Male reproductive success in *Pseudotsuga menziesii* (Mirb.) Franco: the effects of spatial structure and flowering characteristics

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Reproductive patterns and effective pollen dispersal were investigated in a Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) clonal seed orchard using 11 isozyme loci as genetic markers. Progenies from 94 mother trees were analysed by the mixed-mating and the neighbourhood models and outcrossing rates were estimated as 0.96 and 0.97, respectively. The proportion of offspring resulting from pollination by males located outside the neighbourhood of each mother tree (30 m in radius) was estimated as 0.54, with $\approx$43 per cent of matings resulting from outcrossing with nearby males (within neighbourhoods). The effects of distance and direction of individual males from mother trees, pollen fecundity and phenological synchronization were all significant in determining outcross mating patterns within neighbourhoods. Generally, male reproductive success increased with proximity and phenology overlap with mother tree and pollen fecundity. The effect of different factors influencing male reproductive success varied among the three groups of mother trees divided according to the time of female receptivity (early, intermediate and late). Phenology was important in the early and intermediate groups, whereas fecundity was important in the intermediate and late phenology groups. Both distance and directionality effects were significant in the early and late classes. The directionality effect could be partially explained by the wind patterns existing during the pollination period. The mean effective number of males mating with each female within neighbourhoods was 10.95 (49.31 per cent of males within neighbourhoods).

Keywords: mating system, neighbourhood model, pollen dispersal, *Pseudotsuga menziesii*, reproductive success.

Introduction

The mating system plays a central role in determining the genetic structure of plants. Patterns of pollen dispersal, gene flow between populations and differential reproductive success influence the levels of inbreeding, as well as effective population size and distribution of genetic diversity between and within populations. In addition to their importance to population genetics theory, mating patterns are also of great practical value in agriculture and forestry (Adams & Birkes, 1991; Snow & Lewis, 1993). Variation in male fertility is common among plant species and it is expected that several factors, including distance, fecundity and flowering phenology, may affect individual reproductive success and patterns of pollen dispersal (Broyles & Wyatt, 1991; Adams *et al.*, 1992b; Devlin *et al.*, 1992; Snow & Lewis, 1993; Burczyk *et al.*, 1996). For theoretical and practical reasons it is important to identify these factors and the best way to achieve this is the application of genetic estimation techniques using selectively neutral genetic markers (Smouse & Meagher, 1994). Apparently, if an effect of a possible factor influencing reproductive success is to be calculated from individual fertilities, paternity methods are not very suitable for determining its significance if exclusion probabilities are not very high (Devlin *et al.*, 1988; Adams *et al.*, 1992b). Otherwise, the parameter esti-

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mates based on these methods can be substantially biased (Adams, 1992). Estimation of individual fertilities may also be difficult if many potential fathers exist, unless a large number of progeny is examined (Roeder *et al.*, 1989).

If specific mating system parameters are of interest, a natural approach is to use probability models designed to account for observed frequencies of multilocus genotypes in pollen gametes of offspring samples, and then estimate these parameters using either least squares (LS) or maximum likelihood (ML) statistical methods. This *mating model approach* was employed to investigate male fertilities based on LS procedures in a white spruce (*Picea glauca* (Moench) Voss) seed orchard (Schoen & Stewart, 1986). A more general model for estimating male fertilities in angiosperms using ML procedures was presented by Roeder *et al.* (1989). However, both methods assume that mating occurs only within populations. Adams & Birkes (1989, 1991) proposed the *neighbourhood model*, which takes into account the possibility of mating with distant males located outside a local population, and is particularly useful for predominantly outcrossing, especially wind-pollinated species. Additionally, the neighbourhood model allows individual male reproductive success to be related to various factors that may influence it (Burczyk *et al.*, 1996).

Seed orchards are artificial forest tree populations established to promote the production of genetically improved seeds used for reforestation purposes and they are important for breeding programs of several conifers. In order to attain expected genetic gains, several assumptions about mating patterns must be met. From this perspective, mating system studies in seed orchards are of particular interest (Adams *et al.*, 1992a; Wheeler & Jech, 1992). Coniferous clonal seed orchards have several features which make them very useful for investigations of male reproductive success (Adams *et al.*, 1992a). Additionally, in conifers the pollen (paternal) contribution to each embryo can be determined based on codominant genetic markers by assaying the megagametophyte and the diploid embryo.

