
# signif. distortions		6	3	2	4	1	1	17	
# heterozyg. loci		7	3	7	5	2	1		31

[1] No deviation from 1:1 segregation was found for six paternal parents heterozygous at one of the loci *Aco, Got-2* and *F-Est* (not listed)

Freezing damage and seedling heights

The full-sib families having clone BD 1012 (N) as the common paternal parent were significantly ($p < 0.001$) more frost hardy and shorter than the full-sib families with the same maternal parent and either of the two southern paternal parents (Table 8). This fact, however, was not reflected in the pollen mix crosses. The pollen mix families of AC 2003, BD 1004 and BD 1011 had a very close resemblance to their component full-sib families involving the two southern clones as paternal parents (Table 8). This result was consistent across the two crossing locations for the families involving AC 2003 as the maternal parent,

which was the only orthogonal comparison that could be made (not shown). Similar results were shown by clone BD 2009 in crosses with MIX 4 and its two constituent males (Table 9), even though the differences for frost damage were significant only for the Röskär crosses (p = 0.01 and 0.26, respectively, for injuries and $p < 0.001$ for heights). The pollen mix families after crosses with MIX 2 performed similarly to the full-sib families involving AC 2003 as the paternal parent, whose competitive index is 2.6 (not shown).

The results from the freezing tests and height measurements and the results from the paternity analyses support each other and give consistent results concerning paternal success.

Table 7 Segregation at the locus Pgi-2 in pollen mix crosses involving the clone AC 2003 either as the maternal parent (MIX 1) or as the paternal parent (MIX 2). Crosses were performed both at Röskär and at Lillpite

MIX 1 ♀	Allele	♂ P 3017		Sum
		4	5	
AC2003	1	13	13	26
Röskär	2	28	7	35
AC2003	1	11	14	25
Lillpite	2	23	15	38
Sum		75	49	124
MIX 2 ♀	Allele	♂ AC 2003		Sum
		1	2	
AC1008	2	11	15	26
Röskär	3	5	36	41
AC1008	2	10	30	40
Lillpite	3	1	34	35
Sum		27	115	142

DISCUSSION

Segregation distortion

Significant deviations from regular segregation were found for male gametes in 17 of 31 possible cases, but were generally absent in the female gametes. The distortions were in most cases consistent across several maternal parents and between two crossing sites. They were particularly frequent for the loci *Pgi-2*, *Pgm-2* and *Lap-2*. At these loci, specific alleles

Table 8 Mean freezing damage (scale from 0 = no damage to 11 = dead) and height of full and pollen mix families (MIX 1) from crosses in Lillpite. Origin of male parents S: southern, N: northern

♂ ♀	BD1012	P3017 S	T 2004 S	MIX 1
Freezing damage				
AC2003	3.1	9.7	9.1	9.6
BD1004	2.6	9.0	8.4	8.4
BD1011	3.0	10.4	10.9	10.5
Height growth (mm)				
AC2003	83	101	102	101
BD1004	74	93	90	94
BD1011	79	96	97	93

Table 9 Mean freezing damage (scale from 0 = no damage to 11 = dead) and height growth of full-sib and pollen mix families (MIX 4) from crosses at Röskär and Lillpite with clone BD 2009 as the maternal parent. Origin of male parents S: southern N: northern

Site	BD 200x N	R 1003 S	MIX 4
Freezing damage			
Röskär S	3.1	4.6	4.8
Lillpite N	3.1	3.8	4.0
Height growth (mm)			
Röskär S	71	89	86
Lillpite N	75	97	96

were systematically over- or underrepresented in several matings (Table 6). At *Pgi-2*, allele 4 had the higher frequency in all of the five segregations where it occurred. At *Lap-2*, allele 1 was strongly underrepresented, while allele 3 always occurred with the higher frequency.

PAULE *et al.* (1993) estimated allelic frequencies of 174 Norway spruce plus trees of northern and southern Swedish origin, grafted in five seed orchards, the present parents included. Comparisons of these allelic frequencies with their over-/underrepresentation in the segregations show that the favoured allele 4 at *Pgi-2* occurs at intermediate frequencies among both southern and northern clones (0.19 and 0.12, respectively). At *Lap-2*, however, the favoured allele 3 is the most common allele, occurring at frequency of 0.70, while the disfavoured one, allele 1, is rare (frequencies 0.03 and 0.06). Similarly, the disfavoured allele 1 at *Pgm-2* occurs at low frequencies. At the

latter two loci there is a tendency for the most common allele to be favoured and the rare alleles to be disfavoured in the segregations, but not at *Pgi-2*. Recently, ADAMS *et al.* (1990) have shown in Douglas-fir that the most common allele was always deficient in gamete segregations, while STRAUSS and CONKLE (1986) found the most common alleles in knobcone pine to be overrepresented.

