Mating system in a clonal Douglas fir (*Pseudotsuga menziesii* (Mirb) Franco) seed orchard. II. Effective pollen dispersal

D Prat *

Laboratoire INRA-ENGREF de sciences forestières, 14, rue Girardet, 54042 Nancy cedex, France

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Summary — Genetic variation of effective pollen received by individual ramets in a Douglas fir clonal seed orchard was not representative of the genetic variation of this clonal seed orchard. This deviation was not due to selfing rate. Effective pollen dispersal might be responsible for this deviation. It was then assessed around 3 individual ramets by comparing genotypes of effective pollen with those of pollinator and surrounding trees located in a circle or in an ellipse focused on the receptor tree. The best prediction of genetic diversity of effective pollen was obtained considering a maximum pollination distance of about 20–30 m and an elliptical pollen dispersal. Effective pollen dispersal took place preferentially along rows of trees and probably depended on the wind. As the flowering period was short and particularly synchronous in the year of seed collection, reproductive phenology probably did not favour some peculiar crosses. Matings between a few neighbour trees was probably the major cause of the homozygosity level in seed crop.

Résumé — Régime de reproduction dans un verger à graines de Douglas (*Pseudotsuga menziesii* (Mirb) Franco). II. Dispersion du pollen. La diversité génétique du pollen reçu par des ramets individuels d’un verger clonal à graines de Douglas n’était pas représentative de la diversité génétique de ce verger. Ceci ne résultait pas du taux d’autofécondation, comme l’ont montré des études antérieures. Une dispersion réduite du pollen pouvait en être la cause. Aussi, la dissémination du pollen a été étudiée autour de 3 ramets particuliers en comparant la diversité génétique du pollen efficace qu’ils ont reçu à la diversité génétique des arbres proches situés dans un cercle ou une ellipse. La meilleure prédiction de la composition du nuage pollinique efficace a été obtenue en considérant les arbres pollinisateurs situés à une distance maximale de 20–30 m et en pondérant leurs flux polliniques à l’aide d’un modèle de dispersion du pollen en ellipse. La dissémination du pollen a eu lieu principalement le long des lignes de plantation mais les vents dominants paraissent aussi avoir une

* Present address: INRA, station d’amélioration des arbres forestiers, Ardon, 45160 Olivet, France
influence. Les décalages phénologiques entre arbres ne semblent pas responsables de croisements préférentiels car, l'année de la récolte, la période de floraison a été particulièrement brève et synchrone. Les croisements, qui ont lieu principalement entre les quelques arbres voisins, sont vraisemblablement la principale cause des excès d'homozygotie observés dans la semence commerciale.

**dispersion du pollen / effet de voisinage / isoenzyme / Pseudotsuga menziesii = Douglas / régime de reproduction**

**INTRODUCTION**

In a clonal seed orchard, the genetic quality of the crop is mainly affected by the genetic value of clones and by the mating system, especially selfing (Sorensen and White, 1988). Various analyses of mating systems in seed orchard have thus been carried out in diverse tree species (Barrett et al, 1987; El-Kassaby et al, 1988; Paule et al, 1993).

The mating system was studied in one of the first Douglas fir clonal seed orchards to produce seed for afforestation in France. In this Bout seed orchard (located near Moulins), the selfing rate was estimated by using isozymes as genetic markers, and all clones were genetically characterised (Prat and Caquelard, 1995). The selfing rates were low enough (about 4%) not to alter the genetic quality of seed but the level of homozygosity was higher than that expected according to the selfing rate. A lack of heterozygosity can result from a limitation of gene flow in the orchard as produced by relatedness, floral phenology or pollination distance. In the Bout seed orchard, no relationships between trees in the orchard were expected since it consists of an orchard planted with clones selected from different stands. Phenology often influences mating systems (El-Kassaby and Ritland, 1986; El-Kassaby et al, 1988; Erickson and Adams, 1989). However in the year of study of mating system in Bout seed orchard, all clones flowered simultaneously (Prat and Caquelard, 1995). Moreover, genetic variation of male gametes received by a single ramet was not representative of that expected according to the seed orchard genetic composition. A study of pollen dispersal was required to explain these unexpected patterns of paternity.

The aim of the present study was to describe pollen dispersal patterns in a clonal seed orchard in order to explain the genetic structure of the seed crop. The analysis of pollen dispersal included a study of pollination distance. Previous studies in Douglas fir showed that little pollen was dispersed beyond about 30 m (Erickson and Adams, 1989), but this could be influenced by the plantation density. Pollination distance was also estimated in a Bout seed orchard.

