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Title: The Influence of Distance and Floral Phenology on Pollen Gene Flow and Mating System Patterns in a Coastal Douglas-fir Seed Orchard.

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Isozyme analysis was used to investigate pollen gene flow and mating system patterns in a 15 year-old Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) seed orchard. The specific objectives of the study were to: (1) determine the relative influence of distance and floral phenology on cross-pollination patterns, and (2) compare the rates of outcrossing among maternal parents with differing phenological characteristics.

Unique single-locus allozyme marker alleles occurring in two seed orchard clones were employed to study pollen dispersion from designated source trees to surrounding mother trees at varying distances and stages of phenological development. Among the sampled females, the estimated proportion of ovules fertilized by a marker ramet varied considerably (range 0 - 71.4 %). Multiple

regression analysis indicated that 66 percent of the variation in the frequency of marker gametes in the progeny of receptor females was explained by three variables: (1) the phenological likelihood of mating, a measure of the combined effects of male and female floral synchrony and the amount of competition from other seed orchard pollen sources, (2) the direction of floral synchrony (i.e., whether marker pollen shed was before or after peak female receptivity), and (3) a first order interaction between distance and the phenological mating likelihood.

Individual tree outcrossing estimates ($\hat{t}_{m_i}$) were significantly heterogeneous among the five maternal parents sampled, with $\hat{t}_{m_i}$ ranging from 0.50 to 1.07. The highest proportion of selfed progeny (50 %) was produced by an extremely early-flowering clone whose receptivity period was highly synchronous with the release of self-pollen. Clones that became receptive during the height of the pollination season and/or had little within-tree overlap in the timing of male and female flowering produced higher proportions of outcrossed progeny.

Overall, the data suggested that mating behavior in the seed orchard was far from random. Effective pollen exchange, rather, appeared to be strongly regulated by floral phenology, relative pollen fecundity, and the distance between mates.

The Influence of Distance and Floral Phenology on Pollen Gene Flow and Mating System Patterns in a Coastal Douglas-fir Seed Orchard

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In loving memory of my grandmother,
Nellie Miller Erickson

TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	7
Seed Orchard Description	7
Seed Crop Description and Sampling Procedures	8
Floral Phenology and Fecundity Surveys	10
Floral Phenology and Fecundity Analysis Procedures	13
Pollen Dispersion Analysis Procedures	15
Mating System Analysis Procedures	21
RESULTS	26
Orchard Pollen Production and Phenology Patterns	26
Pollen Dispersion Analysis	29
Mating System Analysis	38
DISCUSSION AND CONCLUSIONS	42
LITERATURE CITED	69

LIST OF FIGURES

Figure		Page
1.	Location of the Everett Seed Orchard block in Weyerhaeuser Company's Douglas-fir seed orchard complex south of Olympia, Washington.	54
2.	Geographic source of vegetative materials used in the establishment of the Everett Seed Orchard block.	55
3.	Assumed frequency distribution of mature seed-cone or pollen-cone flowers over time on an individual tree basis.	56
4.	Location of receptor ramets and other non-sampled ramets in the section of the Everett Seed Orchard surrounding the marker pollen source, a ramet of clone 635 carrying unique allele Gdh-2.	57
5.	Location of receptor ramets and other non-sampled ramets in the section of the Everett Seed Orchard surrounding the marker pollen source, a ramet of clone 616 carrying unique allele Idh-3.	58
6.	Illustration of overlap in the normal probability density functions of female receptivity with pollen shedding of a donor male.	59
7.	Histogram of the average pollen production classes among clones in the Everett Seed Orchard block.	60
8.	Histogram of the male and female midpoint Julian flowering dates among clones in the Everett Seed Orchard block.	61
9.	Histogram of the number of days between peak female flowering and the release of self-pollen among clones in the Everett Seed Orchard block.	62

Figure		Page
10.	Cumulative frequencies of gametes contributed by male marker clones 635 and 616 to pollen pools of receptor females with dates of peak flowering occurring before (-) and after (+) maximum pollen shed of the marker clone.	63
11.	Relationship between the predicted frequency of marker gametes (P_m) and the phenological likelihood of mating (LM_{mf}) as the distance from the pollen source increases from 7.5 m to 22 m.	64

LIST OF TABLES

Table		Page
1.	Estimated frequencies of gametes from male marker 635-5-18 in the pollen pools of receptor ramets (P_m), distances of receptors from the marker ramet, and differences between receptors and the marker in floral phenology.	65
2.	Estimated frequencies of gametes from male marker 616-5-56 in the pollen pools of receptor ramets (P_m), distances of receptors from the marker ramet, and differences between receptors and the marker in floral phenology.	66
3.	Fitted regression equation for explaining variation in the frequency of male marker gametes in the pollen pools of receptor females based on the distances between mates (DIST), and on the magnitude (LM_{mf}) and direction (PHEN) of their floral synchrony.	67
4.	Estimated individual tree outcrossing rates, $\hat{t}_{m_i}$, pollen fecundity classes, and within-tree differences in floral phenology for five mother trees in the Everett Seed Orchard.	68

The Influence of Distance and Floral Phenology on Pollen Gene Flow and Mating System Patterns in a Coastal Douglas-fir Seed Orchard

INTRODUCTION

Controlling parentage through the establishment and management of seed orchards has become an important means of producing reforestation seed of a desired genetic constitution. For seed orchards to achieve their potential, however, and be fully efficient from a genetic perspective, it's necessary that both the genetic superiority and broad genetic base of the orchard parents be reflected to a high degree in the seed progeny (Adams and Joly 1980, El-Kassaby et al. 1984). In theory, this requirement would be fulfilled if mating among orchard parents occurred at random, with all clones and mating combinations contributing approximately equally to the gene pool.

In wind-pollinated orchards, one factor influencing the degree to which panmictic conditions are achieved involves the extent and patterns of within-orchard pollen movement. Measurements of pollen dispersal in natural populations have indicated that the majority of the pollen dispersed from a point source is deposited at fairly short distances (Bateman 1950, Colwell 1951, Gregory 1945, Silen 1962, and Wright 1952). While few direct estimates are

available, the extent of pollen movement within seed orchards may also be quite limited, with preferential mating occurring within small neighborhoods. For instance, Shen et al. (1981), using allozyme markers to obtain estimates of pollen dispersion, found that as much as 31 percent of the pollen effective in fertilizing ovules of a given ramet can come from immediate neighbors if wind directions and flowering times are favorable. A pollen flight model developed by Sorensen (1972) predicted that 51.7 percent of the outcrossed ovules of a ramet are fertilized by the nearest eight trees in evenly-spaced orchards.

Since the replication and randomization of individual clones or families throughout a seed orchard may ultimately allow for the realization of all possible mating constellations (Sweet 1975, Muller-Starck 1982), a more significant factor affecting seed orchard cross-pollination patterns may be the amount of variability among orchard parents in the timing and duration of pollen release and female receptivity. For example, one study of early and late flowering individuals in a Douglas-fir seed orchard revealed differences in the midpoint dates of seed-cone and pollen-cone bud burst of up to 29 days (El-Kassaby et al. 1984). In terms of the duration of flowering, the bulk of pollen shedding and female receptivity for individual

Douglas-fir trees usually occurs within a 4 to 9 day period under normal climatic conditions (Owens et al. 1981, Barner and Christensen 1962). These data, in combination with findings of minor phenological differences among individuals within clones or families (Jonsson et al. 1976; El-Kassaby et al. 1984; Wheeler, unpublished data), indicate that many seed orchard parents may have little or no overlap in flowering times.

As an illustration of the importance of considering reproductive phenology in seed orchard designs, Fashler and Sziklai (1980), simulating an orchard layout utilizing clones with widely ranging flowering times (37 days between earliest and latest flushing clones), determined that 36 percent of the total number of crosses had a high probability of occurrence, while 60 percent had little or no chance of occurring. The remaining 4 percent of the crosses had moderate degrees of phenological overlap.

Under natural (wind-pollinated) seed orchard conditions, large differences in floral phenology among individual clones or families may result in the production of a number of breeding sub-populations. The consequences of a phenologically subdivided breeding population are potentially quite significant, and include: (1) reductions in the genetic diversity of seed crops, (2) increased levels of inbreeding due to selfing or mating among sibs of

the same family, and (3) increased pollen contamination from unselected sources. Seed orchard efficiency may be further impaired by large amounts of clonal variability in strobilus production, which may amplify phenological differences and lead to the maintenance of orchard parents which either do not contribute or are disproportionately represented in the gene pool of harvested seed crops. This clonal imbalance may particularly be a problem in younger Douglas-fir seed orchards, where pollen production tends to be more variable and somewhat limited.

For those clones or families flowering out of synchrony with the majority of the orchard parents, there is an increased probability of mating among ramets of the same clone or sibs of the same family. The detrimental effects of selfing, in particular, on the quantity and genetic quality of Douglas-fir seed crops have been well documented, and include low self-embryo viability (Sorensen 1982), severe reductions in filled-seed proportions (Duffield 1950, Orr-Ewing 1954, Orr-Ewing 1957, Sziklai 1966, Piesch and Stettler 1971, Rehfeldt 1978), and reductions in seed germination, survival, and growth rate (Allen 1942, Orr-Ewing 1954, Orr-Ewing 1957, Rehfeldt 1978, Sorensen and Miles 1974, Sorensen and Miles 1982). Because of the severe depression in the survival and growth of progeny resulting from self-fertilization, it is important

to determine whether selfing rates vary among different phenological classes of orchard parents. This information may be useful, for example, in evaluating seed orchard designs or assessing the need for special management practices to enhance cross-pollination (e.g., supplemental mass pollination or the use of water spray applications to promote greater floral overlap among orchard parents).

Although there is some evidence that the proportion of viable selfed progeny can vary considerably among seed orchard parents (Shaw and Allard 1982, Omi and Adams 1986), very little is known about the influence of reproductive phenology on the level of outcrossing. In a recent Douglas-fir seed orchard study, El-Kassaby and Ritland (1986) estimated that the outcrossing rates of early and intermediate reproductive classes were significantly higher relative to the level of outcrossing of late flowering females. The authors associated the low outcrossing rate of the late flowering class with low levels of outcross pollen available at that time either within the orchard, or from surrounding natural stands.

The overall goal of the research described in this thesis was to investigate pollen gene flow and mating system patterns in a first generation Douglas-fir seed orchard. The specific objectives of the study were to: (1) determine the relative influence of distance and floral

phenology on cross-pollination patterns, and (2) compare the rates of outcrossing among maternal parents with differing floral characteristics. Electrophoretic techniques were utilized to achieve these objectives, with both unique single allele and multiple allelic combinations used as pollen markers.

MATERIALS AND METHODS

Seed Orchard Description

The study was conducted in the Everett Seed Orchard block, located in the southwest corner of Weyerhaeuser Company's Douglas-fir complex south of Olympia, Washington (Figure 1). The land surrounding the orchard is natural prairie, although there are scattered Douglas-fir trees approximately 1/2 km from the complex. A large contiguous Douglas-fir stand occurs 2 km to the southwest, the direction of prevailing winds. Within the orchard complex, individual blocks are separated by roads or grass buffers varying in width from 10-70 m.

The 5.3 ha Everett Seed Orchard block was established in 1970 with vegetative material collected from 120 field selected parent trees located throughout the Everett low-elevation (0-610 m) breeding zone in western Washington (Figure 2). The original orchard layout was according to a randomized complete block design with 20 replications, and a single grafted ramet of each clone (parent tree) in each block. Plant density of ramets was approximately 448 trees/ha, with ramets of the same clone separated by a minimum of 27.4 m. In 1983, when materials for the study were collected, spacing within the orchard was no longer uniform due to the loss of individuals from graft

incompatibility, thinning operations, and genetic roguing. At age 13, the 1010 ramets of 111 clones remaining in the Everett Seed Orchard had an average spacing of 56 m^2 (184 trees/ha) and averaged 10 m in height. The mean number of ramets per clone was 9, ranging from 1 to 17.

Seed Crop Description and Sampling Procedures

In the fall of 1983, cones for the study were collected from a total of 32 ramets chosen because of their proximity to, and distribution around, a selected ramet of either of two clones carrying a unique marker gene. Fifteen cones per ramet were collected by Weyerhaeuser harvesting crews from the midcrown portion of the tree on the side facing the designated marker ramet. Cones and seed were kept separate by female ramet throughout collection, processing, and storage.

Seed-cone and pollen-cone production in the Everett orchard block was generally good in 1983, with ramets responding to both favorable climatic factors and various flower induction treatments applied the previous year. The 1983 seed crop represented the highest yields (1.2 kg/ha) recorded in the Everett Orchard's six-year production history. Although the sampled seed crop was the result of wind-effected pollination, natural pollen dispersal patterns were modified by the use of pollen boosting

techniques in spring 1983. The treatment, applied every other day, subjected the lower crowns of ramets in alternate orchard rows to an "Orchard Master" air blaster. The effect of the blaster was to spray large volumes of air in an upward and outward direction, increasing air movement and assisting in pollen dispersion. The blast of air produced by the equipment used in this procedure extended approximately 11 m beyond the treated tree, and as much as 20 m in a vertical direction (personal communication, D. Hodgins, Weyerhaeuser Company).