Selfing in conifers usually accounts for less than 10 per cent of seeds and a large proportion of fertilizations in seed orchards result from gene flow (contamination) from distant pollen sources (Muona, 1990; Adams & Birkes, 1991; Savolainen, 1991). However, the information on the outcrossing patterns, including reproductive success and effective pollen dispersal within seed orchards, is rather limited (Wheeler *et al.*, 1993). In this paper we demonstrate the application of the neighbourhood

model to the progeny arrays in a Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed orchard using isozymes as genetic markers, and illustrate the effects of several factors (distance, direction, flowering fecundity and phenology) in determining mating success of individual males.

Materials and methods

The seed orchard studied was established in 1978 and laid out in Lavercantière in a broad-leaved forest area (near Cahors, France, 1°20′E, 44°37′N). The orchard (14 ha in size) consists of 70 Douglas-fir clones selected phenotypically by INRA (France) and Danish State Forestry for height growth, stem straightness and then wood density from a provenance trial established in Denmark. All trees included in the provenance experiment originated from the Darrington provenance in Washington State, U.S.A. Clonal ramets were planted at 8×6 m spacing and randomly distributed in the orchard. At the time of sampling there were 1128 growing ramets. Flowering has taken place for several years. However, gibberellins $A_{4/7}$ treatment and girdling were practised on some trees in order to enhance flowering and seed production (Bonnet-Masimbert & Webber, 1995). No Douglas-fir stands are located around the studied seed orchard.

Seeds were collected in autumn 1990 from one or two ramets of each clone. The progeny of 94 ramets (mother trees) growing at least 25 m inside the orchard borders were sampled. All mother trees (with their progeny) were divided into three groups of early-, intermediate- and late-flowering clones, according to the mean day of female receptivity (23, 36 and 35 ramets, respectively). The analyses of mating system were executed for each reproductive phenology class separately, as well as for the total population. For each ramet, about 10 seeds (pairs of megagametophyte and embryo tissues, 925 seeds in total) were analysed electrophoretically using standard procedures (Conkle *et al.*, 1982) based on 11 gene loci: *Aat-2, Est-1, Fdh, G6pdh, Glydh, Idh, Lap-1, Mdh-1, Mdh-3, 6Pgd* and *Sod.* Enzyme systems were stained following various procedures (Conkle *et al.*, 1982; Adams *et al.*, 1990; Lewandowski & Mejnartowicz, 1992). The multilocus genotypes of all 70 clones present in the orchard were also obtained.

The standard estimates of single- (t_s) and multilocus (t_m) outcrossing rates were calculated based on a mixed-mating model using the maximum likelihood procedures that were developed for conifers (Ritland & El-Kassaby, 1985), after which the neigh-

bourhood model was applied following Adams & Birkes (1989, 1991). In this model it is assumed that pollen that successfully fertilizes viable embryos of a mother tree comes from three sources: self-fertilization (with probability s); outcrossing with trees growing outside an arbitrary specified area around a mother tree (with probability m; the area around a mother tree is the neighbourhood); and outcrossing with trees located within the neighbourhood (probability $1-m-s$), with relative mating success (i.e. fertility) of the jth outcross male in the neighbourhood equal to ϕ_j. In our analyses the neighbourhood size was set to be 30 m in radius, which already has been found to be sufficient in the case of seed orchards (Adams et al., 1992a). The probability of observing multilocus genotype g_i, among pollen gametes of offspring from a mother tree is:

$$P(g_i) = sP(g_i \,|\, M) + (1-m-s) \sum_{j=1}^{r} \phi_j(g_i \,|\, F_j) + mP(g_i \,|\, B),$$