In an anemophilous species like Douglas fir, pollen could diffuse around pollinator tree with a possible preferential direction due to wind. Circle and elliptical areas of pollen dispersal were tested to identify the actual number of pollinator trees of a given receptor tree. Seeds of several ramets in the seed orchard were analysed with isozymes in order to determine the position of their respective pollinator trees and consequently the pollen dispersal. At the seed level, only effective pollen (resulting in viable seed production after fecundation) could be detected. In the case of competition and selection as suggested by Apsit et al (1989) the complete pollen flow could not be assessed.

**MATERIALS AND METHODS**

**Plant material**

Pollen flow was studied in a Douglas fir clonal seed orchard, the Bout seed orchard, located in
central France. It consists of 60 clones. The distance between ramets (all grafted) was 5 m in all direction at plantation establishment. Each of the 20 blocks in the orchard contained 1 ramet of each clone. The survival rate of trees was about 50% the year of this study. All 60 clones were identified by unique multilocus genotypes at 9 enzyme loci (Prat and Caquelard, 1995). Seeds were collected in 1987, the first year of large seed production in this orchard, which was planted in 1966.

Three ramets from 2 clones were chosen for the study: clone 64 (ramet 1 and ramet 2) and clone 95 (ramet 1) because they bore rare alleles (Got-1, and G6pdh6 in clone 64, Mdh-3, in clone 95) and most of their gametes were identifiable according to their multilocus genotype (all gametes from clone 95 were identifiable). The selfing rates of these ramets were already estimated for the same year of seed collection (Prat and Caquelard, 1995). Pollen genotypes of trees surrounding studied ramets can be thus determined.

**Pollen dispersal**

The flowering period was very short the year of seed collection, because of a late frost period. Consequently, floral phenology was not taken into account in the present study although it can be one of the major components of the mating system in Douglas fir seed orchard when flowering stretches over a longer period (El-Kassaby et al, 1988; Erickson and Adams, 1989).

The pollination distance and the pollen flow direction were assessed from the position of trees pollinating the studied ramets. The genotypes of male gametes providing embryos in the collected seeds were used to determine fatherhood and clone location. The ramets analysed were sufficiently distant (more than 40 m) from any other ramet of the same clone to avoid noticeable pollination between ramets of same genotype. This minimal distance between 2 ramets was chosen according to the results of Erickson and Adams (1989) so as to be higher than the pollination distance that these authors observed in a Douglas fir seed orchard in the United States. Different pollen dispersal simulations were tried, which led to an allelic composition of pollen received by a receptor tree. The pollen dispersal model with the lowest and non-significant difference between observed and expected allelic frequencies in pollen ($\chi^2$ test) was presumed the best.

**Circle**

As a first step, the pollen dispersal was presumed to be without preferential direction around each pollinator surrounding the receptor tree. Various pollination distances, up to 40 m, were tested. In the simulations, only male-flowering ramets were considered. Their contribution was weighted according to their distance to the receptor tree and to their flowering abundance (noted in 3 classes: low, intermediate, high; respective weight: 1, 2, 3).

**Ellipse**

If the allelic frequencies observed in the pollen remained significantly different from the expected one whatever the pollination distance, pollen is presumed to be dispersed in a preferential direction. Such preferential directions of pollination have already been studied by Baradat et al (1984) and Erickson and Adams (1989). In the presence of a single factor (such as dominant wind) inducing a preferential direction of pollen dispersal, pollen should be distributed within a upwind/downwind stretched ellipse instead of a circle; the pollinator tree is at the focus of this ellipse. The pollination thus occurred preferentially on one side of the pollinator tree. In this condition, the relative pollen flow can be assessed in each direction by the distance from the focus to the point of the ellipse circumference in the corresponding direction (segment OA' in fig 1). The relative length of segment OA' depends on the orientation ($\theta - \phi$) of the point A' to the long axis of the ellipse and on its flatness $\alpha$ (width/length) (fig 1). The pollen dispersal was determined by 3 parameters: the orientation $\phi$ of the ellipse, its flatness $\alpha$ and the maximal distance $D$ of pollination. When the same force (direction and intensity) was supposed to act on the pollen of each tree, the pollen flow received by a tree was dependent on the same 3 parameters, as if the receptor tree was located at the downwind focus of the ellipse. The pollen flow was proportional to the distance from the receptor tree to the point of the ellipse circumference in the considered direction. The orientation $\phi$ and the flatness $\alpha$ of the ellipse were tested to find the best relationship between the expected and observed allelic frequencies in pollen. Trees surrounding the receptor and located within the considered ellipse or located under a maximal distance $D$ from the receptor were taken into account; their distance was also tested for weighting.
Pollen allelic frequencies were deduced from megagametophyte and embryo genotypes of the same seed at several enzyme loci (G6pdh, Mdh-1, Mdh-2, Mdh-3, α-Est, Lap-1 and Lap-2) according to Prat and Caquelard (1995). More than 400 seeds were analysed for each studied ramet. Only outcrossed seeds were considered in the present study. The pollen genotypes at the most polymorphic locus (G6pdh) or at all loci analysed (multilocus analysis) were taken into account.

