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Variation in reproductive phenology in a *Pinus patula* seed orchard and risk of genetic contamination from nearby natural stands

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Abstract
Genetic variation in reproductive phenology among clones in a seed orchard affects the genetic efficiency of the orchard. Our objective was to evaluate genetic variation in reproductive phenology of *Pinus patula* clones in a seed orchard and the amount of overlap with pollen dispersal in natural stands. In 2014 and 2015, phenology of female and male strobili was recorded on 31 clones in the orchard, and phenology of male strobili was measured on 10 trees in each of four nearby natural stands along an elevational transect. Onset and end dates, and length of female receptivity (F_onset, F_end and F_length) and pollen dispersal (M_onset, M_end and M_length) were calculated, and genetic parameters were estimated. Differences between years in M_onset were larger in the natural stands than in the orchard, but there was a large overlap between the orchard and natural stands. A negative linear relationship with elevation was found for M_end and M_length in natural stands along the elevation transect. Genetic variation was detected for most reproductive phenology traits in the orchard. Genetic control was stronger for M_onset and M_length ($H_c^2 \geq 0.54$) than for female receptivity traits ($H_c^2 \leq 0.38$). Most phenological traits showed high genetic stability in both years ($r_B \geq 0.76$). We found a positive genetic correlation ($r = 0.67$) between F_onset and M_onset, suggesting there is a risk of selfing among clonal ramets. Moreover, the overlap between female receptivity in the orchard and pollen dispersal in neighboring natural stands indicates a risk of genetic contamination in the orchard, particularly for late-phenology clones.

Keywords Reproductive phenology · Genetic variation · Heritability · Genetic contamination

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Introduction

Seed orchards are the main source of germplasm required for establishing forest plantations of most economically important conifer species (Askew 1988). The design and management of these germplasm production units are intended to ensure higher genetic value and diversity than seed from seed areas or natural stands (El-Kassaby et al. 1984). Maximum genetic diversity of the seed orchard crop occurs when all the parents have the same probability of crossing (panmixia) and contribute equally to the harvested seed (Kang et al. 2001; Moriguchi et al. 2007).

To achieve maximum genetic value of seed in an open-pollinated seed orchard, the following conditions must be met (Eriksson et al. 1973): (a) all clones supply equal numbers of male and female gametes to the seed crop, (b) receptivity of ovules is synchronized with pollen dispersal of all clones, (c) all clones supply equal numbers of seed to the seed crop, (d) all possible crosses among clones are equally compatible, (e) all clones have the same self-fertility, and (f) the orchard is isolated from natural stands of the same species to avoid contamination from external pollen flow.

Studies on reproductive phenology and synchronization in conifer seed orchards have revealed that panmixia is difficult to achieve (El-Kassaby et al. 1984; Askew and Blush 1990). Differences in reproductive phenology affect gene exchange among clones, excluding some crosses, and even preventing the contribution of some clones to the seed crop (Matziris 1994; Alizoti et al. 2010). Phenological variation among clones reduces the effective population size of the orchard, promotes selfing and increases risk of contamination by non-orchard pollen (Chaisurisri and El-Kassaby 1993; Burczyk and Chalupka 1997). For instance, significant genetic variation in flowering among clones of *Pseudotsuga menziesii* Mirb. Franco (El-Kassaby et al. 1984) and *Pinus radiata* D. Don (Codesido et al. 2005) has been reported. Moreover, reproductive phenology and the proportion of male and female strobili per clone can vary among years and tree ages (Nikkanen and Velling 1987), affecting genetic efficiency of the orchard.

Seed orchards are preferably established with clones from the same geographic region to homogenize reproductive phenology and guarantee adaptation (Pakkanen and Pulkkinen 1991). However, when clones in the orchard are from the local provenance, flowering might overlap with that of neighboring stands, increasing the risk of contamination by external pollen, as shown in orchards of *Pinus sylvestris* L. (Pakkanen and Pulkkinen 1991; Pulkkinen 1994). The risk of pollen contamination in a seed orchard depends upon the onset and ending dates of flowering. It also depends on the duration of female receptivity and pollen dispersal of clones in relation to the timing of pollen dispersal in nearby natural stands (Nikkanen 2001). This information is fundamental for making wise management decisions regarding orchard activities such as thinning, complementary pollination, and/or controlled crosses (Blush et al. 1993).