$$(1)$$

where $P(g_i\,|\,M)$, $P(g_i\,|\,B)$, and $P(g_i\,|\,F_j)$ are the transition probabilities (Devlin et al., 1988; Adams & Birkes, 1991) that the pollen gamete with genotype g_i is produced by the mother tree, by pollen sources outside the neighbourhood and by the jth (out of r) outcross male in the neighbourhood, respectively. It is assumed that the mating success of each outcross male in the neighbourhood (ϕ_j) follows an exponential relationship (Adams & Birkes, 1991; Burczyk et al., 1996). In this study we related male mating success to four factors:

$$\phi_j = \frac{e^{\beta d_j + \gamma f_j + \varphi p_j + \delta \cos(\alpha_0 - \alpha_j)}}{\displaystyle\sum_{k=1}^{r} e^{\beta d_k + \gamma f_k + \varphi p_k + \delta \cos(\alpha_0 - \alpha_k)}},$$

$$(2)$$

where d_j, f_j and p_j indicate distance (m) from the mother tree, fecundity and phenology scores (see below), respectively, and $\cos(\alpha_0 - \alpha_j)$ is the angle cosine between the presumed prevailing direction of effective pollen dispersal and the location of the jth male within a neighbourhood (Burczyk et al., 1996). The parameters β, γ, φ and δ are the estimated parameters that describe the effects of the respective factors.

Mating system parameters included in various models were estimated by fitting the models to multilocus pollen gametic arrays in the offspring of individual mother trees using maximum likelihood (ML) methods (Rao, 1973; Roeder et al., 1989). This was carried out using the NEIGHBOR computer

program (Burczyk et al., 1993). Details on statistical procedures have been reported elsewhere (Burczyk et al., 1996; documentation of NEIGHBOR program).

Given estimates of the mating system parameters, fertilities of individual males within a neighbourhood (ϕ_j) can be obtained from eqn (2) (Burczyk et al., 1996). These fertilities were used to calculate the effective number of pollen parents in the neighbourhood siring offspring of an individual mother tree and the mean distance of effective pollen dispersal between mother trees and their outcross mates within neighbourhoods (Adams & Birkes, 1989; Burczyk et al., 1996).

Observations of male flowering intensity and male and female flowering phenology were made in spring 1990 prior to the seed collection. For each clone, from two to 11 grafts (six on average) were investigated. For each graft one of four male fecundity scores (0, 1, 2 or 3) was assigned, where 0 and 3 indicated the absence of and the greatest male flowering, respectively. Additionally, the timing of the start and end of male and female flowering was noticed for each ramet. Individual ramet data were used to obtain average clonal estimates. However, ramets with zero fecundity scores were excluded as potential fathers in the neighbourhood analyses. Phenology scores (eqn 2), indicating the degree of flowering overlap between a mother tree and potential outcross males in the neighbourhood were calculated using the NEIGHBOR program, following procedures originally developed by Adams and co-workers (Adams & Birkes, 1989; documentation of NEIGHBOR program). The standard deviation of the flowering period, σ, for each clone was estimated according to Erickson & Adams (1989). In the present study we assumed that 10 per cent and 90 per cent of reproductive strobili had shed pollen or become receptive to pollen when the start and end of flowering was observed. Thus σ was estimated as: $\sigma = (x_{90} - x_{10})/2z$, where $z = 1.28$ (Snedecor & Cochran, 1980).

Heterogeneity of mating system parameters was investigated by Fisher's heterogeneity χ^2_{HET} test (Rao, 1973). The significance of the null hypotheses, that $t = 1$ and that $m = 0$, was determined by analysing the limits of confidence intervals. The significance of other mating system parameters in the neighbourhood model (s, β, γ, φ, δ) was tested using the likelihood ratio test (Rao, 1973). Finally, in order to verify the validity of mating system estimations based on the neighbourhood model we calculated exclusion probabilities (EP) within each neighbourhood using an empirical approach (Roeder et al., 1989) following 5000 simulations. The

EP is the probability of being able to exclude potential males on the basis of genetic incompatibility alone (Adams, 1992). High estimates of EP suggest high precision of mating system parameter estimates.