RESULTS

No preferential direction of pollen dispersal

A single locus analysis (G6pdh locus) was carried out for the 2 ramets of clone 64. For the ramet 1 of clone 95, the analysis was either single locus (α-Est or G6pdh) or multilocus. The observed allelic frequencies (selfing excluded) in the pollen for the 3 ramets were significantly different from those of the seed orchard and from those around the ramet used as female whatever the pollination distance considered up to 35 m. The best concordance was observed when pollinator trees were located not more than 25 m from the receptor as for instance in ramet 2 of clone 64 (table I). The same situation was observed for ramet 1 of clone 64, but allele G6pdh1 was observed in the pollen received by this ramet at the frequency 0.011, and no tree up to 45 m bore this allele.

Weighting by the distance d (by $d^{-1}$ or $d^{-2}$) between receptor and pollinator trees did not improve the expected frequencies, while the male-flowering intensity did a little. The observed and expected allelic frequencies in the pollen were different in all

Fig 1. Representation of pollen dispersal model. The pollen flow from tree O, located at the focus of an ellipse, in the direction of a tree A, is that of direction towards A' when distance has no effect, A' being a point of the ellipse in direction of A, and is proportional to the length of segment OA'. It depends thus on the orientation $\theta - \phi$ of the point A' to the long axis and on the flatness $\alpha$ (width/length) of the ellipse, and was:

$$OA' = \alpha^2 L / 2(1 - \sqrt{1 - \alpha^2 \cos(\theta - \phi)})$$.
Table I. Comparison of allele frequencies in pollen received by ramet 2 of clone 64, and those expected according to the genotypes of surrounding trees located at a maximal distance from the receptor tree (evenly weighted trees, self-pollen, 5% excluded) at the locus G6pdh (492 outcrossed seeds).

<table>
<thead>
<tr>
<th>Maximal distance of surrounding trees (m)</th>
<th>Expected allelic frequency in pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>0.029**</td>
</tr>
<tr>
<td>20</td>
<td>0.018</td>
</tr>
<tr>
<td>25</td>
<td>0.013</td>
</tr>
<tr>
<td>30</td>
<td>0.008</td>
</tr>
<tr>
<td>35</td>
<td>0.006</td>
</tr>
<tr>
<td>40</td>
<td>0.006</td>
</tr>
<tr>
<td>Observed frequencies</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Significant deviation at the 0.05*, 0.01** and 0.001*** levels, respectively.

Fig 2. Distribution of the G6pdh alleles surrounding the ramet 2 of clone 64 (G6pdh2 or G6pdh3). ---→ high pollen flow; .......→ low pollen flow; -; common allele (G6pdh2 or G6pdh3).
the tested conditions. The distance of efficient pollination was about 15–30 m.

**Preferential direction of pollen dispersal**

Although intensely male-flowering, certain ramets close to the tested female fertilized relatively few seeds. In contrast some low male-flowering and more distant ramets fertilized many seeds (fig 2). The pollen dispersal could not be considered as isotropic around the trees. Pollen dispersal according to an elliptical area was then considered.

When only pollinator trees located inside an ellipse (length of long axis, $L$, corresponding to a maximal pollination distance, $D$) were taken into account, dramatic variations of the expected allelic frequencies in pollen were observed according to $\varphi$, $\alpha$ or $L$. Variation of these parameters induced changes (presence or absence) of the pollinators taken into account and consequently in their genetic diversity. The orientation $\varphi$ of the ellipse appeared as a major factor. The expected allelic frequencies of pollen remained significantly different from the observed ones.