In 2003, a clonal seed orchard of *Pinus patula* Schiede ex Schltdl. *et Cham.* was established in the Sierra Norte de Puebla as part of a regional program of genetic improvement to satisfy the needs for genetically superior germplasm for planting (Castaños-Martínez and Castro-Zavala 2014). The orchard is located within the species' natural distribution at an elevation of 2860 m, so it allows us to study factors that affect reproductive phenology and the risk of genetic contamination in seed orchards. The objectives of the study were to (a) determine the degree of overlap in reproductive phenology between the *P. patula* clones in the orchard and nearby natural stands of the species and (b) evaluate the level of genetic variation in phenology traits among the seed orchard clones and the heritability and

stability of these traits among years. Information of this nature is scarce for most subtropical pines, including *P. patula*.

Materials and methods

Study area

The study was conducted in a 10-year-old clonal seed orchard of *P. patula* established by grafting at the Multi-functional Forest Reserve "El Manantial" in the municipality of Aquixtla, Puebla, Mexico (19°43′13″N; 97°59′20″W; 2860 m elevation). The orchard covers an area of 1.2 ha, was established in a completely random design with 3 × 3 m spacing, and contains a variable number of ramets per clone (1–22, average = 8, median = 7). At the beginning of the study, the orchard had 660 ramets belonging to 82 clones. The study also included natural even-aged stands of the species located along an elevation transect (2807–3011 m), 150 to 1000 m from the center of the orchard.

Evaluation of reproductive phenology

In 2014 and 2015, we selected a sample of 31 clones with three ramets per clone (93 trees) for study. These clones were selected based on their above-average cone production in 2012. The phenological progress of female (upper part of crown) and male (lower part of crown) strobili was monitored on five branches per ramet from January to March in 2014 and 2015. Data were taken every 3 to 4 days until all pollen was released and the female strobili were no longer receptive. Observations were made on the same trees during both years, but not necessarily on the same branches.

We also measured the phenological progress of male strobili on adult (sexually mature) *P. patula* trees at four sites in nearby natural stands along an elevational transect. At each site, we selected ten trees producing male strobili that could be easily observed. The phenological evaluation was carried out with the same methodology used in the orchard during the same periods.

To define the phenological stages of the strobili, we used the methodology of Matziris (1994) for female strobili and that of Codesido and Merlo (2001) for male strobili, modified for *P. patula* by Hernández-Zaragoza et al. (2016). We measured female strobili using four stages. In stage 1, the female bud is increasing in size, but still covered by bud scales. In stage 2, the female strobili becomes visible and elongates; the ovules are not receptive, but pollen can get inside the scales, and could remain viable to fertilize the ovule when it reaches stage 3. For this reason, receptivity is considered to be 20% at stage 2. Maximum receptivity is reached at stage 3, which occurs when the scales gradually separate until they form a right angle with the strobilus axis. At this stage, pollen can penetrate easily between the scales and reach the ovules, so receptivity is considered to be 100%. In stage 4, the scales increase in size and thickness, so no pollen grains can penetrate between them. For male strobili in stage 1, the male bud is covered with bud scales. In stage 2, the male strobili emerge from the bud scales and elongate. In stage 3, the male strobili start shedding pollen, and stage 4 begins when pollen-shedding ends and pollen sacs dry and drop off. Thus, pollen release occurs only at stage 3, when the strobili elongate and acquire a yellow coloring. When the marked twigs had more than one female strobilus, the receptive period was counted from the date on which the first

strobilus on the branch was receptive until the last strobilus completed stage 3. The same criterion was used for pollen release from twigs with multiple male strobili.

Data analysis

The female and male onset dates (F_onset, M_onset) and end dates (F_end, M_end) were measured as number of days after January 1. Then, the lengths of female receptivity and pollen dispersal periods (F_length, M_length) were determined from phenological data of each sampled branch for each ramet and clone using SYNCHRO software (Zas et al. 2003). These variables were analyzed using the MIXED procedure of the SAS statistical software (SAS Institute 2002). In the first step, M_onset, M_end and M_length in the seed orchard was compared with that of neighboring natural stands in both years using the following model (Eq. 1). All effects were considered fixed, except the residual error:

$$Y_{ijk} = \mu + R_i + P_j + R_iP_j + e_{ijk} \tag{1}$$

where Y_{ijk} is the mean value of the kth tree (mean of the five branches sampled) in the jth population [seed orchard (SO) or natural stand (NS)] in the ith year; μ is the general mean; R_i is the effect of the ith year; P_j is the effect of the jth population; R_iP_j is the interaction effect of the ith year and jth population; and e_{ijk} represents the random error. For the seed orchard, a randomly chosen ramet from each clone was included in this analysis to avoid underestimating within-population variation from using all the ramets per clone. A Tukey test was used to compare LS-mean values of phenology traits between populations. In a second step, we tested for a linear effect of elevation on M_onset, M_end and M_length in the natural stands sampled along the elevation transect using a linear regression model. The regression model was run for the pooled data across years.