Pollen production in the natural Douglas-fir trees surrounding the orchard complex was quite light in 1983. Background pollen catch data indicated that the influence of contaminating pollen sources on the 1983 Everett seed crop was fairly low, with less than 15 percent of the seed produced by Everett Seed Orchard parents estimated to have been fertilized by pollen from surrounding natural stands (Wheeler and Jech 1986). Although not quantified, the opportunity for pollen contamination from other seed orchard blocks within the complex was likely reduced by cone induction treatments, which greatly enhanced pollen production in the Everett Seed Orchard relative to other untreated blocks. The impact of other-orchard pollen should have been further diminished by: (1) prevailing wind patterns, which would have carried contaminating pollen

away from the Everett block, and (2) phenological observations which indicated that in 1983, clones in the Everett Seed Orchard flowered 7 to 8 days earlier relative to parents in the other three orchard blocks (personal communication, N. Wheeler, Weyerhaeuser Company).

Floral Phenology and Fecundity Surveys

During the 1983 pollination season, Weyerhaeuser personnel collected data from a single ramet of each orchard clone to obtain information on clonal variation in the timing and duration of pollen shed and female receptivity. Ramets were selected on the basis of their large crown size and overall vigor, and on the availability of male and female strobili. The phenological status of floral buds was monitored up to three and four days per week throughout the flowering period (ca. March 22 - May 9). At each census, the male and female strobili of selected ramets were visually assessed to determine the dates at which 25, 50, and 75 percent of the female strobili had become receptive or male strobili had dispersed pollen. Conelets were considered receptive when they had emerged approximately one-half of the way out of their bud scales (the "B+O" stage, according to Owens et al. 1981). Pollen-cones were considered mature when the

pollen sacs had begun to split and the slight jarring of male strobili resulted in the release of pollen grains.

It should be noted that the phenology data obtained from the one selected ramet per clone were not necessarily acquired from the same ramets included in the present study. In analyses involving these data, however, it was assumed that the phenological observations were accurate reflections of the floral bud development of all other ramets of the same clone. Evidence from previous studies involving both Scots pine (Jonsson et al. 1976) and Douglas-fir (N. Wheeler, unpublished data) appear to substantiate this assumption. In examining the flowering phenology of 4 ramets of each of 15 Scots pine clones over four pollination seasons, Jonsson et al. (1976) reported non-significant within-clone variance ratios for the onset of pollen release, as well as for the duration of both pollen shedding and peak female receptivity periods. Analysis of variance results indicated that the onset of the peak receptivity stage in Scots pine was somewhat more variable, however, with two of the four flowering seasons showing weakly significant ($P < 0.10$) within-clone variation.

Wheeler (unpublished data) investigated the patterns and extent of phenological variation within and among Douglas-fir clones in the Everett and Cascade blocks of the

orchard complex during the 1983 flowering season. Analysis of variance of the timing of male and female flowering indicated that the variation attributable to ramets within clones, although statistically significant, was extremely small relative to the clonal source of variation. Within versus between clone variation was 2.6 versus 59.6 percent for the timing of male flowering, and 4.7 versus 69.0 percent for the timing of female flowering. The remainder of the variation in floral phenology was attributed to vertical crown position and interactions between crown position and clones, and ramets within clones.

All ramets in the Everett Seed Orchard were assessed and scored for pollen production in mid-April, 1983. During the field surveys, the abundance of male flowering was based on a four-class scheme, with individual ramets classified as heavy, medium, light, or very-light pollen producers. For analysis purposes, the relative level of pollen output was approximated for each orchard parent by converting the subjective field scores to a quantitative scale. Ramets originally classified as heavy pollen producers were assigned a score of 4; medium, light, and very-light pollen producers were assigned relative production values of 3, 2, and 1, respectively. There was no metric relationship between fecundity classes.

Floral Phenology and Fecundity Analysis Procedures

Observations involving the dates of 25, 50, and 75 percent flowering were used to estimate the mean and standard deviation of each orchard clone's flowering period. These parameters were then used to specify density distribution functions that described the progress of pollen anthesis and female-cone receptivity over time. For individual clones, the assumption was made that the frequency distribution of mature flowers closely approximated a normal probability distribution (Figure 3). Although normality of the phenology data used in the present study could not be tested, evidence for the assumption is provided by a phenological study conducted in 1983 (Wheeler, unpublished data) involving three ramets of each of nine clones represented in the Everett and Cascade orchard blocks. Chi-square goodness of fit tests (Snedecor and Cochran 1980) were performed by this author on the data from these nine Douglas-fir clones to compare the observed frequency distribution of receptive seed-cone buds with the theoretical Gaussian distribution. Chi-square test statistics were non-significant for all nine clones ($P < 0.05$), indicating that female flowering over time may be approximated by a normal probability distribution. Although involving other species, floral phenology data presented in Eriksson et al. (1973) and Jonsson et al.

(1976) also suggest that for individual clones, the progression of pollen dissemination and female receptivity is approximately normal.

For each clone in the Everett Seed Orchard, the date that 50 percent of the reproductive strobili had shed or become receptive to pollen was used to estimate the date of mean or peak flowering, μ. The standard deviation of a clone's flowering period, σ, was approximated from the 25 and 75 percent flowering dates using the properties of the standardized normal distribution. For example, assuming normal phenology data, the number of days to 25 percent flowering (x_{25}) and 75 percent flowering (x_{75}) is equal to $\mu - z\sigma$ and $\mu + z\sigma$, respectively, where $z = 0.67$ (Table A3, Snedecor and Cochran 1980). Solving for σ in each case and taking the mean, the computing formula used to estimate σ for each clone was:

$$\hat{\sigma} = \frac{(x_{75} - x_{25})}{2(0.67)} \quad . \qquad (1)$$

Once the mean and standard deviation of the flowering periods were specified for each orchard parent, the equation for the standardized normal distribution was employed to generate a probability density curve for describing the distribution of receptive seed-cones or shedding pollen-cones over time. Thus, the density of

mature male or female flowers at time x for the i^{th} clone was estimated according to the distribution function:

$$Y = \frac{1}{\hat{\sigma}\sqrt{2\pi}} e^{[-\frac{1}{2}(x-\hat{\mu}/\hat{\sigma})^2]}, \quad (2)$$

where Y = height of flowering density curve corresponding to an assigned value of x.

$\hat{\sigma}$ = estimated standard deviation of length of male or female flowering period.

$\hat{\mu}$ = estimated date of peak (50%) pollen shed or female receptivity.

x = Julian date during the 1983 pollination season.

Pollen Dispersion Analysis Procedures

General Approach. Unique single-locus allozyme marker alleles occurring in two seed orchard clones were employed to study pollen gene flow patterns from a point source to surrounding mother trees at varying distances and stages of phenological development. Floral observations were used to estimate the phenological likelihood of mating between male marker ramets and receptor females. These data, along with information on the mapped positions of neighboring ramets, were then used in regression analyses to examine the relationships between distance between mates and their coincidence in flowering, and the frequencies of marker

gametes in sampled pollen pools (pollen effective in fertilizing ovules of resulting filled seeds).

Seed sampling and electrophoretic methods. While conducting electrophoretic surveys of Everett orchard parents, Jech and Wheeler (1984) identified clone 616 as being heterozygous for a unique allele (*Idh-3*) at the *Idh* (isocitrate dehydrogenase) locus, and clone 635 as heterozygous for a unique allele (*Gdh-2*) at the *Gdh* (glutamate dehydrogenase) locus. Although these two alleles occurred in no other parents in the Everett block, they were both present at very low frequencies in the Twin Harbor Seed Orchard block to the north. Neither allele has been observed in trees from the surrounding natural stands (personal communication, N. Wheeler, Weyerhaeuser Company).

For the purpose of the study, one ramet of each clone, 635-5-18 (clone-row-column) and 616-5-56, was selected as a marker pollen source. These individuals were chosen primarily on the basis of their overall vigor and heavy pollen production relative to other ramets of clones 616 and 635. Seed crops were sampled from 17 neighboring ramets of marker 635-5-18 (Figure 4), and from 21 trees at varying distances and directions from ramet 616-5-56 (Figure 5).

To estimate gametic contributions of marker pollen parents, embryos of seed were assayed electrophoretically for the presence of the specific marker alleles (Gdh-2 or Idh-3). Tissue preparation and electrophoretic procedures followed those outlined by Neale et al. (1984) and Merkle and Adams (1987). Overall, 5586 seeds were assayed; the number of seed sampled from each receptor averaging 147 (range 66-206).

For each sampled maternal parent (receptor), the observed frequency of the marker allele in the pollen pool was computed by summing the number of unique Idh-3 or Gdh-2 alleles and dividing by the total number of embryos assayed. As both marker clones were heterozygous at the locus under consideration, only one-half of the embyros fertilized by pollen from the marker clones were expected to carry the unique allele. Therefore, to estimate the proportion of embryos resulting from fertilization by marker clones, the observed frequency of embryos with marker alleles was multiplied by two.

It was assumed during data analysis that all unique marker alleles originated from the designated marker ramets. The contribution of pollen gametes from alternative ramets of clones 635 and 616 was considered minimal due to the combined effects of the seed sampling methods and the design of the Everett Seed Orchard block.

For instance, while the distances to the 17 receptors surrounding marker 635-5-18 ranged from 6.1 to 38.1 m ($\bar{x}$ = 18.7 m), the distances separating the other 16 ramets of clone 635 from these females was much greater. Other than the marker ramet, the closest ramet of clone 635 to any sampled receptor was 18.2 m away (ramet 635-1-19, Figure 4). In sharp contrast to the designated marker ramet, this individual was classified in field surveys as a very-light pollen producer, further reducing its reproductive influence on the pollen pools of receptor females. The average distance of sampled females from the second marker pollen source, ramet 616-5-56, was 24.3 m (range 9.5 - 40.9). Ten other grafts of clone 616 occur in the Everett Seed Orchard; one light pollen producer (ramet 616-10-54, Figure 5) was 6.1 m from a receptor individual. The distances of the nine remaining ramets of clone 616, however, were all greater than 30 m from any of the females assayed for Idh-3 contributions. Cone collection procedures which restricted sampling to the side of a maternal tree facing the designated marker ramet should also serve to reduce the impact of other pollen sources carrying the unique Idh or Gdh alleles.

Estimation of the phenological likelihood of mating between male marker ramets and receptor females. The phenological likelihood of each marker-receptor crossing

combination was estimated based on: (1) the relative pollen fecundity of the marker ramet, (2) the amount of congruence between male marker and female receptor flowering periods, (3) the relative pollen fecundity of all other ramets occurring in the Everett Seed Orchard, and (4) the amount of phenological overlap of the female receptor with all other competing pollen sources within the Everett Orchard. The phenological likelihood of female receptor f mating with male marker ramet m was estimated as:

$$LM_{mf} = \frac{C_{f(j=m)} \times F_{(j=m)}}{\sum_{j=1}^{r} (C_{fj} \times F_j)}, \qquad (3)$$

where F_j = relative pollen fecundity of the j^{th} ramet in the Everett orchard.

C_{fj} = proportion of the receptivity period of female f coincident with the pollen shedding period of ramet j.

r = total number of ramets in the Everett Orchard.

F_j was estimated by the pollen production score of the j ramet obtained in the flowering survey. C_{fj} was estimated by the area under the normal probability density function for female receptivity of f that was overlapped by the pollen density function of male j (Figure 6).

Regression Models. Multiple regression methods were employed to examine the relationship between the

estimated frequency of male marker gametes (P_m) in the pollen pools of receptor females and various phenological and spatial parameters. The data sets representing the two groups of receptor females were pooled and analyzed collectively. Phenological data for the receptivity periods of females 718-5-16 and 701-5-62 were not available and, as a consequence, only 36 observations were included in the regression analysis. Prior to analysis, the P_m for each receptor ramet was transformed using an angular transformation (Snedecor and Cochran 1980). Stepwise variable selection procedures in the SYSTAT computer statistical package were utilized for model building, with the criteria for variable entry and exclusion set at probability levels of 0.05 and 0.10, respectively. The variables included in the full linear model were:

$$y_m = b_o + b_1 DIST_{mf} + b_2 LM_{mf} + b_3 (LM_{mf} \times DIST_{mf}) + b_4 PHEN_{mf} + b_5 SIN\theta_{mf} + b_6 COS\theta_{mf} ,$$

where y_m = arcsin $\sqrt{P_m}$

$DIST_{mf}$ = distance (m) between male marker m and female receptor f.