Results

Multilocus outcrossing rate (t_m) was estimated to be 0.963 for the total population, which means that only 3.7 per cent of embryos resulted from self-fertilization $(s = 1 - t)$ (Table 1). The estimates of outcrossing did not vary among phenology classes, although the highest value was observed for the intermediate group. Single-locus outcrossing rates (t_s) varied considerably and were statistically heterogeneous across the studied loci for all groups except the intermediate class (data not shown). The mean values of single-locus outcrossing rates within groups were similar to the respective multilocus estimates. The multilocus estimate for the total population, as well as several single-locus estimates, were significantly different from unity $(t \neq 1$; Table 1).

On average, there were ≈ 29 grafts growing within the neighbourhoods of individual mother trees, although only 22.2 grafts produced male flowers and were considered as potential fathers. EP calculated for each neighbourhood ranged between 0.648 and 0.834, with a mean of 0.756, thus, on average, 16.8 of 22.2 males within the neighbourhoods could be excluded as potential fathers of a given offspring, based on genetic incompatibility. The mean standard error of EP estimates was 0.003.

Selfing estimates were generally low (0.016–0.042), although selfing was a significant factor in all groups except the early phenology class (Table 1). The proportion of successful pollen coming from outside of the neighbourhood was found to be 0.544 for the total population, and decreased from the early to the late phenology classes. Consequently, the proportion of outcrossing with local males (within the neighbourhood) increased from 0.307 in the early to 0.485 in the late phenology class. The total population accounted for 42.6 per cent of local outcross matings.

Generally, all factors expected to influence mating success of individual males (distance, fecundity, phenology and directionality) were significant in describing outcross mating patterns within the neighbourhood, although each factor affected the mating success to different degrees. The strongest effect was observed for the components related to flowering characteristics. Both fecundity and phenology were highly significant for the total population $(P < 0.0001$; Table 2), but their importance changed among phenology classes. For the early group phenology but not fecundity was important, whereas the opposite situation occurred in the late group. Both factors were very important in the intermediate class. The differential influence of fecundity and

Table 1 Estimates of mating system parameters and the most likely directions of pollen influx within the neighbourhood, calculated for different phenology classes of *Pseudotsuga menziesii* from the mixed-mating and neighbourhood models (standard errors in parentheses)

Mating system parameter	Reproductive phenology class			Total population
	Early	Intermediate	Late	
Mixed-mating model				
Outcrossing (t_m)	0.952	0.963	0.954	0.963[a]
	(0.034)	(0.026)	(0.028)	(0.015)
Neighbourhood model				
Selfing (s)	0.0162	0.0419	0.0336	0.0299
	(0.0153)	(0.0185)	(0.0195)	(0.0105)
	$P = 0.0643$	$P = 0.0013$	$P = 0.0283$	$P < 0.0001$
Background pollination (m)	0.6766	0.5868	0.4810	0.5440
	(0.0807)	(0.0609)	(0.0530)	(0.0348)
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Local outcrossing $(1 - s - m)$	0.3072	0.3713	0.4854	0.4261

[a]Estimate significantly different from $t = 1$ at $P < 0.05$ level.

phenology may be clearly observed if relative mating success (ϕ_j) is plotted against the fecundity and phenology scores for different phenology classes (Fig. 1). In the intermediate class, generally, only males with the highest fecundity and the greatest flowering phenology overlap with mother trees could be successful in mating.

The distance and directionality effects were not significant in the intermediate group, but they were important in the early and the late phenology classes (Table 2). Distance was also significant for the total population. Although the mean physical distance between any mother tree and potential fathers within the neighbourhood was ≈ 20.5 m, the mean distance of effective pollen dispersal varied among different phenology groups because of the variable distance effect (β) and was calculated to be 14.75,

21.28 and 16.52 m for the early, intermediate and late classes, respectively (Table 2). The values, lower than the mean physical distances, indicate that mating occurs mainly between near-neighbours. The relationship between distance and relative mating success is presented in Fig. 2. Finally, only distance and fecundity effects were found to be significantly heterogeneous among phenology classes (Table 2).