For the seed orchard clones, we used an analysis of variance of phenology traits (F_onset, F_end, F_length, M_onset, M_end, and M_length) to estimate variance components. For variables associated with female receptivity and pollen release, we estimated broad-sense heritability for clonal mean values (H_c^2=clonal repeatability) using the following statistical model:

$$Y_{ijk} = \mu + R_i + C_j + R_iC_j + e_{ijk} \tag{2}$$

where Y_{ijk} is the mean value of the kth ramet of the jth clone in the ith year; μ is the general mean; R_i is the fixed effect of the ith year, C_j is the random effect of the kth clone ~ NID (0, σ_c^2); R_iC_j is the random effect of interaction between the ith year and the jth clone ~ NID (0, σ_{rc}^2); and e_{ijk} is the error ~ NID (0, σ_e^2). Broad-sense heritability (H_c^2) was calculated with the following formula:

$$H_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + \left(\frac{\sigma_{rc}^2}{a}\right) + \left(\frac{\sigma_e^2}{ra}\right)} \tag{3}$$

where σ_c^2 is the variance among clones; σ_{rc}^2 is the variance due to the interaction between years and clones; σ_e^2 is the variance due to error; a is the number of years and r is the number of ramets per clone.

From the variance components for clones (σ_c^2) and from the interaction between years and clones (σ_{rc}^2), type-B genetic correlations (r_B) were estimated for the phenological traits between years, using the following equation:

$$r_B = (\sigma_c^2)/(\sigma_c^2 + \sigma_{rc}^2) \tag{4}$$

Table 1 Statistical significance (*P*) for phenological traits associated with pollen dispersal in the seed orchard and natural stands of *P. patula*

Source of variation	M_onset[a]	M_end[a]	M_length[a]
Year	< 0.001	0.813	< 0.001
Population[b]	< 0.029	< 0.001	< 0.001
Year × Population	0.052	< 0.001	0.772

[a]"M_onset" and "M_end" are the onset and end dates of pollen dispersal; "M_length" is the length (in days) of the pollen dispersal period

[b]The seed orchard and the natural stands are considered two different populations

Table 2 LS-means for phenological traits associated with pollen dispersal in the seed orchard and in natural stands of *P. patula* in two consecutive years

Year	Population	M_onset[1] (days after January 1)	M_end[1] (days after January 1)	M_length[1] (days)
2014	Seed orchard	72.4 b[2]	92.0 a	19.6 a
	Natural stands	77.0 a	89.2 b	12.2 b
Average 2014:		74.1	90.6	15.9
2015	Seed orchard	70.9 a	93.7 a	22.8 a
	Natural stands	71.2 a	87.1 b	15.9 b
Average 2015:		71.1	90.4	19.4

[1]"M_onset" and "M_end" are the onset and end dates of pollen dispersal; "M_length" is the length (in days) of the pollen dispersal period

[2]LS-means for each trait followed by the same letter are not statistically different between populations in a given year ($P < 0.05$)

Phenotypic and genotypic correlations were also estimated between male and female phenological traits for the orchard clones. For the phenotypic correlations, we calculated Pearson´s correlation coefficient between the two-year mean clonal values of traits. For genetic correlations we used clone variances (σ^2_{xc}, σ^2_{yc}) and covariances between traits (σ_{xyc}), according to the equation described by Falconer (1989). Clone covariances were estimated using the procedure described by White and Hodge (1989).

Results

Pollen dispersal in the seed orchard and natural stands of *Pinus patula*

Most traits were significantly different ($P \leq 0.05$) between years and populations (seed orchard *vs*. natural stands), except for M_end between years (Table 1). There was also a significant year × population interaction for M_end (Table 1). On average, pollen dispersal began three days earlier in 2015 than in 2014, but ended on the same date in both years. Therefore, pollen dispersal lasted three days longer in the second year (Table 2). In both years, pollen dispersal in the orchard began earlier and ended later than in the natural stands, lasting 7 d longer in the orchard (Table 2). M_onset was more stable between years in the seed orchard than in natural stands (Table 2). That is, pollen dispersal in the seed

orchard began at about the same date in both years, but in natural stands began about six days earlier in 2015 (Table 2).