LM_{mf} = phenological mating likelihood for male marker m and female receptor f.

$PHEN_{mf}$ = 1 if the date of peak female receptivity (μ_f) occurred after the date of maximum marker pollen shed (μ_m), 0 otherwise.

$SIN\theta_{mf}$ = sine of the azimuth angle between male marker m and female receptor f.

$COS\theta_{mf}$ = cosine of the azimuth angle between male marker m and female receptor f.

Mating System Analysis Procedures

General approach. Multilocus genetic information from the seed progeny of five maternal parents was used in conjunction with mating system models to estimate, on an individual tree basis, the proportion of progeny due to self-fertilization. Phenological models presented earlier provided a method for assessing each maternal parent's phenological potential for selfing. Descriptive measures were then employed to examine the correspondence between observed floral characteristics and the estimated proportion of viable selfed progeny.

Seed sampling and electrophoretic methods. Of the 32 ramets from which seed was originally collected, the maternal parents selected for mating system analysis included: 643-7-19, 695-5-13, 673-6-20, 639-8-14, and 634-5-28. These particular mother trees were chosen primarily on the basis of seed availability and their different phenology patterns. Ramets 634-5-28 and 643-7-19 were included because their floral phenology suggested a high potential for selfing (i.e., high correspondence between time of self-pollen release and female receptivity). To the extent possible given seed

constraints, females were also selected to represent different phenological classes.

For each of the five mother trees, estimates of the proportion of viable progeny due to self-fertilization were based on the multilocus electrophoretic analysis of germinated seed progeny. Two types of information were required for the estimation of outcrossing rates from electrophoretic data: (1) the multilocus genotype of each mother tree, and (2) the multilocus genotypes of the pollen gametes successful in the fertilization of viable embyros. Multilocus genotypes of the five maternal parents were obtained from Jech and Wheeler (1984), but were later confirmed by observing the segregation of alleles in the megagametophytes of each ramet. The multilocus genotype of each pollen gamete effective in fertilization was inferred by assaying both the haploid megagametophyte and the diploid embryo of each seed progeny. Using electrophoretic methods described in Merkle and Adams (1987), an average of 90 seeds (range 86-96) per parent were assayed, for a total of 425 offspring.

The genotypes of seed tissues were determined at 11 allozyme loci: Pgm (phosphoglucomutase); Lap1 and Lap2 (leucine aminopeptidase); Gdh (glutamate dehydrogenase); Got 1, Got2, and Got3 (glutamate-oxaloacetate transaminase); Cat (catalase); 6-PGD (6-phosphogluconate

dehydrogenase); Idh (isocitrate dehydrogenase); and Dia (diaphorase). Details of the banding patterns of these allozymes and analysis of their Mendelian genetics are found in Neale et al. (1984) and Merkle and Adams (1987).

Individual-tree multilocus estimates of outcrossing. The multilocus estimator described in Green et al. (1980) and applied in Neale and Adams (1985) and Omi and Adams (1986) was used in determining proportions of outcrossed progeny for individual maternal parents ($\hat{t}_{m_i}$). This method is based on the mixed mating model of Fyfe and Bailey (1951) which, as applied here, assumes that: (1) each viable seed is the result of either random outcrossing (with probability $\hat{t}_{m_i}$) or self-fertilization (with probability $1-\hat{t}_{m_i}$), (2) the probability of an outcross is independent of the genotype of the maternal parent, and (3) no selection occurs between germination and the census of seed tissue. In this estimation procedure, the multilocus genotype of a pollen gamete is compared to the genotype of the maternal parent, with the mating then assigned to either a detectable outcross class or an ambiguous class. Detectable outcrosses arise when a pollen gamete carries an allele at any one or more loci that could not have been contributed by the maternal parent. The ambiguous class includes progeny resulting from either: (1) self-fertilizations, or (2) fertilizations by pollen

parents which carry alleles identical to those carried by the maternal parent, over all loci scored. The outcross pollen pool allele frequencies used in the calculation of $\hat{t}_{m_i}$ were obtained using the single-locus estimation procedure and computer programs described in Neale (1986). A separate set of pollen pool allele frequencies was estimated for each of the five mother trees. Differences in outcrossing among the maternal parents were tested using Fisher's heterogeneity chi-square test (Rao 1973).

Estimation of the phenological likelihood of selfing. For each of the five mother trees, the phenological likelihood of mating of self-pollen relative to all other pollen sources in the Everett Seed Orchard was estimated as:

$$LM_{mf} = \frac{C_{f(j=f)} \times F_{(j=f)}}{\sum_{j=1}^{r} (C_{fj} \times F_j)}, \qquad (4)$$

where F_j = relative pollen fecundity of the i^{th} ramet in the Everett Orchard.

C_{fj} = proportion of the receptivity period of female f coincident with the pollen shedding period of ramet j.

r = total number of ramets in the Everett Orchard.

Similar to the phenological mating likelihood derived earlier for marker and receptor parents, F_j was estimated

by the pollen production score of the j^{th} ramet obtained in the flowering survey. $C_{f(j=f)}$ was estimated by the area of overlap between the density function describing the receptivity period of female f and the normal probability density for self-pollen release.

RESULTS

Orchard Pollen Production and Phenology Patterns

Pollen production in the Everett Seed Orchard was generally good in 1983, with 105 of the 111 orchard clones (95 %) producing male strobili. Calculations of the average pollen fecundity among clones (Figure 7) indicated that the majority of orchard parents produced appreciable amounts of pollen in 1983. For instance, a total of 24 and 69 percent of the clones were represented by average pollen production scores that corresponded to medium and heavy fecundity classes, respectively. Less than 1 percent of the clones produced light or very light pollen crops.

Observations on the floral phenology of the Everett Orchard clones indicated that the 1983 flowering season was quite extended. Among the 105 producing clones, the overall period of pollen release was approximately 42 days, extending from March 22 to May 3. The total length of the receptivity period of the 101 producing females was slightly shorter (37 days), with flowering continuing from March 21 through April 28.

Ranges among clones in the midpoint (50 %) anthesis and receptivity dates were also quite large, reaching 26 days for females and 28 days for males (Figure 8). The average midpoint flowering date was April 7 (day 97) for

females, and April 9 (day 99) for males. The duration of individual flowering periods was relatively short, with standard deviations averaging 2.4 days for seed-cone receptivity (range 1.5 to 4.4) and 2.7 days for pollen release (range 1.5 to 5.9).

Approximately 62.9 percent of the orchard males and 64.4 percent of the females reached peak flowering within 1σ of the average midpoint date of pollen release or seed-cone receptivity, indicating that the majority of the orchard parents were intermediate in their flowering phenology. The maximum proportion of clones reaching peak flowering on a given date, however, never exceeded 12 percent (Figure 8). This percentage was greatly reduced in the early and late stages of the flowering season, revealing that the bulk of the dispersing pollen and mature seed-cones during these times were contributed by a very restricted number of clones.

The male and female flowering periods of orchard clones were somewhat offset, with the proportion of females reaching peak receptivity exceeding the proportion of pollen shedding males throughout the early to mid-stages of the flowering season (Figure 8). This trend then reversed itself in the latter part of the season. Because the majority of female strobili had already been pollinated, FIgure 8 suggests that very late-flowering males were not

well-represented in the orchard pollen pool. In the case of extremely early-flowering females, it's possible that a large portion of the available pollen cloud consisted of either self-pollen and/or contaminating pollen from non-orchard or other-orchard sources.

Observations involving the timing of pollen shed and seed-cone receptivity on the same tree indicated that the overlap of male and female flowering periods varied considerably among the Everett Seed Orchard clones in 1983. For some of the parents, the timing of seed-cone receptivity and the release of self-pollen was widely divergent, with differences between sexes in the midpoint flowering date reaching up to 15 days (Figure 9). Twelve of the 101 clones which produced both male and female strobili had anthesis and receptivity periods that were completely overlapping, however, and 61 percent of the Everett clones had male and female midpoint flowering dates that were less than 3 days apart. The average amount of floral overlap between the sexes within a tree was estimated to be 55 percent over all clones. In general, seed-cone receptivity within a clone generally preceded pollen shed. In 60 percent of the Everett clones, the midpoint date of seed-cone receptivity occurred prior to the date of peak pollen shed.

Pollen Dispersion Analysis

Phenological likelihood of mating between male marker ramets and receptor females. The two clones used as pollen sources in the study differed in both the timing and duration of pollen shed. Clone 635 peaked in pollen dispersal 11 days earlier than clone 616, and had a anthesis period only one-half that of clone 616 (i.e., σ = 3.0 vs. 6.0). Both marker ramets were classified as heavy pollen producers in 1983.

The range in the dates of peak female receptivity was 23 days among the receptor ramets sampled around marker 635-5-18, and 18 days among the neighboring females of marker 616-5-56. In general, the duration of female flowering for an individual receptor was quite brief relative to the range among clones, with a mean standard deviation of 2.2 days (range = 1.5 to 3.7).

As a result of the early anthesis period of clone 635, maximum female receptivity of receptors generally lagged behind peak pollen shed of the marker ramet by 3 to 16 days (Table 1). Two exceptions to this general pattern were receptors 656-2-18 and 634-5-28, with maximum receptivity occurring 3 and 7 days prior to peak pollen shed of the 635 marker.

The proportion of the female receptivity period in receptors that was coincident with pollen dispersal in marker clone 635 (C_{fm}) ranged from 0 to 100 percent (Table 1). In half of the cases C_{fm} was greater than 40 percent, indicating that the potential for clone 635 to contribute to pollen pools was relatively high. Actual contributions were probably lowered, however, by competition from pollen produced by other clones in the orchard. The phenological likelihood of mating (LM_{mf}) provided a measure of the combined effects of floral overlap and pollen competition. LM_{mf} values for marker ramet 635-5-18, ranging from 0 to 0.0049 (Table 1), indicated that if only these two factors determined mating success, gametes from this ramet would not be expected to comprise more than 0.5 percent of the pollen pools of receptor females.

In contrast to the first group of receptors, the females surrounding 616-5-56 were predominantly early flowering, with the date of maximum receptivity occurring as early as 14 days prior to peak marker pollen shed (Table 2). Despite the apparent asynchrony, male and female flowering density curves frequently coincided, as evidenced by C_{fm} values that ranged from 10.64 to 98.87 percent. For these mating combinations, floral synchrony was attributed primarily to the extended pollen shedding period of clone 616. Phenological likelihoods of mating (LM_{mf}) in this

second group of marker and receptor pairs ranged from 0.0007 to 0.0039 (Table 2).

Observed pollen dispersion from marker ramets. Estimated proportions of embryos resulting from fertilization by marker ramet 635-5-18 ranged widely (0 - 71.4 %) among the 17 receptor ramets sampled (Table 1). In general, higher frequencies of marker alleles were observed among the progeny of receptors which had higher LM_{mf} values and that occurred in close proximity to the marker ramet. Receptor ramet 662-4-18, having the highest estimated frequency of progeny sired by the marker ramet, was located a distance of only 7.3 meters away and coincided completely in flowering period with the pollen marker. The next largest gametic contributions from the 635 marker were observed in the pollen pools of ramets 643-7-19 (P_m = 0.2233), and 645-2-23 (P_m = 0.1037). Although these two female receptors had the same high degree of floral synchrony with the marker (C_{fm} = 45.86 %), the difference in P_m may largely be explained by the distances which separated them from the marked pollen source (14.9 vs. 26.7 m). It should be emphasized, however, that electrophoretic results refer only to successful gametes and viable seed. As a consequence, the confounding impacts of gametic or zygotic viability selection, as well as incompatibility problems or other

mating specific effects, may possibly be included in the results.

In most cases, the detected frequencies of pollen from the marker ramet were considerably higher than that predicted based on LM_{mf}. Because the computation of LM_{mf} assumed that the competition from alternative pollen sources was dependent only on the degree of phenological overlap and relative pollen production of ramets in the orchard, the results indicated that distance may be an additional important factor in determining mating success. Moreover, the higher than expected representation of marker 635-5-18 in the pollen pools of sampled receptors suggests that sources of effective pollen within the orchard may be restricted to males within fairly limited distances from females. The effective number of mates for each female, therefore, would be less than the total number of ramets in the orchard, with the orchard comprised of a series of overlapping neighborhoods within which mating occurs preferentially. This finding agrees with the observation that no unique Gdh alleles were detected in receptors more than 30 m away from the marker pollen source.

Finally, the data provided little evidence for directional pollen flow; examination of the map locations of sampled females (Figure 4) indicated that the detection of Gdh-2 was somewhat greater in receptor ramets to the N

and NE of 635-5-18 than in other directions. Ramet 643-7-19, however, was located almost due south of the marker ramet and had an estimated 22 percent of its ovules fertilized by ramet 635-5-18.