Pollinator trees were then only considered when their distance to the receptor tree was less than the maximal pollination distance $D$. The relative pollen flow from each pollinator was weighted according to its orientation $\varphi$. A weighting by the distance $d (d^{-1}$ or $d^{-2}$) between receptor and pollinator trees, or by the male-flowering intensity did not improve the expected allelic frequencies in pollen. The orientation $\varphi$ of the ellipse greatly influenced the expected fre-

<table>
<thead>
<tr>
<th>Maximal distance of surrounding trees (m)</th>
<th>Flatness ($\alpha$)</th>
<th>Ellipse orientation ($\varphi$)</th>
<th>Expected allelic frequencies in pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>0.40</td>
<td>0</td>
<td>0.008</td>
</tr>
<tr>
<td>15</td>
<td>0.40</td>
<td>$\pi/2$</td>
<td>0.005</td>
</tr>
<tr>
<td>15</td>
<td>0.20</td>
<td>0</td>
<td>0.003*</td>
</tr>
<tr>
<td>15</td>
<td>0.20</td>
<td>$\pi/2$</td>
<td>0.002*</td>
</tr>
<tr>
<td>25</td>
<td>0.40</td>
<td>0</td>
<td>0.003*</td>
</tr>
<tr>
<td>25</td>
<td>0.40</td>
<td>$\pi/2$</td>
<td>0.003*</td>
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<td>35</td>
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<td>$\pi/2$</td>
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</table>

Observed frequencies: 0.014 | 0.504 | 0.362 | 0.190 | 0.010

Significant deviation at the 0.05*, 0.01** and 0.001*** levels, respectively.
Fig 3. Concordance of observed allelic frequencies of pollen gametes (selfing excluded) and those expected from the ellipse model of pollen dispersal applied to ramet 2 of clone 64 in the Bout seed orchard at G6pdh locus, according to (a) the orientation $\phi$ of the ellipse ($\alpha = 0.05$) and (b) to the flatness $\alpha$ of the ellipse ($\phi = \pi/2$) when each tree surrounding the receptor (within a maximal distance $D$ to pollinator tree) was taken into account (degrees of freedom: 2 for $D = 15$ m and $D = 20$ m, 4 for other distances). $\cdots \cdots \alpha = 15$ m; $\cdots \cdots \alpha = 20$ m; $\cdots \cdots \alpha = 25$ m; $\cdots \cdots \alpha = 30$ m; $\cdots \cdots \alpha = 35$ m.
quencies especially when the maximal distance of pollination was reduced to 15 m (table II). Pollen dispersal occurred along rows of trees. This preferential direction was the same whatever the considered distance of pollination up to 35 m (fig 3a). The flatness $\alpha$ of the ellipse also significantly influenced the expected frequencies up to a pollination distance of 25 m (fig 3b). The best agreement between expected and observed frequencies was obtained with a flatness of about 0.4 and a distance of pollination of 15–20 m (ramet 2 of clone 64 and ramet 1 of clone 95) and 30 m (ramet 1 of clone 64). Allelic frequencies expected from the ellipse model were close to those observed for each allele (table II).

In these conditions, ramets received pollen essentially from a small number of pollinators (less than 10 trees: 5–7 according to the ramet). The number of live trees was higher around ramet 1 of clone 64 (101 trees at less than 37 m) than around the ramet 1 of clone 95 (74 trees at less than 37 m). A higher density of trees did not seem to reduce the pollination distance.

The multilocus analysis applied for ramet 1 of clone 95 allowed a more precise analysis because of the possible identification of some clones according to their gametes. In fact, only 10% of gametes allowed identification of unique parental clone. None of the gametes from these parental clones could be identified and trees with a large pollinic contribution could not be located. Several trees on the same side did not produce any progeny in spite of their large production of pollen. About 10% of the identified pollen came from clones more than 25 m away. A larger part (20%) of the pollen might not be produced by the clones according to their assessed genotypes. It probably came from flowering rootstocks (in 7 clones out of 21, the ramets of the same presumed clone did not show one single genotype; Prat and Caquelard, 1995).

In the 3 ramets analysed, the overrepresented pollen came from the same direction and could be attributed to westerly dominant wind. Wind might be more important for pollen dispersal than the tree distance.