Pollen dispersal in natural stands along an elevational transect

All phenological traits showed significant differences ($P \leq 0.05$) between years, but only M_end and M_length had a significant linear relationship with elevation (Table 3). In 2015, pollen dispersal began 6 d earlier and ended 2 d earlier than in 2014, with a 4 d longer duration (Table 2). Pooled across years, pollen dispersal ended earlier as site elevation increased, reducing the dispersal period (Fig. 1).

Genetic variation in reproductive phenology of seed orchard clones

In the seed orchard, no significant differences were found between years for F_onset and M_onset, but there were differences in F_end, F_length, M_end and M_length (Table 4). F_length and M_length were 2 to 3 d longer in 2015 than in 2014 (Table 4). In both years, F_length lasted 8 to 9 d longer than M_length, beginning 12 to 13 d earlier and ending 3 to 4 d earlier than pollen dispersal.

At the clone level, genetic variation was found for all phenological traits, except for F_length (Table 5). The proportion of variance due to the year × clone interaction was relatively low (less than 33% of the clonal variation) for most traits, except for M_end. Thus, most traits showed high type-B (r_B) genetic correlations, except F_length and M_end (Table 5). With the exception of F_length and M_end, all other phenological traits exhibited moderate to high broad-sense heritabilities, with H_c^2 values varying between 0.36 and 0.60 (Table 5). Pollen dispersal generally showed stronger genetic control than female receptivity.

The genetic correlations involving F_length were null (Table 6) because no genetic variation was found for this trait (Table 5). M_onset and M_end were positively and highly correlated with one another ($r_g = 0.96$), but they were negatively correlated with M_length ($r_g = -1.00$) (Table 6). F_onset also had a moderately positive genetic correlation ($r_g = 0.67$) with M_onset. At the phenotypic level, the correlation between F_onset and F_end was stronger ($r_p = 0.83$) than between M_onset and M_end ($r_p = 0.49$). In both cases, however, F_onset and M_onset were strongly and negatively correlated with F_length and M_length, respectively ($r_p \leq -0.85$). On the other hand, phenotypic correlations between female and male phenology traits varied from moderately negative to moderately positive ($-0.61 \leq r_p \leq 0.60$) (Table 6).

Table 3 Statistical significance (*P*) and slope estimates for phenological traits associated with pollen dispersal in natural stands of *P. patula* along an elevational transect

Trait	*P*- value		Slope estimate ± se
	Year	Elevation	
M_onset[a]	< 0.0001	0.8225	0.00153 ± 0.00680
M_end[a]	0.0283	0.0266	−0.01205 ± 0.00533
M_length[a]	< 0.0001	0.0003	−0.01358 ± 0.00358

[a]"M_onset" and "M_end" are the onset and end dates of pollen dispersal; "M_length" is the length (in days) of the pollen dispersal period