The primary direction of effective pollen dispersal (α_0), which gave the best fit of the model (the greatest log-likelihood), varied widely among phenology classes (Fig. 3). The estimates of α_0 were found to be (in degrees from north) 172 (nearly south) for the early, 306 (nearly north-west) for the intermediate, and 358 (nearly north) for the late group (Table 2). The direction for the total population was estimated to be 353 (nearly north). However, a significant

Table 2 Estimates of parameters of outcross mating success within the neighbourhood calculated for different phenology classes of *Pseudotsuga menziesii*. The significance of individual estimates (P) was calculated from the log-likelihood ratio test (N_e, effective population size; N_e/N, proportion of effective to actual population size; standard errors in parentheses)

Parameters of outcross mating success	Reproductive phenology class			Total population
	Early	Intermediate	Late	
Main parameters				
Fecundity effect (γ)[a]	0.2635	3.0699	1.7454	1.7195
	(0.5092)	(1.0283)	(0.3740)	(0.2675)
	$P = 0.596$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Phenology effect (φ)	13.4152	19.0795	3.3057	13.9109
	(3.8115)	(8.1443)	(7.6357)	(2.4420)
	$P < 0.0001$	$P = 0.0037$	$P = 0.6835$	$P < 0.0001$
Distance effect (β)[a]	-0.1503	0.0024	-0.0833	-0.0497
	(0.0587)	(0.0235)	(0.0225)	(0.0147)
	$P = 0.0008$	$P = 0.9296$	$P = 0.0003$	$P = 0.0011$
Directionality effect (δ)	1.0757	0.4046	0.4627	0.2962
	(0.6239)	(0.2594)	(0.2288)	(0.1679)
	$P = 0.0252$	$P = 0.0916$	$P = 0.0335$	$P = 0.0654$
Estimates calculated from main parameters				
Main direction of pollen influx (α_0) (degrees from north)	172	306	358	353
Mean physical distance (m)	20.43	20.53	20.61	20.54
Mean effective dispersal distance (m)	14.75	21.28	16.52	18.54
N_e	5.68	7.87	10.24	10.95
N_e/N (%)	26.07	35.60	45.39	49.31

[a]Estimates significantly heterogeneous across reproductive phenology classes.
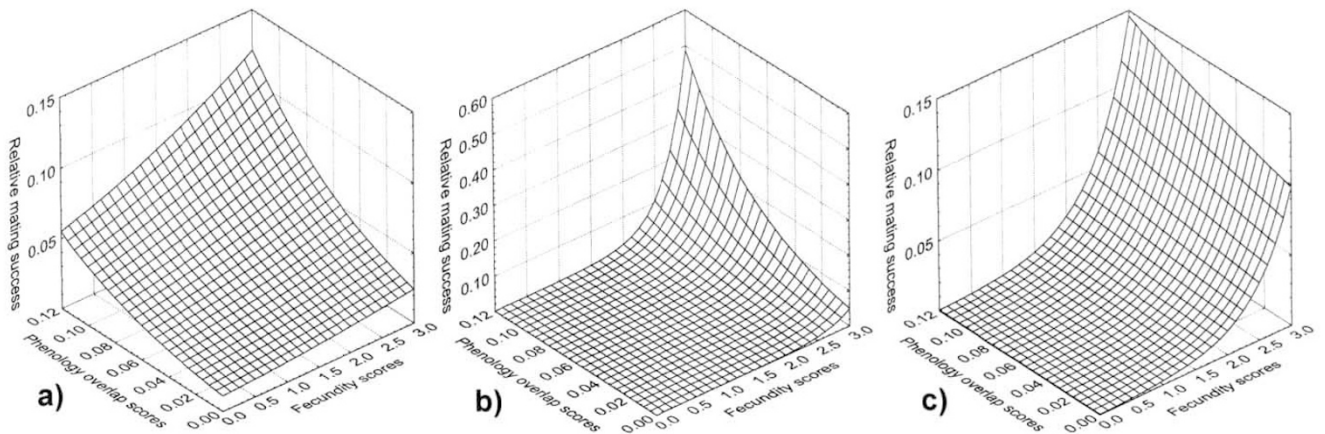
Fig. 1 The joint effects of pollen fecundity and phenological overlap on relative reproductive success ($\hat{\phi}$) of males within neighbourhoods of different phenology groups of *Pseudotsuga menziesii* (a, early; b, intermediate; c, late), when other effects are ignored. The plots are based on ϕ values over all neighbourhoods within each group (23, 36, 35, respectively, for early, intermediate and late) and are specific for the observed distributions of fecundity and phenology overlap scores of male trees in these neighbourhoods.