With respect to the second group of maternal parents, the unique Idh allele of clone 616 was detected infrequently among the embryos assayed (Table 2). Moreover, 11 out of a total of 21 screened pollen pools lacked detectable gametic contributions from this marker pollen source. The greatest pollen contribution from marker 616, 14.81 percent, was observed in the progeny of ramet 701-5-62, a clone for which, unfortunately, no phenology data were available. The second highest gametic contribution, 13.1 percent, was observed in the progeny of receptor 704-3-58. This ramet was located 15.8 m from the pollen source and was one of the five receptor ramets whose peak flowering period occurred after the date of maximum marker pollen release. Females that had 616 pollen gametes in their progeny reached peak receptivity -3.8 days on average relative to peak marker pollen shed. In the case of females where no unique Idh alleles were detected, the average number of days separating peak male and female flowering was -8.0.

With the exception of one highly synchronous female, 682-5-68, no unique marker alleles were again detected in

pollen pools further than 30 m from the designated source (Table 2). Also similar to the first group of females was the impression that pollen movement was somewhat random with respect to direction. Corresponding to prevailing wind patterns, however, was the observation that the two females receiving the highest marker gametic contributions, ramets 701-5-62 and 704-3-58, were located to the N and NE of the marker ramet (Figure 5).

The results presented in Tables 1 and 2 suggested that in addition to the degree of synchrony between male and female flowering periods, mating success may also be dependent on the direction of the synchrony. For instance, the paucity of detectable 616-5-56 pollen gametes may have been due, in part, to the predominantly earlier flowering times of surrounding females. Further, because the anthesis period of clone 616 occurred at a time when the frequency of peak pollen shed among orchard clones was at a maximum (day 104, Figure 8), the heavy competition from alternative pollen sources may have also contributed to the marker ramet's lack of pollination success.

To illustrate the importance of phenological synchrony and, in particular, the direction of the synchrony to mating success, cumulative frequencies of pollen gametes contributed by male markers to receptor females were plotted relative to the number of days between peak marker

pollen shed and maximum female receptivity (Figure 10). The number of days between the culmination of male marker and female receptor flowering periods ranged from -12 to +16 (negative values correspond to females that reached maximum receptivity prior to the date of peak marker pollen shed). A separate cumulative frequency distribution was plotted for each male marker. Receptors 718-5-16 and 701-5-62 were excluded from the calculations for lack of phenology data.

Although other important variables such as distance and direction to the pollen source were ignored, a general trend apparent in Figure 10 was that of a sharp rise in both of the curves in the incidence of donor pollen as the dates of peak male and female flowering approached coincidence. Further, despite a vast difference in the shapes of the two curves, the data suggested that the reproductive success of both donor ramets tended to be much higher among synchronous females whose receptivity period occurred on or shortly after the date of peak pollen release.

Multiple regression analysis. Stepwise regression of the arcsin-transformed frequencies of male marker gametes in the pollen pools of receptor ramets (y_m) on the various phenological and spatial parameters showed that mating success of the marker ramets could primarily be explained

by three variables: (1) the phenological likelihood of mating (LM_{mf}), (2) the direction of floral phenology between receptors and pollen marker (peak pollen shed before/after female receptivity, PHEN), and (3) a first order interaction term calculated as the product of distance between mates and the phenological likelihood of mating (LM_{mf} x DIST) (Table 3). Distance, as a main effect, was not significant ($P > 0.10$) in the model. Spatial variables representing compass direction between receptor and marker ramets (sine and cosine of azimuth angle between mating pair) also lacked significance. The model R^2 was 0.66.

As was evident from the independent variables remaining in the model (Table 3), phenological attributes appeared to be the more important variables for explaining variation in y_m. The regression relationships indicated that the successfulness of a mating combination was largely dependent on the degree of floral synchrony between male and female flowering periods. The direction of the synchrony was also important, explaining over 8 percent of the variation in y_m. For a given female, the regression equation predicted that other things being equal, the most successful pollinators were males with anthesis periods that directly coincided or were just prior to maximum female receptivity (i.e., PHEN = 1). This result was

consistent with observations on the development of pollination mechanisms in Douglas-fir (Ho 1980, Owens et al. 1981), where the first pollen to arrive was found to have an advantage over equally viable pollen arriving later. This phenomenon was the basis of the "first come, first served" hypothesis (Franklin 1974, Jonsson et al. 1976) which, as applied here, postulates that the probability of fertilization by late-flowering males was lowered as a result of other pollen grains which had already reached the pollen chamber of receptive ovules.

In examining the regression model (Table 3) it was interesting to note the highly significant interaction between phenological likelihood of mating and distance between mates, a term which, by itself, explained nearly 40 percent of the variation among receptor pollen pools in y_m. This interaction indicated that the magnitude of the distance effect depended on the degree of floral synchrony between mating pairs. For example, predicted values of y_m (original scale of measure) plotted against LM_{mf} for two selected distances (7.5 m and 22 m) showed that with good floral synchrony (i.e., high L_{mf} values), mating success was expected to be much higher for males and females in close proximity. As the phenological likelihood of mating decreased, however, mating success was predicted to

decrease rapidly among near neighbors, whereas more distant mating pairs were less affected.

Mating System Analysis

Differences among the five sampled ramets in the proportion of outcrossed progeny ($\hat{t}_{m_i}$) were quite large and significantly heterogeneous, with $\hat{t}_{m_i}$ ranging from 0.50 to 1.07 (Table 4). Ramet 673-6-20 had an estimate that was greater than 1.00, which can occur if the observed proportion of discernible outcrosses is greater than that expected based on the model's ability to detect actual outcross events (Neale 1984).

Outcrossing rates may vary from tree to tree for a number of reasons, including: (1) differential self-fertility, and (2) differential rates of self-fertilization as affected by (a) the proximity of male and female strobili within a crown, (b) the amount of overlap between female receptivity and self-pollen shed, and (c) the degree of competition from outcross pollen sources, which involves the relative amounts of pollen produced by self and outcross sources, as well as the degree of floral synchrony of other (nearby) trees. It is important to note that in the present study, variation in $\hat{t}_{m_i}$ can be explained only in terms of (2b) and (2c) above.

Despite the small sample size (n = 5), estimates of $\hat{t}_{m_i}$ appeared to correspond well with estimates of the amount of overlap between female receptivity and self-pollen shed and the degree of competition from outcross pollen sources. In general, higher outcrossing rates were associated with lower CF_{fm} and LM_{mf} values.

Ramet 634-5-28, the female with the lowest $\hat{t}_{m_i}$, reached peak receptivity on day 86, at least 10 days before the other assessed females (Table 4). Moreover, Figure 8 indicated that during the peak receptivity period of clone 634, the degree of competition from other seed orchard pollen sources was negligible, with less than 2 percent of the Everett orchard clones dispersing appreciable amounts of pollen. Examination of the mapped positions of orchard trees surrounding ramet 634-5-28 revealed that the two closest pollinators with any degree of floral synchrony (C_{fm} = 67.91 and 32.91) occurred 23 and 31 m away. The potential for clone 634 to produce selfed progeny was further enhanced by the high degree of synchrony (67.9 %) between female receptivity and the release of self-pollen (Table 4).

Clones 639 and 643 both reached peak receptivity at a time when pollen production in the Everett Seed Orchard was rapidly increasing (day 96, Figure 8). It is interesting to compare the outcrossing rates of ramets 639-8-14 and

643-7-19 since, although they were receptive during the same timeframe, the clones had anthesis periods that were quite different. Within ramets of clone 639, for example, there was a large divergence in the time of female strobilus receptivity and pollen shed, with less than 22 percent overlap between male and female flowering periods (Table 4). This is in contrast to clone 643, where approximately 75 percent of the female flowering period was coincident with the release of self-pollen. As expected, $\hat{t}_{m_i}$ of female 639-8-14 was higher, with 88 percent outcrossed progeny as opposed to 76 percent for ramet 643-7-19 (Table 4).

The relatively low outcrossing rate of ramet 695-5-13 (0.84, Table 4) indicated that inbreeding can be a problem even when conditions appear unfavorable. For example, female strobili of clone 695 reached peak receptivity approximately 10 days prior to the maximum release of self-pollen. Within-tree floral overlap for this clone was extremely low, 5.19 percent, as was the phenological likelihood of self-mating (LM_{mf} = 0.0002, Table 4).

Peak receptivity of the remaining female, a ramet of clone 673, occurred on day 102 during the height of the pollination season (Figure 8). Fecundity data indicated that no pollen was dispersed from 673-6-20 in 1983 and, as

a result, LM_{mf} for this ramet was 0 (Table 4). The lack of self-pollen may largely explain the fact that this mother tree had the highest $\hat{t}_{m_i}$ of any of the sampled females, with no selfed progeny detected among the analyzed seeds (Table 4).

DISCUSSION AND CONCLUSIONS

The flowering patterns of the Everett Seed Orchard parents provides evidence that mating was far from random in 1983, with clones differing widely in both flower production and floral phenology. Although there appeared to be much phenological overlap for the majority of clones, the lack of floral synchrony between early and late flowering parents indicates that many potential crosses occurred infrequently, if at all. The implications of the observed flowering patterns include possible reductions in the genetic quality and variability of harvested seed crops, as well as increased levels of inbreeding and/or contamination from unselected pollen sources. The impact on the genetic gain potential of the orchard could be considerable, particularly if asynchronous clones have high breeding values.

Although the phenology data represent a single pollination season, investigations involving other species (Eriksson et al. 1973, Jonsson et al. 1976), as well as Douglas-fir (Fashler and Sziklai 1980, El-Kassaby et al. 1984), have found comparable differences among clones or families in the timing of seed-cone and pollen-cone bud burst. In the literature, this variation has been interpreted to reflect adaptations of the reproductive cycle to local climatic factors at the place of tree origin

(Ebell and Schmidt 1964, Sarvas 1967, Koski 1970, Chung 1981). While the high degree of genetic control over reproductive bud flush suggests that the order of male and female flowering may not change significantly from year to year, external environmental conditions may strongly influence bud development to the extent that the entire flowering period may be condensed or extended (Stanley and Kirby 1973, Jonsson et al. 1976). For this reason, the phenological patterns in 1983 may have been unusual in some respects due to a set of climatological conditions that were somewhat uncommon for western Washington. Floral bud emergence, for instance, was approximately two weeks ahead of schedule due to a very warm winter (Wheeler, unpublished data). Then, as buds first emerged, the orchard experienced a very wet spell with cool temperatures that slowed pollen bud dehiscence but only minimally affected the maturation of seed-cones. It's possible, therefore, that the amount of variation in the timing of pollen shed and female strobili receptivity may have been less under more normal climatic conditions.

As main effects in regression analysis, however, phenological attributes of the mating pairs were the major factors determining mating success in 1983. Distance between mates was also important to successful pollen exchange, however the relationship was strongly dependent

on phenology. For example, although mating probabilities always decreased with increased distances between mates, the magnitude of the effect was greatest when phenology was highly conducive to mating (i.e., high degree of floral synchrony and/or little competition from neighboring pollen sources). It should also be noted that beyond 30 m, the frequency of observed mating decreased rapidly. Marker progeny were observed in only one receptor ramet of the 10 located at distances greater than 30 m from male markers.

The strong observed relationship between effective pollen exchange and floral synchrony of mating pairs is consistent with the results of Shen et al. (1981) who, in a study of pollination patterns in a Scots pine seed orchard, reported a marker ramet that fertilized 31 percent of the ovules of a highly synchronous neighboring tree. It was also concluded by Shen et al. (1981) that in addition to the coincidence of male and female flowering, pollen dispersal was dependent on the distances between the source and mother trees, as well as the prevailing wind direction during flowering.

Although the effects of wind direction and floral phenology were not examined in an otherwise analogous pollen dispersal study, Muller-Starck (1982) found no consistent relationship between cross-pollination patterns and distance. Unlike the Everett Seed Orchard, however,

the Scots pine seed orchard studied by Muller-Starck (1982) contained a large number of ramets of the marker clone in a relatively small area within the orchard. Although a model was applied to account for this fact, it did not consider wind direction or fecundity differences among the ramets dispersing marked alleles. As a result, it's conceivable that in Muller-Starck's investigation, gametic contributions to receptor females did not necessarily originate from the assumed pollen source (the nearest ramet of the marker clone), thereby possibly eliminating or severely confounding any influence of distance on the degree of effective pollen dispersal.

The data in the present study provide evidence for preferential mating among highly synchronous mates in close proximity. Gametes from the marker clones, however, generally represented only a small percentage of the pollen pools of the sampled females. Based on these results, it appears that the combined net effect of variable floral synchrony, floral productivity, and distances between mates may be to promote cross-fertilization among clones, with a number of clones (presumably) comprising the pollen pools of any single clone. It might be further postulated that limited floral asynchrony actually tends to "break-up" the tendency for preferential mating between nearest neighbors.