Both pre- and post-zygotic factors can cause segregation distortions. They can occur during meiosis (meiotic drive) and cause unequal representation of the gametes in the pollen. Alternatively, the gametes may have unequal ability to survive and compete until fertilization is completed, causing male gametic viability selection or differential male gametic mating success, respectively (GILLET and GREGORIUS 1992). Post-zygotic viability selection occurs due to selective death of the diploid embryos. It has been argued that interpopulation or wide crosses can cause meiotic aberrations with deleterious effects, which may appear as segregation distortions (THOMPSON and WOODRUF 1978; STRAUSS and CONKLE 1986). In the present experiments, pollen of both northern and southern clones was produced at a southern locality and their mixed pollen was used in pollinations at both a southern and a northern site. However, there were no differences in the frequency of segregation distortions among the northern and southern paternal clones nor between the two localities. This makes it unlikely that directed meiotic disturbances caused by the changed environment should be an important factor. One attractive interpretation of segregation distortions is that factors like rates of pollen germination and tube growth are determined by single genes in the pollen grain. The distortions then represent linkages to such genes.

On the other hand, there are errors and uncertainties connected to isozyme analyses, both in identification and chemistry (STRAUSS and CONKLE 1986). Such errors are magnified in paternal analyses, as the paternal alleles are the differences between the a diploid genotype and a haploid maternal genotype. Thus, experimental errors cannot be ruled out as one part of the explanation of the segregation distortions.

The results from the reciprocal crosses with the clone AC 2003 (male segregation distortion only and different female fertilization distributions) indicate that also the maternal gametes may be a factor in these non-random segregations (GILLET and GREGORIUS 1992). A specific allelic combination that was not observed in the parents, was strongly deficient. This could be due to a male-female interaction in fertilization or caused by post-zygotic selection against this gametic combination. As we do not have the fertilization distributions in the other non-random segregations, it is not possible to decide between the factors gametophytic incompatibility, selective fertilization or post-zygotic viability selection.

Paternal success

Significant differences in paternal reproductive success were found between the males in four pollen mixes. The differences were in most cases consistent across several maternal parents, and between a northern and a southern crossing site. The differences were verified by both genetic markers and quantitative traits, which were in close agreement (Tables 2 – 5, 8 – 9). Similar variations in male reproductive success in pollen mixes have been demonstrated both for the same species (CHELIAK *et al.* 1987; SCHOEN and CHELIAK 1987) and for other conifer species (MORAN and GRIFFIN 1985; APSIT *et al.* 1989; EL-KASSABY and RITLAND 1992; NAKAMURA and WHEELER 1992). In order to enhance competition in the present experiment, pollen of males from different latitudes and with assumedly different adaptive properties was mixed. Single crosses were also made with the individual members of the pollen mixes.

One of the male parents in each of MIX 2 and MIX 3 (BD 1006 and AC 1013, respectively) produced only few viable seedlings in single crosses with the same maternal parents. The low paternal efficiency of BD 1006 in MIX 2, in particular, may therefore be due to low pollen viability for technical and non-genetic reasons, which may be caused by the forcing of male strobili and pollen handling. It may therefore not reflect low competitive ability under more natural conditions. The other male parents with low reproductive success indices, however, fathered substantial numbers of seedlings in single crosses with the same maternal parents. This shows that paternal reproductive success may depend on the competitive environment offered during or after pollination with a mixture of pollen from several males. This effect was independent of the maternal parent in all but one cross. It was also rather consistent across the two crossing sites, even if some discrepancies could be found. More southern than northern clones had high indices. However, as the tested number of clones was small and the trend was not consistent, no clear conclusions can be made.

The competitive ability of pollen from different males may depend on pollen traits such as size and respiration rate. In Douglas-fir, NAKAMURA and WHEELER (1992) found no correlations between paternal success and respiration rates, but they found a positive correlation with pollen size in one of two seed orchards. In the present experiment mixtures

were made of equal weight units of pollen. Clonal differences in the mean weights of individual pollen grains could therefore influence the relative frequency of pollen grains from each male in the mix. The size of spruce pollen grains vary between trees and origins (ANDERSSON 1954), but not so much that it seems likely that pollen weight variations can explain the magnitude of the RS indices found.

Selective embryo abortion rather than pollen competition has in most cases been the explanation of differential paternal success (e. g. EL-KASSABY and RITLAND 1992; NAKAMURA and WHEELER 1992). However, the influences of other genetic factors such as pollen size and number, variations in pollen viability and fertility, and male-female interactions, cannot be ruled out. The high frequency of paternal segregation distortions observed here is taken as an indication of selection among the pollen of each male. This may also be a reason for selection among pollen from the paternal parents in the mix. Therefore, the observed segregation distortions can be one of the factors contributing to the large differences in male fertility. Other forces, however, must also be present. Combining the information from male segregations and between male preferences (Table 6, Tables 2 – 5), it is seen that males containing alleles that are at a disadvantage within a cross may have a reproductive success equal to or above the average, i. e. clones T 2004 in MIX 1.

The presented results stress the need for a better understanding of the sexual reproductive process in conifers. This is needed both for drawing the correct conclusions from population genetics studies, for the production of seeds in seed orchards and for understanding the importance of this process in the evolution of the species.

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