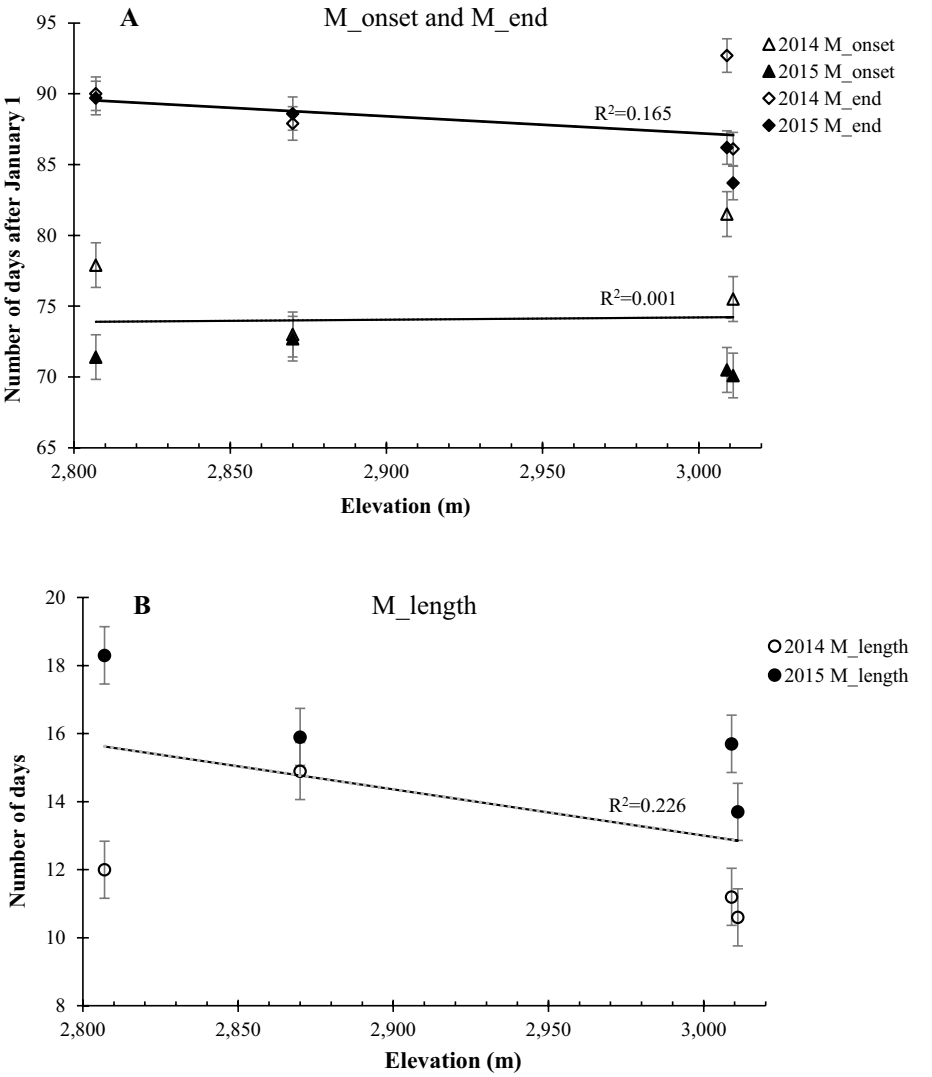

Fig. 1 **A** Onset and end dates (M_onset and M_end), and **B** length (M_length) of the pollen dispersal period in natural stands of *P. patula* along an elevational transect in two consecutive years

Discussion

Year-to-year differences in reproductive phenology of *Pinus patula*

In 2015, pollen dispersal in natural stands began earlier, but ended on the same dates as in the previous year. Thus, pollen dispersal lasted longer in 2015 (Table 2). This trend was not observed for female receptivity or pollen dispersal in the seed orchard. There were no differences between years in F_onset and M_onset (Table 4). In addition, both periods ended later the second year in the orchard, resulting in longer periods. These year-to-year

Table 4 LS-means per year for female and male phenological traits in the *P. patula* seed orchard in two consecutive years

Year	Female phenology			Male phenology		
	F_onset[1]	F_end[1]	F_length[1]	M_onset[1]	M_end[1]	M_length[1]
	(days after Jan. 1)		(days)	(days after Jan. 1)		(days)
2014	58.2 a[2]	88.0 b	29.8 b	71.2 a	91.4 b	20.2 a
2015	58.5 a	90.7 a	32.2 a	70.8 a	94.3 a	23.5 b

[1]"F_onset" and "F_end" are the onset and end dates of female receptivity; "F_length" is the length (in days) of the female receptivity period. "M_onset" and "M_end" are the onset and end dates of pollen dispersal; "M_length" is the length (in days) of the pollen dispersal period

[2]LS-means for each trait followed by the same letter are not statistically different ($P < 0.05$)

Table 5 Variance components, σ^2_{rc}/σ^2_c ratio, broad-sense heritability of clone means (H^2_c) and type-B (r_B) genetic correlations for female and male phenological traits in the *P. patula* seed orchard measured in two consecutive years

Parameter	Female phenology			Male phenology		
	F_onset[a]	F_end[a]	F_length[a]	M_onset[a]	M_end[a]	M_length[a]
σ^{2b}_c	3.93	1.22	0.00	12.00	0.34	8.34
$\sigma^{2\,b}_{rc}$	0.77	0.00	1.04	1.87	1.49	2.70
σ^{2b}_e	36.87	13.24	28.83	42.95	15.85	34.07
σ^2_{rc}/σ^2_c	0.20	0.00	–	0.16	4.38	0.32
H^2_c	0.38	0.36	0.00	0.60	0.09	0.54
r_B	0.83	1.00	0.00	0.87	0.19	0.76

[a]"F_onset" and "F_end" are the onset and end dates of female receptivity; "F_length" is the length (in days) of the female receptivity period. "M_onset" and "M_end" are the onset and end dates of pollen dispersal; "M_length" is the length (in days) of the pollen dispersal period