The extent to which the results of the study were influenced by the use of the air blaster during the 1983 pollination season is largely unknown. It's likely, however, that pollen was dispersed to greater distances relative to seed orchards in which pollen transfer is completely wind-effected. The lack of evidence for directional pollen dispersion in the orchard may also be related to the use of the air blaster.

It was assumed during data analysis that all unique marker alleles originated from the designated pollen source. The degree to which this assumption is violated could conceivably influence the importance and effect of both distance and spatial orientation as independent variables in regression models. As mentioned earlier, it was determined a priori that the influence of alternative ramets of the two marker clones would be negligible due to the combined effects of seed sampling methods, fecundity differences, and the number and distribution of the marked ramets within the Everett Seed Orchard. Observations on the detection frequency of marker alleles in the pollen pools of receptor females support this initial assumption. For example, a ramet of clone 616, classified in field surveys as a light pollen producer in 1983, was only 6.1 m from a receptor individual. However, neither this receptor, nor any other sampled female in close proximity

received detectable pollen contributions from a ramet carrying the unique Idh allele of clone 616. Support for the assumption also comes from the isozyme study of Shen et al. (1981), where the proportion of marker gametes detected in seeds collected on the side of a mother tree facing the pollen source was over 1.4 times the amount detected on the tree's corresponding opposite side.

In assessing the potential for self-fertilization, phenological observations of the Everett Seed Orchard parents generally indicated that in 1983 many of the clones possessed flowering characteristics considered highly conducive to selfing. Estimates of the proportions of viable outcrossed progeny for individual trees, although quite variable, corresponded well with the variables measuring the phenological potential for selfing (C_{fm} and LM_{mf}). In one extremely early flowering female, for example, over 50 percent of the viable progeny were estimated to have resulted from self-fertilization, a proportion considered exceptionally high for Douglas-fir.

While average outcrossing rates have been found to be high (> 90 percent) for Douglas-fir in both natural stands and seed orchards (El-Kassaby et al. 1981, Shaw and Allard 1982, Neale and Adams 1985), the range among individual trees can be quite large. For example, Sorensen (1973)

observed outcrossing rates that ranged from 50.5 to 100 percent among 19 sampled trees. Shaw and Allard (1982) reported values of $\hat{t}_{m_i}$ for four "highly-outcrossed" and 5 "highly-selfed" seed orchard families (classification based on previous surveys in the orchard) that varied from 0.56 and 1.13, a range nearly identical to that found in the present study. In an investigation where clones were chosen based on similarity in floral phenology (Omi and Adams 1986), the outcrossing rates of six females ranged from 0.70 to 1.01. The average outcrossing rate of these 6 clones (upper crown) was substantially higher than that that found in the present study ($\hat{t}_{m_i}$ = 1.00 vs. 0.81).

From a pollen availability and phenological standpoint, the outcrossing rates of the Everett Seed Orchard parents seemed somewhat low, especially for those maternal parents without particularly good male/female synchrony. Although the sample size was small and there are other reasons for low outcrossing, such as high self-fertility, one factor possibly contributing to these results could have been the use of the air blaster during the 1983 pollination season. During the blasting operation, for instance, much of the propelled air is moved in an upward or partly upward direction. Since the air source is only a few feet above ground level, large amounts of dislodged pollen from the lower branches of a tree may

be driven up through the crown to receptive female flowers, thereby increasing the potential for self-pollination.

Further and more extensive investigations are necessary to determine the relationship between the use of the air blaster and inbreeding, as well as the degree to which the estimated $\hat{t}_{m_i}$ values represent the true variability among diverse phenological classes of orchard clones. It would also be of future interest to consider the influence of both background and other-orchard pollen sources on mating system patterns, since inflated outcrossing estimates have been found in association with higher levels of contamination, especially among early or late flowering clones (El-Kassaby and Ritland 1986, El-Kassaby et al. 1986).

Although it was not possible in this study to examine the influence of pollination and mating patterns on the size of the effective breeding population, it is important to consider the impact that phenology may have on the effective number of males contributing to the pollen pools of individual females. For example, to estimate N_e for the dispersion of pollen gametes only, the formula of Levin and Kerster (1974) can be modified as follows:

$$N_e = 2\pi d\hat{t}_{m_i}\sigma^2 , \qquad (4)$$

where d is the effective plant density, $\hat{t}_{m_i}$ is the individual tree outcrossing rate, and σ^2 is the variance of pollen dispersal distances. If estimates of effective plant density were calculated for each maternal parent as the number of phenologically synchronous males per unit area, a 50 percent reduction in the effective number of synchronous mating combinations would result in a commensurate reduction in the effective male population size. (This reduction may be offset by increases in the pollen dispersal variance, however, since effective pollen would now be contributed by more distant males.) If the outcrossing rate of asynchronous females is significantly reduced, to 50 percent for example, then the combined effect of asynchrony and low outcrossing could result in the reduction of 75 percent of the effective breeding size. While these estimates are purely speculative, they point to the need for further studies to determine more fully the role that phenology and other factors play in restricting random mating among seed orchard parents.

Several aspects of the research described in this thesis are of relevance to the design and management of both existing and future seed orchards. For example, the influence of air blasters on seed orchard pollen dispersal and mating patterns is of interest, particularly if this type of procedure is to become common practice in Pacific

Northwest seed orchards. In addition, differential flowering phenology and pollen production, along with widely varying outcrossing rates among orchard clones portend the need to consider special management practices to enhance the genetic efficiency of conifer seed orchards.

In addressing problems with the mating system, outcrossing rates may possibly be improved by bloom delay techniques (Silen and Keane 1969, Fashler and Devitt 1980), which attenuate the effects of asynchronous flowering classes by reducing the range of phenological differences among orchard parents. The use of waterspray-cooling as a method for delaying flowering would also promote a greater overlap in the flowering times of orchard parents, thereby increasing the amount of cross-pollination and the effective population size. Reporting results of research conducted over a 10 year period, El-Kassaby et al. (1986) determined that the use of overhead cooling in a Douglas-fir seed orchard compacted the pollination period, resulting in reduced contamination rates and similar seed yields among the various phenological classes.

Supplemental mass pollination (SMP) is an additional management tool that has been proposed for use in operational seed orchards (Woessner and Franklin 1973, Denison and Franklin 1975). Although the practicality and benefits of this treatment must be assessed for each

individual orchard, the associated costs and necessary special equipment may be justifiable in certain instances. Several studies have concluded that SMP, particularly in very early or late-flowering clones, may greatly increase seed yields and enhance genetic efficiency by increasing outcrossing rates and reducing the amount of contamination from foreign pollen sources (Daniels 1978, Wheeler and Jech 1985, El-Kassaby and Ritland 1986). Genetic gain may also be increased by SMP if asynchronous clones have high breeding values.

Finally, in making roguing decisions in existing seed orchards, it may be prudent to amass phenology information on the reproductive dynamics of orchard parents over several pollination seasons. These data could be used, for example, to rogue from orchards those clones or families that due to their floral characteristics, contribute little to the orchard gene pool. The phenology data could also be used to remove or manage separately those individuals that appear to have a high potential for selfing.

With respect to the design of future seed orchards, it has been suggested that floral phenology data be used to guide the placement of clones so that neighboring individuals are ensured greater overlap in flowering times (Fashler and Sziklai 1980). In most cases, the required phenology data could only be obtained from the original

parent trees in the wild or perhaps representatives in progeny test sites. Even if these sources were available and of reproductive age, the information derived from them would not necessarily be an accurate reflection of the material's floral tendencies under seed orchard conditions. It may be worthwhile, instead, to explore more fully the relationship between Douglas-fir floral and vegetative bud flush. Vegetative phenology data obtained from immature seed orchard trees could be extrapolated and used, for example, to move individuals from temporary to permanent seed orchard positions that would maximize their crossing potential. Unfortunately, in the single study that has apparently addressed this question in Douglas-fir, no significant correlations were found between the dates of floral and vegetative bud flush (Sorensen and Campbell 1971).

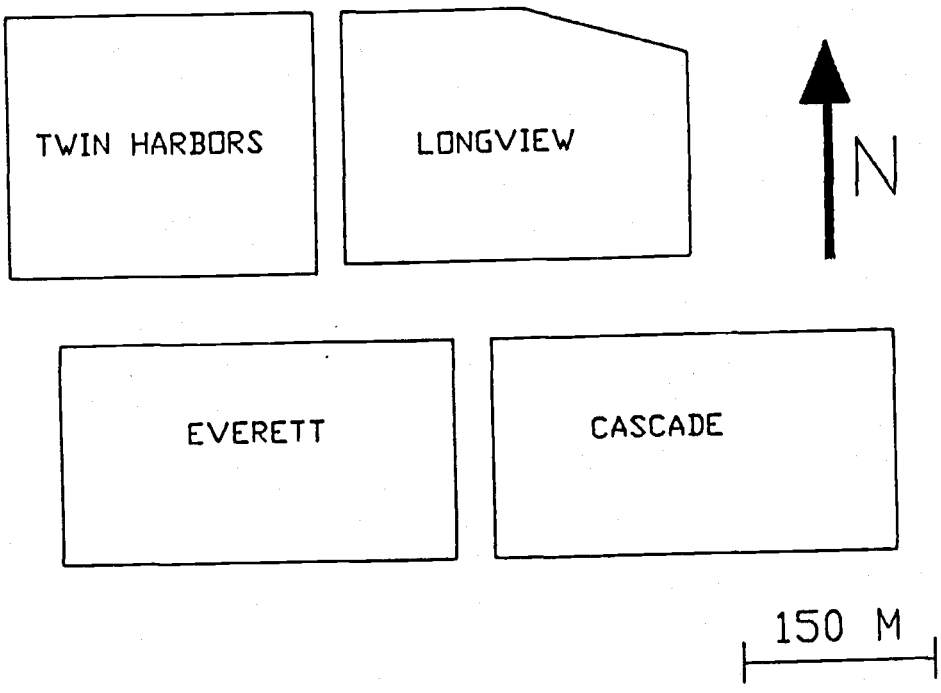

Figure 1. Location of the Everett Seed Orchard block in Weyerhaeuser Company's Douglas-fir seed orchard complex south of Olympia, Washington.

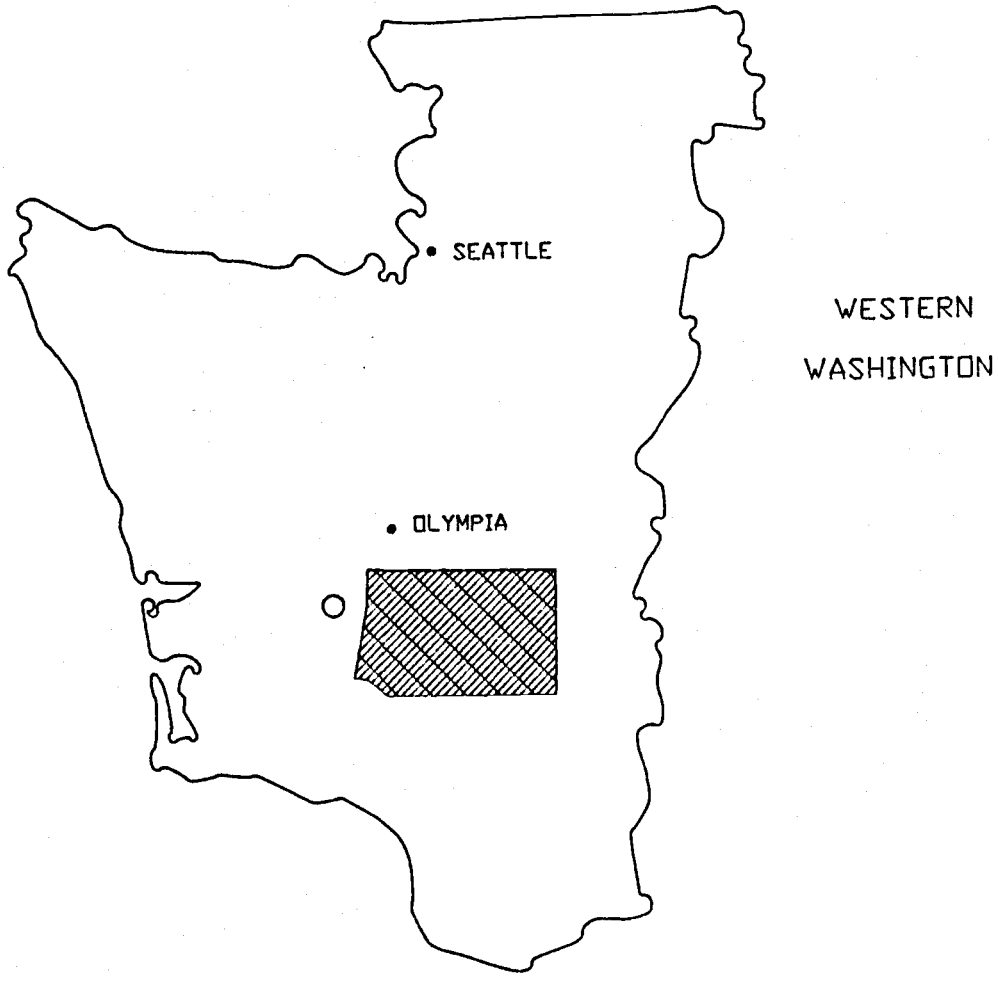

Figure 2. Geographic source of vegetative materials (▨) used in the establishment of the Everett Seed Orchard block (○).