directionality effect was observed only for the early and late phenology classes.

Differential mating success of individual males results in reduced effective population size (N_e) of male parents compared to the census population. The effective population size of male trees in the neighbourhood, based on the individual fertilities obtained from eqn (2), was found to be 10.95 individuals for the total population, with each individual having an equal probability of mating with a mother

tree. The N_e estimates increased through time from 5.68 in the early, through 7.87 in the intermediate, to 10.24 individuals in the late phenology group (Table 2).

Discussion

The influence of several environmental (distance and direction) and biological (fecundity and phenology) factors on male reproductive success was
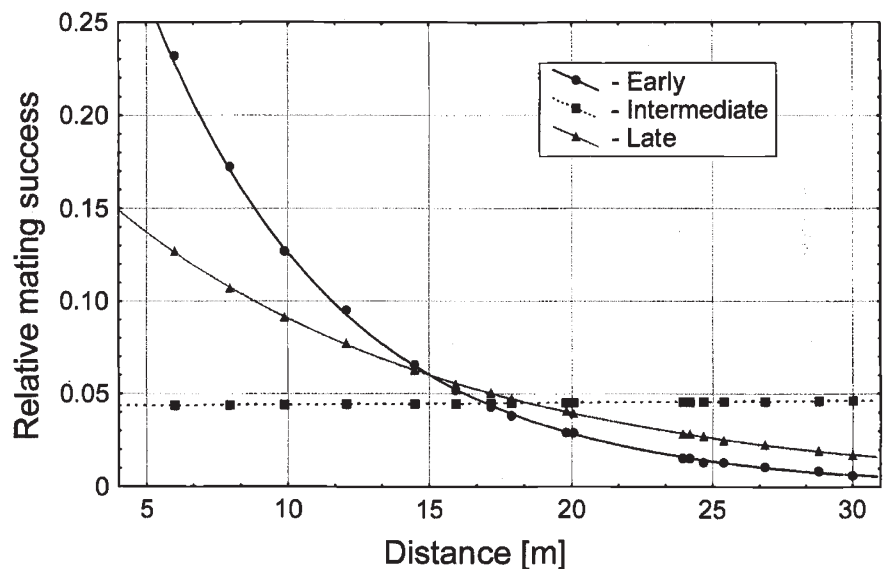
Fig. 2 The effect of distance from mother tree on relative reproductive success ($\hat{\phi}$) of males of *Pseudotsuga menziesii* within neighbourhoods of different phenology groups when other effects are ignored. The plot is based on ϕ values over all neighbourhoods within each group and is specific for the observed distribution of distances of male trees in these neighbourhoods.
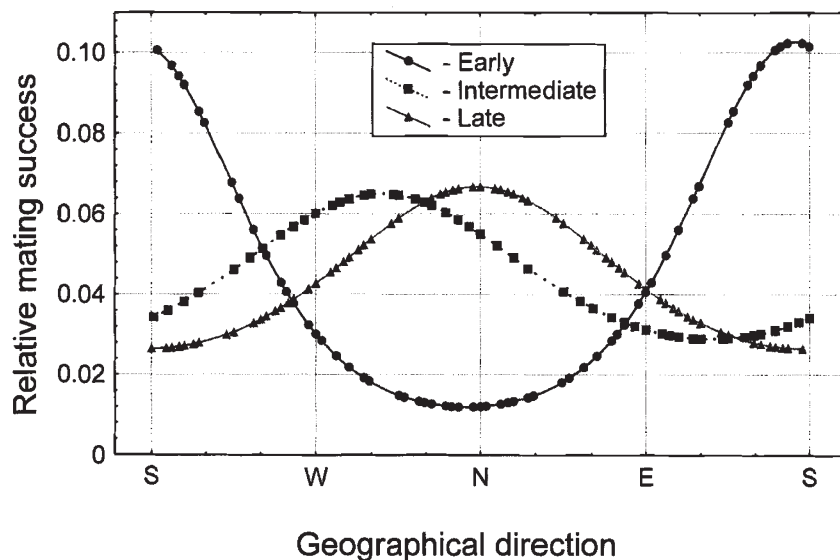
Fig. 3 Relationship between relative reproductive success ($\hat{\phi}$) of males of *Pseudotsuga menziesii* within neighbourhoods and their location relative to that of the mother tree (i.e. direction from the mother trees) for three phenology groups when other effects are ignored. The plot is based on $\hat{\phi}$ values over all neighbourhoods within each group and is specific for the observed distribution of relative locations of male trees in these neighbourhoods.