DISCUSSION

Pollination distance

Erikson and Adams (1989) have shown that very little pollen was effectively dispersed beyond 30 m in a Douglas fir seed orchard in Washington State. In a Pinus pinaster seed orchard the dispersal of pollen was restricted to about 10 m (Baradat et al, 1984). In the present study at least 10% of pollen came from more than 25 m; most of the pollen was dispersed within 20–30 m.

Because of its dilution in the pollen pool, the pollen dispersal was not detected beyond some tens of meters. The pollen produced by a tree might partly diffuse around this tree (where it could be detected) and partly diffuse very far, after suspension in atmosphere (and become undetectable because of dilution). Moreover its viability after a long migration was probably reduced.

Elliptical dispersal of pollen

Baradat et al (1984) previously used an ellipse for representation of pollen dispersal. However in their case, trees were not considered at a focus but at the centre of the ellipse. This could result from the orchard design because the distance between the trees was not the same between and within the rows. In such a design, pollen dispersal was not the same between and within
rows because of the physical presence of trees. In their model, Baradat et al (1984) analysed the possible origin of pollen from different sectors of an elliptical area in order to recognize some preferential directions of pollen flow. The number of parameters required for the complete description of pollen dispersal was higher than in the present study. Baradat et al (1984) showed that pollen flow occurred mainly along the tree rows.

As found from the 3 ramets of the Bout seed orchard, consideration of a preferential direction of pollen flow improved the assessment of allelic frequencies. In ramet 2 of clone 64, considering the ellipse model of pollen dispersal, no significant difference between observed and expected allelic frequencies was noticed for 1 set of parameter values. This was not the case considering a circular pollen dispersal. In ramet 1 of clone 64, when allele G6pdh\(_1\) was not taken into account (no source of this allele up to 45 m), similar results were obtained. This allele may belong to the 10–20% of pollen coming from more than 25 m as observed in ramet 1 of clone 95, or it may come from mislabelled trees since such trees exist in the seed orchard (Prat and Caquelard, 1995).

The direction of pollination was the main effect on the pollen flow in the Bout seed orchard. Even in a regular design, the elliptical area of the pollen dispersal appeared suitable. As in the general situation, phenology is a major component of the mating system of the seed orchard and considerations of the elliptical dispersal of pollen and phenology (as taken into account by Erickson and Adams, 1989) can be combined.

**Preferential crosses**

Apsit et al (1989) suggested that a selection took place to explain the distortion observed in controlled crosses. But Webber and Yeh (1987) observed that the first pollen grain arriving on the ovule was the fertilising one. This does not seem to always be the observed situation (Prat, unpublished results). According to the first-on first-in hypothesis of Webber and Yeh (1987), no gametic selection occurred and the non-random effects (deviation from the expected probability of gamete association) resulted from heterogeneous pollen flow. Preferential crosses observed between more distant trees in the Bout seed orchard might result from non-random mating. The general orientation of the pollen flow is sufficient in the Bout orchard to explain the observations.

The small distance of pollination and the highly oriented pollen flow resulted in a small number of major pollinators (less than 10) per tree. This reduced genetic mixing of pollen and might alter the Hardy–Weinberg equilibrium. The preferential crosses between neighbour trees affected the mating system in the orchard. This might explain the lack of heterozygosity observed in the orchard and not due to selfing.

**Seed orchard design**

The distance between receptor and pollinator trees was not a major factor in the pollen dispersal up to about 25–30 m. The minimal distance of ramets belonging to the same clone in a seed orchard should thus be of the same amount to avoid intra-clonal crosses. When the distance between and within rows is the same, square blocks with a minimal size of 30–35 m would be the best.

In the Bout seed orchard, mating system was mainly affected by crosses between neighbour trees. Since the ramets were distributed randomly in the seed orchard, the surrounding ramets of a clone varied from one block to another and each clone could be statistically pollinated by all other clones. Nevertheless none of the
neighbour effects were suppressed by random distribution of clones and an increase of the homozygosity level in seed crop was observed. An improved orchard design should include all combinations of clone neighborhood in order to maximise panmixia, as suggested by Vanclay (1986, 1991).

CONCLUSION

The model of pollen dispersal according to an elliptical area allows for the detection of the orientation of dispersal and the number of major pollinating trees. This model is more efficient than circular pollen dispersal in prediction of pollen allelic frequencies. Trees were pollinated by few surrounding trees with a large influence of wind direction. The result is a lack of pollen flow between each clone and the occurrence of preferential crosses, and consequently a lack of heterozygosity in the crop, in spite of the random design of clones in every block.

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