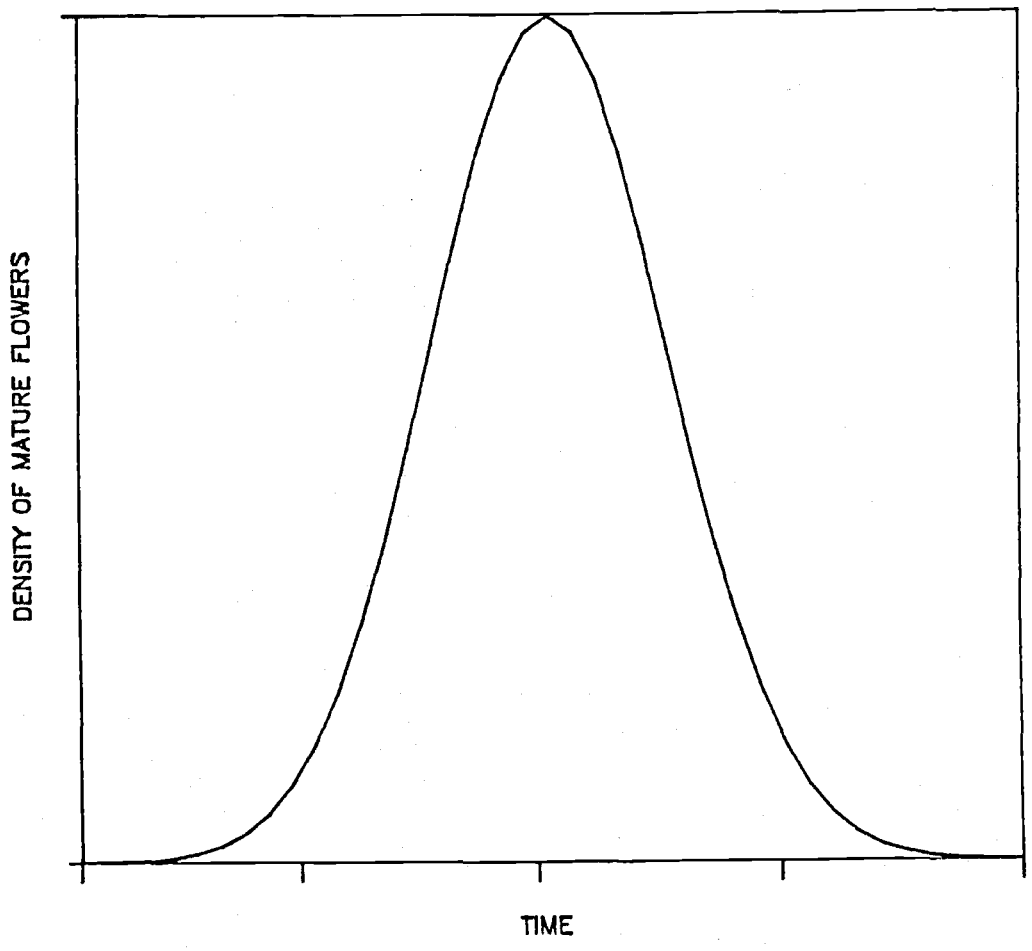

Figure 3. Assumed frequency distribution of mature seed-cone or pollen-cone flowers over time on an individual tree basis.

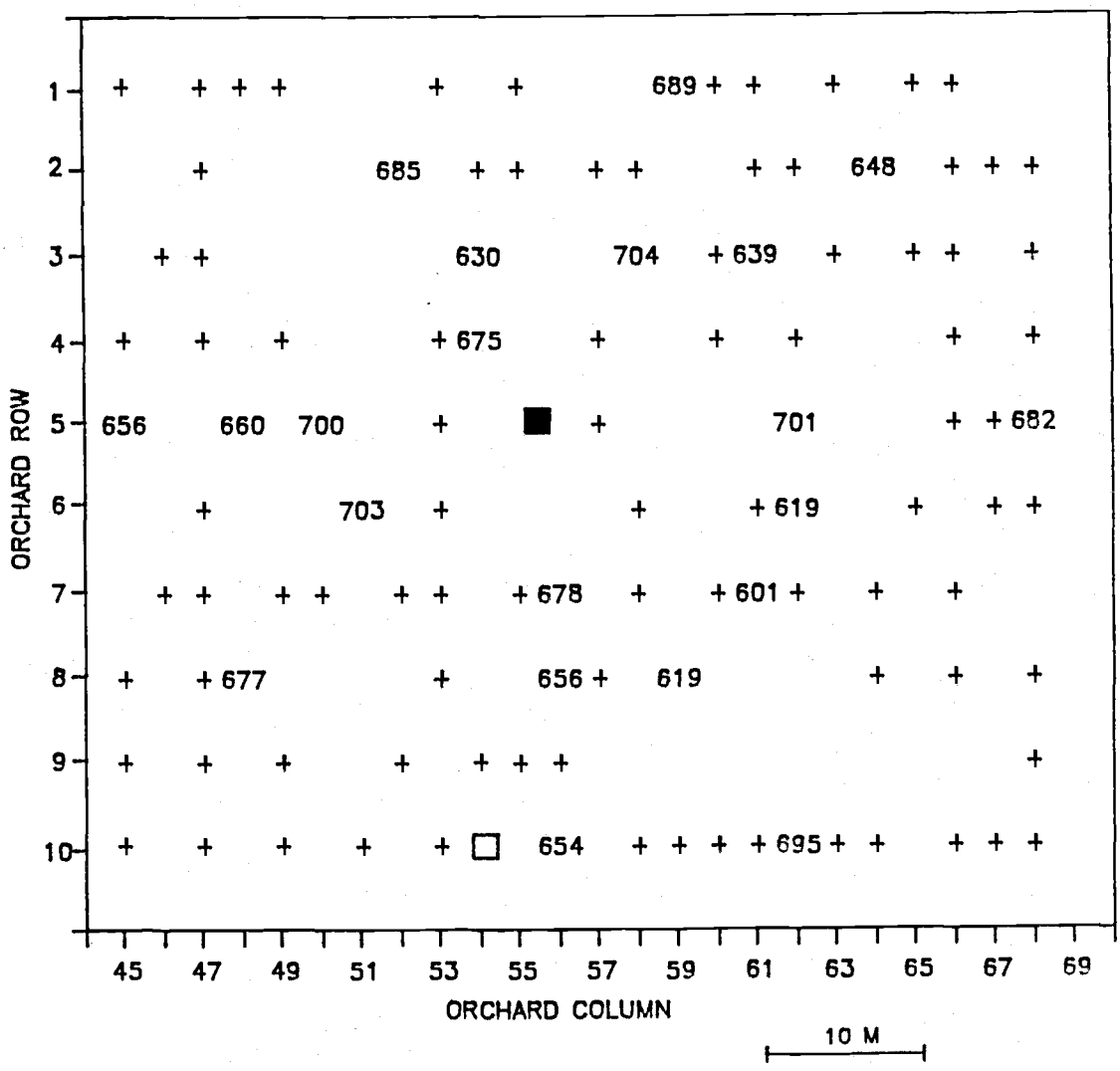

Figure 4. Location of receptor ramets (numbered individuals) and other non-sampled ramets (+) in the section of the Everett Seed Orchard surrounding the marker pollen source (■, a ramet of clone 635) carrying unique allele Gdh-2. (□ is an alternate ramet of clone 635).

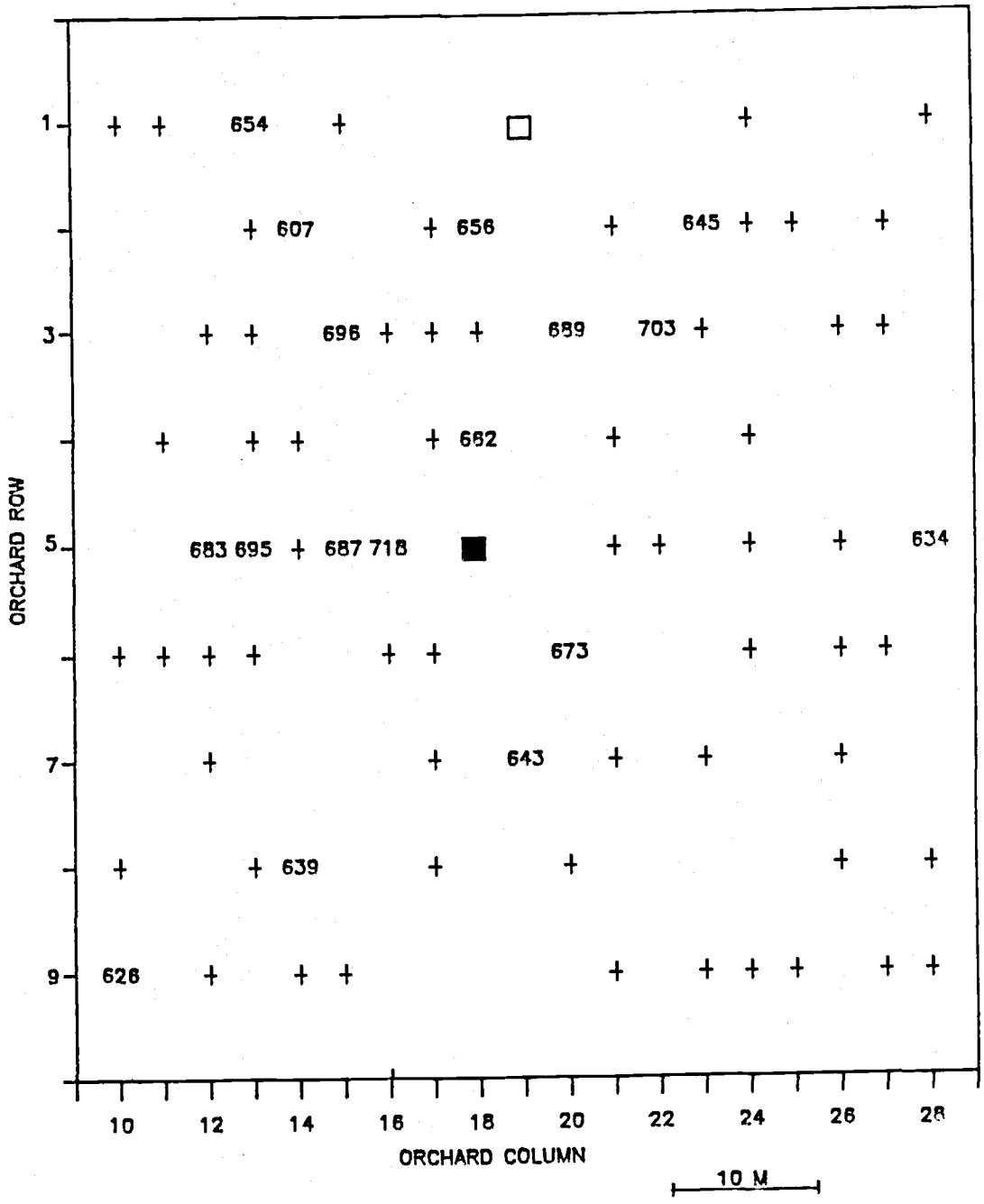

Figure 5. Location of receptor ramets (numbered individuals) and other non-sampled ramets (+) in the section of the Everett Seed Orchard surrounding the marker pollen source (■, a ramet of clone 616) carrying unique allele Idh-3. (□ is an alternate ramet of clone 616).