expected from many past and recent studies, although the exact relationship was not known. In this study we used genetic analysis for detailed investigations of mating patterns and effective pollen dispersal in a Douglas-fir seed orchard.

The levels of outcrossing estimated in this study were almost identical for the mixed mating (0.963) and the neighbourhood (0.970; $t = 1-s$) models. They were generally higher, although similar to the estimates reported in natural and artificial Douglas-fir populations (Neale & Adams, 1985; Ritland & El-Kassaby, 1985; El-Kassaby *et al.*, 1988).

Phenology is probably the single most important influence on outcrossing patterns in seed orchards (Jonsson *et al.*, 1976; El-Kassaby *et al.*, 1988; Erickson & Adams, 1989; Wheeler *et al.*, 1993). The lack of flowering phenology overlap among clones may prevent some pairs of clones producing offspring and the reproductive success of males which tend to flower earlier or later than the majority may be substantially reduced. Clonal variation in pollen (or male strobili) production of many coniferous trees is also large (Jonsson *et al.*, 1976; Burczyk & Chałupka, 1997), which may have further implications for male reproductive success. However, the possibility of clone×year interaction in pollen fecundity may influence the variation of male reproductive success in consecutive years.

The influence of pollen fecundity on mating success was investigated in a white spruce (*Picea abies* Voss) seed orchard using a mating model (Schoen & Stewart, 1986). The male fertility of individual clones varied from 0 to 14.6 per cent and

male reproductive success of a pollen parent was significantly related to its pollen production. From Fig. 1 it can be seen that the reproductive success of a single male observed in this study is low when either its fecundity or phenology overlap with a mother tree is small, independent of other factors. In order to be successful, a male tree must produce a large amount of pollen and must be synchronized in flowering with a mother tree.

Despite the absence of pollen contamination from surrounding stands, more than 54 per cent (on average) of fertilizing pollen gametes came from outside the designated neighbourhoods, from males located greater than 30 m from mother trees. This suggests that mating between distant individuals in the orchard may occur. Consequently, only about 42 per cent of seeds resulted from outcrossing within a neighbourhood. However, the probability of mating between mother trees and potential males within neighbourhoods was decreased with distance, indicating that most of the matings occurred among near-neighbours. This relationship has been previously found based on rare marker analyses (Müller, 1977; Shen *et al.*, 1981; Yazdani *et al.*, 1989). It was also supported by the observation that the majority of pollen released from isolated trees comes to rest within a few tens of metres (Di-Giovanni & Kevan, 1991). Apparently, the distance effect was not significant in two Douglas-fir seed orchards studied using the neighbourhood model (Adams & Birkes, 1991; Adams *et al.*, 1992a); the authors postulated that the effect of distance was probably obscured by clonal differences in pollen

fecundity and phenology. Erickson & Adams (1989) found that the dispersal of rare markers was strongly associated with distance between mating individuals only when floral synchrony among clones was high. In another Douglas-fir seed orchard which was subjected to flower stimulation the distance effect was detected, and was more important in stimulated than in unstimulated orchard blocks (W. T. Adams, J. Burczyk, D. S. Birkes and V. D. Hipkins, unpubl. obs.). Distance appeared to be significant in a natural stand of knobcone pine (*Pinus attenuata* Lemmon.) (Burczyk *et al.*, 1996).

Patterns of directionality of pollen dispersal, based on genetic marker data, have been studied only in a limited way (Shen *et al.*, 1981; Erickson & Adams, 1989; Prat, 1995) primarily using a source-orientated approach (Wheeler *et al.*, 1993). It was generally found that capture of rare marker alleles by female receptor trees was greatest downwind of the male marker (Shen *et al.*, 1981). Prat (1995), applying an elliptical model, indicated that pollen dispersal took place preferentially along rows of trees, but he also suggested a wind effect. The same methods as in this study were used to investigate pollen dispersal in a natural stand of knobcone pine (Burczyk *et al.*, 1996). The mating success was greatest for males located east of mother trees, but it was not related directly to general wind directions.

The method of determining direction of pollen influx presented in this study is efficient only if one prevailing direction exists. This feature probably caused the problems with estimating the prevailing direction for the total population because, during the whole flowering period (17 days), wind patterns (direction and speed) could change several times. Once the different phenology classes were considered separately (each class covered a period of 5–7 days, see Materials and methods) it appeared that the directionality effect was significant for the early and the late phenology groups. The only information about wind patterns during pollination comes from two close meteorological stations (Gourdon and Le Montat) located 14 km north-north-east and 27 km south-south-east from the seed orchard, respectively. The wind speed and direction recorded at 09.00, 12.00 and 15.00 h indicated that both were changing during the flowering period, but they were mainly consistent between the two meteorological stations (data not shown). Generally, it was found that the direction of pollen dispersal calculated from the model was more or less comparable with the wind patterns observed at the beginning of the flowering period of the given phenology class (data not shown).

Although outcrossing (or selfing) rates were fairly constant across phenology classes, background pollination was continuously decreasing from 0.677 to 0.481. This could result from an increasing production of pollen within neighbourhoods, as well as from the observed decreasing wind speed during the pollination period. In consecutive phenology groups different factors influenced male reproductive success to different degrees (Table 2). Spatial distribution of males (distance and direction) had no effect in the intermediate class but was very important in the early and the late groups. The fecundity effect appeared to be very significant in the intermediate and the late phenology classes, when most of the clones were already capable of shedding pollen. Phenology was most important in the early reproductive phenology group, whereas it was not significant in the late one. This suggests that at the beginning of flowering, especially when a deficiency of pollen may occur, phenology overlap among mating individuals is a crucial factor determining male reproductive success. Its importance, however, may be decreasing when pollen from many clones becomes available towards the end of the flowering period. These results support the 'first-come, first-served' hypothesis (Owens *et al.*, 1981). In the intermediate class both fecundity and phenology effects, being very significant, probably obscured the influence of distance and directionality (Erickson & Adams, 1989; Adams & Birkes, 1991; Adams *et al.*, 1992a).

Although in this study we investigated only the influence of distance, directionality, male fecundity and phenology overlap on male reproductive success, a variety of other factors may also be important. However, regardless of the mechanisms generating male fertility variation, the apparent presence of differential male fertilities is of great importance in forest tree improvement programmes, as well as for genetic conservation purposes. The usual assumptions that the parents of open-pollinated progenies have the same contribution (equal fertility among male parents) may lead to either over- or underestimates of additive genetic variance, which, in turn, causes biases in estimates of heritability, genetic gains and other genetic parameters (Squillace, 1974).

The ability to measure differences in male reproductive success in natural and artificial populations is central to a number of important problems in population genetics and population biology (Smouse & Meagher, 1994). In this study we found that the neighbourhood model appeared to be very effective in determining different aspects of mating patterns.

In particular, it allowed several factors influencing male reproductive success to be determined. Because the neighbourhood model approach can incorporate a variety of these factors, it is a promising tool for further studies of mating patterns in plant populations.

Acknowledgements

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