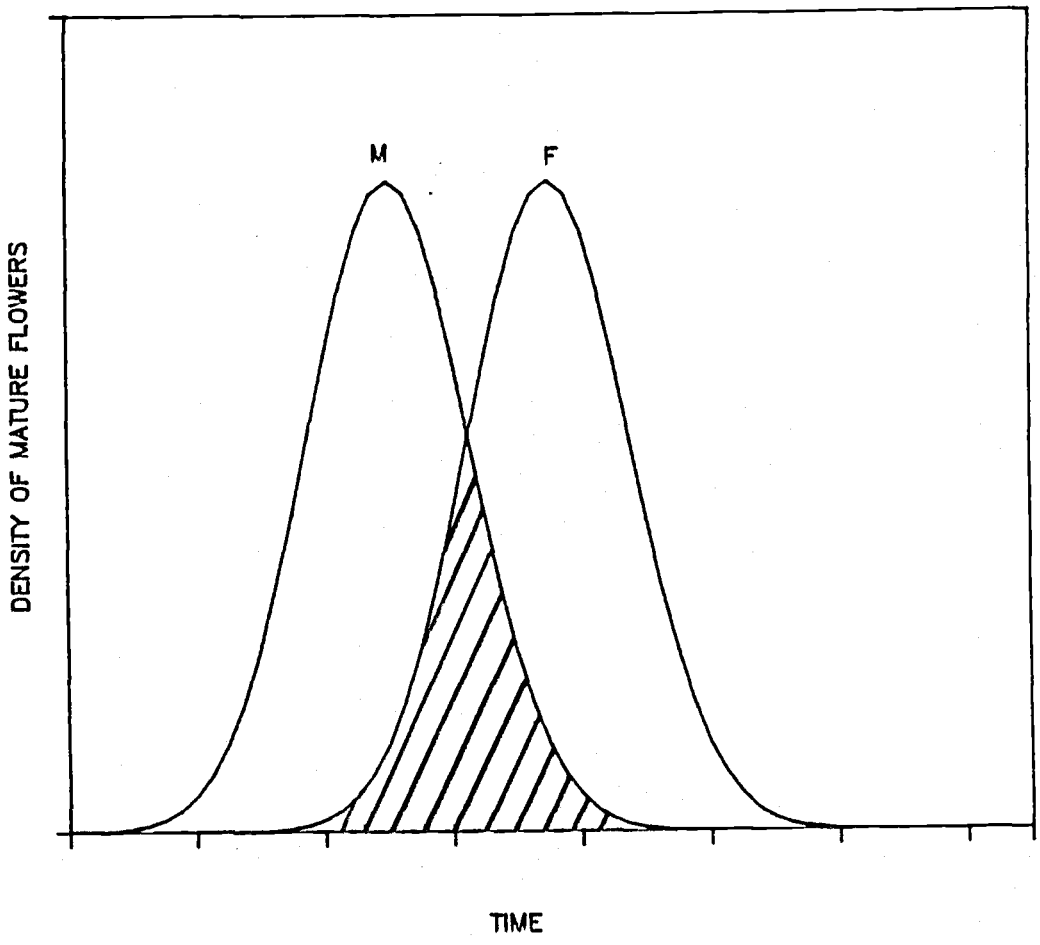

Figure 6. Illustration of overlap in the normal probability density functions of female receptivity with pollen shedding of a donor male.

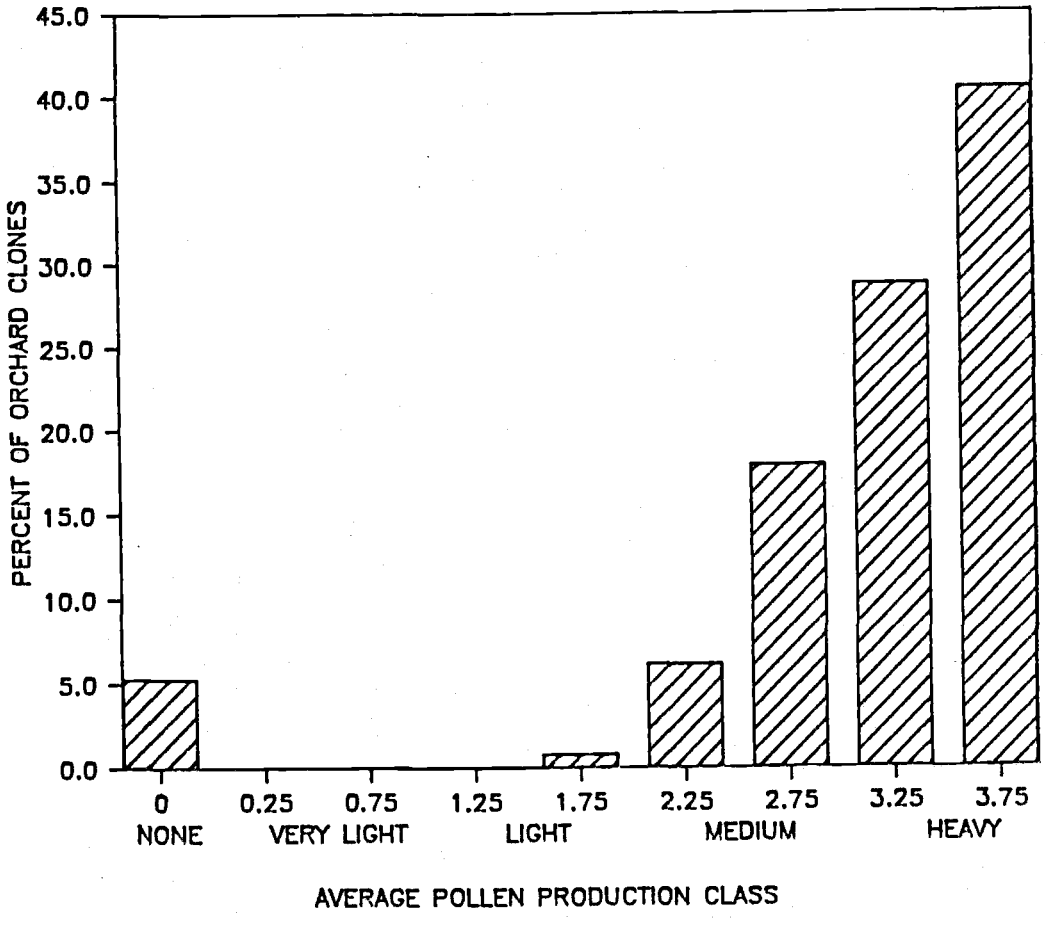

Figure 7. Histogram of the average pollen production classes among clones in the Everett Seed Orchard block.

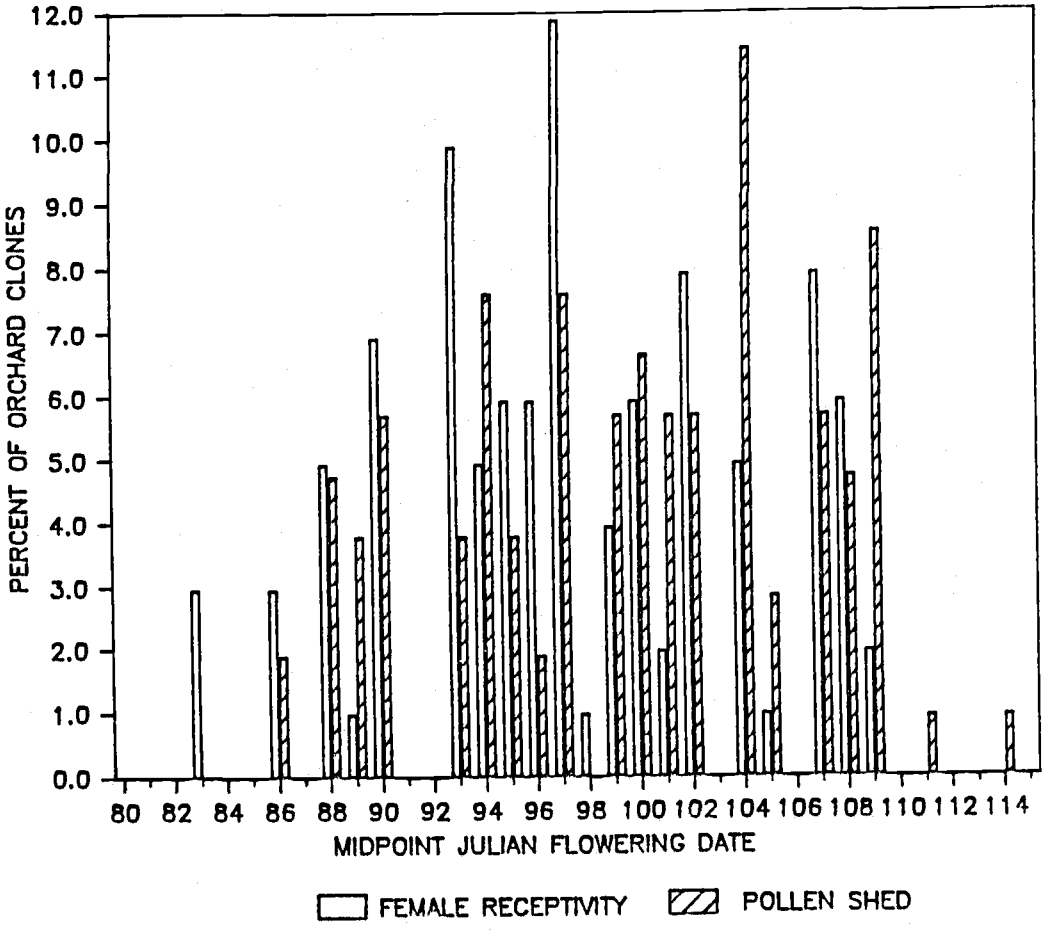

Figure 8. Histogram of the male and female midpoint Julian flowering dates among clones in the Everett Seed Orchard block.

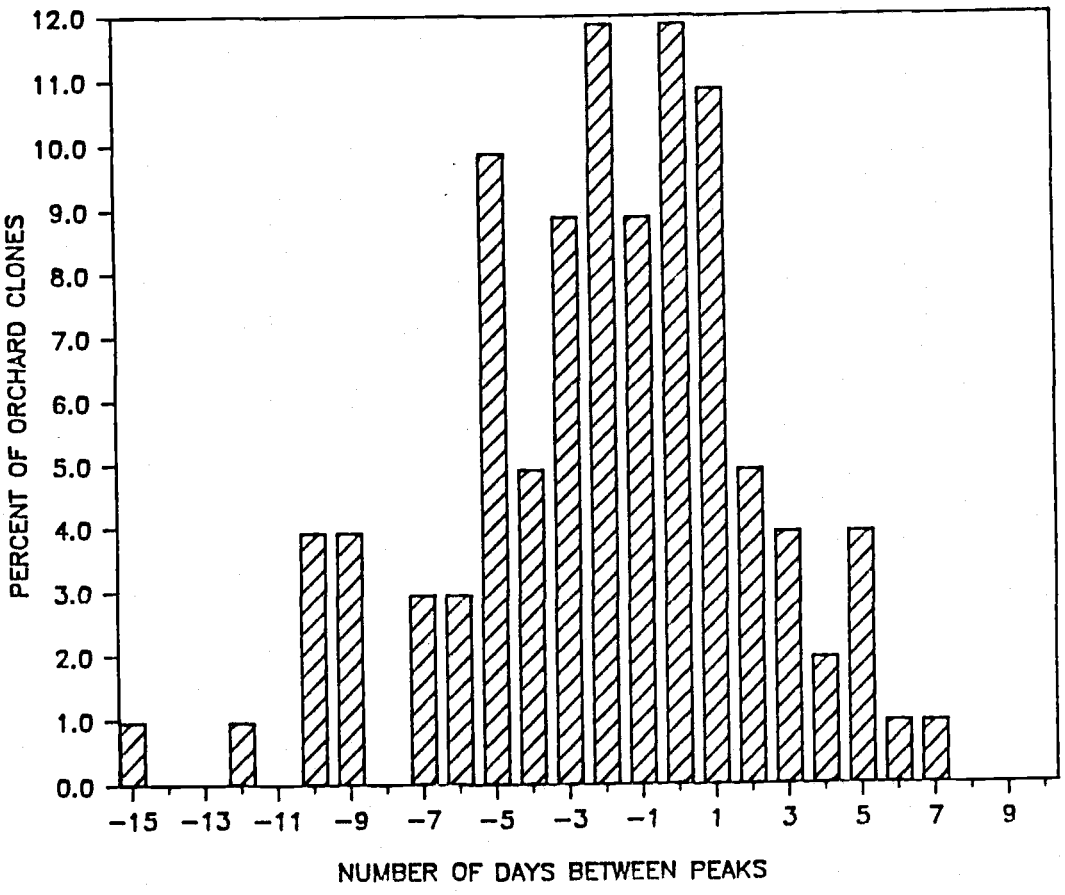

Figure 9. Histogram of the number of days between peak female flowering and the release of self-pollen among clones in the Everett Seed Orchard block. (Negative values correspond to clones in which peak female receptivity occurred prior to maximum pollen shed).

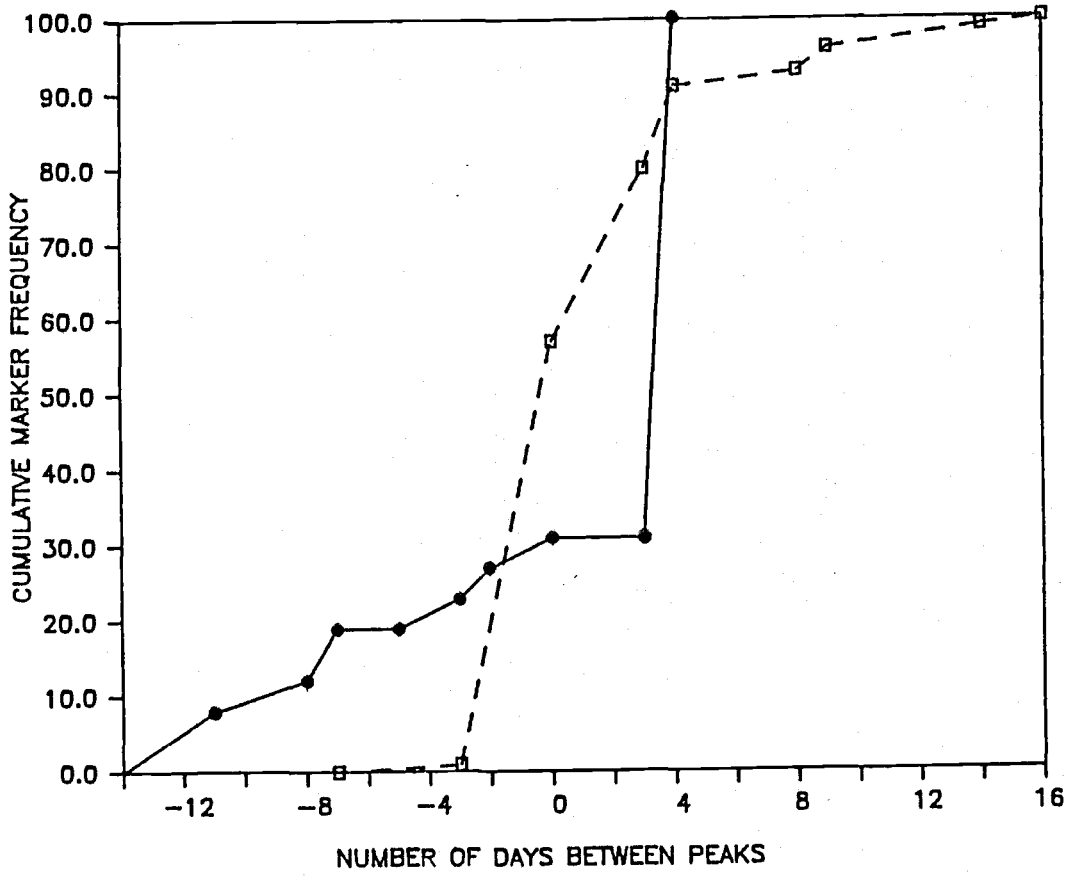

Figure 10. Cumulative frequencies of gametes contributed by male marker clones 635 (□) and 616 (●) to pollen pools of receptor females with dates of peak flowering occurring before (-) and after (+) maximum pollen shed of the marker clone.

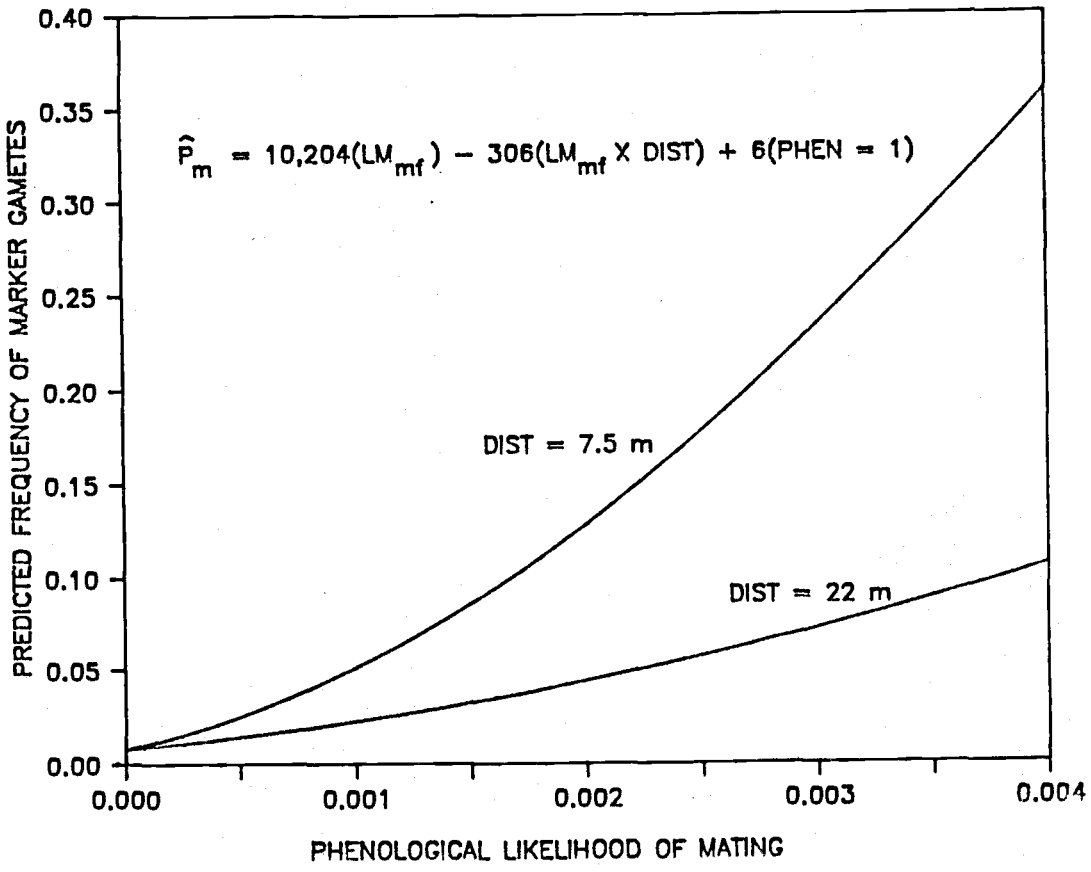

Figure 11. Relationship between the predicted frequency of marker gametes (P_m) and the phenological likelihood of mating (LM_{mf}) as the distance from the pollen source increases from 7.5 to 22 m.

Table 1. Estimated frequencies of gametes from male marker 635-5-18 in the pollen pools of receptor ramets (P_m), distances of receptors from the marker ramet, and differences between receptors and the marker in floral phenology.

Female Receptor Ramet	Number Seed Assayed	(P_m)	Dist.(m) From Marker	Floral Differences $\hat{\mu}_f - \hat{\mu}_m$[a]	C_{fm}(%)[b]	LM_{mf}[c]
718-5-16	164	0.0976	6.1	*	*	*
662-4-18	199	0.7136	7.3	0	100.00	0.0049
687-5-15	179	0.0112	9.1	16	0.00	0.0000
673-6-20	192	0.0208	9.5	9	13.20	0.0005
643-7-19	206	0.2233	14.9	3	45.86	0.0025
695-5-13	200	0.0500	15.2	4	43.19	0.0020
689-3-20	183	0.0437	15.9	14	1.77	0.0001
696-3-15	134	0.0299	17.3	9	13.20	0.0005
683-5-12	184	0.0435	18.3	4	43.19	0.0020
703-3-22	157	0.0382	19.1	8	6.88	0.0003
656-2-18	66	0.0303	22.0	- 3	61.61	0.0038
639-8-14	117	0.0000	25.1	3	45.86	0.0025
607-2-14	183	0.0437	25.1	4	43.19	0.0020
645-2-23	135	0.1037	26.7	3	45.86	0.0025
634-5-28	140	0.0000	30.5	- 7	29.24	0.0030
654-1-13	67	0.0000	33.0	9	8.06	0.0004
626-9-10	193	0.0000	38.1	14	0.50	0.0000

[a] Number of Julian days to peak female receptivity, $\hat{\mu}_f$, minus number of Julian days to maximum pollen shed of marker ramet, $\hat{\mu}_m$. Thus, a negative value indicates that maximum receptivity occurred prior to peak pollen shed.

[b] Proportion of the female receptivity period that was coincident with pollen shed of the marker ramet.

[c] Phenological likelihood of mating between male marker ramet and receptor females (see text for computing formula).

* No phenology information available.

Table 2. Estimated frequencies of gametes from male marker 616-5-56 in the pollen pools of receptor ramets (P_m), distances of receptors from the marker ramet, and differences between receptors and the marker in floral phenology.

Female Receptor Ramet	Number Seed Assayed	(P_m)	Dist.(m) From Marker	Floral Differences $\hat{\mu}_f - \hat{\mu}_m$[a]	C_{fm}(%)[b]	LM_{mf}[c]
675-4-54	121	0.0000	9.5	- 7	33.05	0.0015
678-7-56	178	0.0225	14.6	- 7	33.05	0.0015
704-3-58	183	0.1311	15.8	4	44.60	0.0030
630-3-54	167	0.0000	15.8	- 8	21.84	0.0012
703-6-51	98	0.0204	16.9	- 3	51.26	0.0027
700-5-50	112	0.0000	18.3	0	78.52	0.0037
701-5-62	189	0.1481	18.3	*	*	*
619-6-62	180	0.0111	19.7	-11	19.97	0.0010
639-3-61	142	0.0141	21.1	- 8	21.84	0.0012
601-7-61	85	0.0000	21.1	-14	13.81	0.0008
656-8-56	123	0.0000	21.9	-14	10.64	0.0007
619-8-59	140	0.0143	23.8	-11	19.97	0.0010
660-5-48	136	0.0147	24.4	- 2	72.04	0.0028
685-2-5	180	0.0667	25.1	4	44.60	0.0030
689-1-59	180	0.0000	30.7	3	65.40	0.0031
677-8-48	114	0.0000	32.8	- 5	51.98	0.0020
648-2-64	180	0.0000	32.8	-14	10.64	0.0007
656-5-45	88	0.0000	33.5	-14	10.64	0.0006
682-5-68	98	0.0204	36.6	0	98.87	0.0039
654-10-56	102	0.0000	36.6	- 2	65.19	0.0030
695-10-62	91	0.0000	40.9	- 7	33.05	0.0015

[a] Number of Julian days to peak female receptivity, $\hat{\mu}_f$, minus number of Julian days to maximum pollen shed of marker ramet, $\hat{\mu}_m$. Thus, a negative value indicates that maximum receptivity occurred prior to peak pollen shed.

[b] Proportion of the female receptivity period that was coincident with pollen shed of the marker ramet.

[c] Phenological likelihood of mating between male marker ramet and receptor females (see text for computing formula).

* No phenology information available.

Table 3. Fitted regression equation for explaining variation in the frequency of male marker gametes in the pollen pools of receptor females based on the distances between mates (DIST), and on the magnitude (LM_{mf}) and direction (PHEN) of their floral synchrony.

Independent Variables	$\hat{b}$	S.E. $(\hat{b})$	Standardized b	Prob.	Cumulative R^2
LM_{mf}	10,204.54	1518.88	1.16	<.001	.190
LM_{mf}xDIST	- 306.21	55.76	-0.94	<.001	.576
PHEN	6.37	2.29	0.29	.009	.658
Constant	- 1.20	2.27	0.00	.602	

Table 4. Estimated individual tree outcrossing rates, $\hat{t}_{m_i}$, pollen fecundity class, and within-tree differences in floral phenology for five mother trees in the Everett Seed Orchard.

Ramet	Pollen Fecundity Class[a]	Floral Phenology $\hat{\mu}_f$[b]	$\hat{\mu}_f - \hat{u}_m$[c]	C_{fm}(%)[d]	LM_{mf}[e]	$\hat{t}_{m_i}$ (S.E.)
634-5-28	4	86	- 2	67.91	0.0069	0.50 (.06)
643-7-19	3	96	- 1	74.69	0.0030	0.76 (.09)
639-8-14	4	96	6	21.85	0.0012	0.88 (.06)
695-5-13	4	97	-10	5.19	0.0002	0.84 (.06)
673-6-20	0	102	- 6	16.81	0.0000	1.07 (.04)
Unweighted Mean						0.81 (.06)
Heterogeneity[f]						69.15[g]

[a] Pollen production class: Heavy = 4, Medium = 3, Light = 2, Very Light = 1, None = 0.

[b] Number of Julian days to peak female receptivity, μ_f.

[c] Number of Julian days to peak female receptivity, μ_f, minus number of Julian days to maximum shed of self-pollen, μ_m. Thus, a negative value indicates that maximum receptivity occurred prior to peak pollen shed.

[d] Proportion of the female receptivity period that was coincident with the release of self-pollen.

[e] Phenological likelihood of mating of self-pollen relative to all other orchard pollen sources (see text for computing formula).

[f] Test for heterogeneity among t_{m_i}'s, Chi-square with 4 degrees of freedom.

[g] Significant at 0.01 probability level

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