[b]σ^2_c is the clonal variance, σ^2_{rc} is the year × clone interaction variance, σ^2_e is the error variance, and σ^2_{rc}/σ^2_c is the ratio of the year × clone interaction variance to the clonal variance

differences in pollen dispersal contrast with the results from a previous report (Muñoz-Gutiérrez et al. 2017) which reported that pollen dispersal in both orchard and natural stands began and ended earlier in 2014 than in 2015. However, these differences may be attributed to differences in the evaluation method used for each study. In the previous report, dates of pollen dispersal were estimated based on the capture of pollen grains without identifying their source. In the current study, individual trees were sampled in natural stands and the seed orchard, and the phenology of male and female strobili was measured directly. Therefore, the trees and clones sampled in this study might not have been the first ones dispersing the pollen captured in traps.

In addition, temperature and relative humidity may have hindered the ability to detect the onset of pollen dispersal using traps. In other species, the onset of pollen dispersal is correlated with temperature and the accumulation of degree-days (Luomajoki 1993). Higher temperatures generally promote earlier bud phenology (Torimaru et al. 2013), as has been shown in a *Larix principis-rupprechtii* Mayr. seed orchard (Zhang et al. 2001).

Table 6 Genetic correlations (left of the diagonal line) and phenotypic correlations (right of the diagonal line) for female and male phenological traits in the *P. patula* seed orchard

	Female phenology			Male Phenology		
	F_onset[a]	**F_end[a]**	**F_length[a,b]**	**M_onset[a]**	**M_end[a]**	**M_length[a]**
Female phenology:						
F_onset[a]		0.83	-0.85	0.60	0.37	-0.50
F_end[a]	1.65		-0.42	0.39	0.33	-0.28
F_length[a,b]	-	-		-0.61	-0.30	0.56
Male phenology:						
M_onset[a]	0.67	0.19	-		0.49	-0.90
M_end[a]	1.51	1.73	-	0.96		-0.06
M_length[a]	-0.91	-0.32	-	-1.00	-1.00	

[a]"F_onset" and "F_end" are the onset and end dates of female receptivity; "F_length" is the length (in days) of the female receptivity period. "M_onset" and "M_end" are the onset and end dates of pollen dispersal; "M_length" is the length (in days) of the pollen dispersal period

[b]Genetic correlations involving "F_length" were null because no genetic variation was found for this trait

Also, high relative humidity reduces the pollen dispersal of *Pinus strobus*, *Pinus contorta,* and *Abies amabilis* (Ebell and Schmidt 1964). Our data indicate that male strobili dispersed pollen earlier in 2015 than in 2014. However, in 2014, rains occurred during the first few days of pollen dispersal (Muñoz-Gutiérrez et al. 2017). Therefore, pollen dispersal was intermittent and may not have travelled long distances, affecting the ability to estimate the onset of pollen release for this particular year when pollen traps were used.

When bud phenology was measured directly, year-to-year differences in M_onset were larger in the natural stands than in the seed orchard. This suggests that pollen contamination in the orchard will vary from year-to-year according to the amount of overlap between pollen dispersal and female receptivity in the orchard, plus the amount of pollen generated by the orchard clones. The implications of these phenomena are discussed in greater detail below.

Differences in the pollen dispersal period between the seed orchard and natural stands

In both years, pollen dispersal in the seed orchard began earlier and ended later than in the natural stands. These results are consistent with a previous study by Muñoz-Gutiérrez et al. (2017), and could be partly attributed to the younger age, higher vigor, and greater genetic variation of the trees in the orchard. In conifers, reproductive bud and vegetative bud phenology are correlated (Dick et al. 1990). Furthermore, shoot growth generally begins earlier in younger trees, as shown for *Pinus pinaster* Aiton (Miguel-Pérez et al. 2002). Moreover, in younger, smaller and evenly-spaced trees, sunshine

reaching the ground results in warmer temperature in the lower canopy. This can accelerate bud flush and pollen dispersal because this is where male strobili are located (Lindgren et al. 1995). Nikkanen (2001) mentions that competition among trees also affects male flowering phenology; when trees are more open-grown and receive more sunlight, flowering occurs earlier. Thus, these differences in flowering phenology may be observed regularly between orchards and mature natural stands.

In both years, we found an 8-day overlap in pollen dispersal between the orchard and natural stands. Thus, there is a risk of external pollen reaching the receptive trees in the orchard. The risk of pollen contamination is affected by several other factors, including the size of the orchard, orchard pollen production, phenological variation among clones, and the degree of synchrony between female receptivity and pollen dispersal in the orchard (Di-Giovanni and Kevan 1991). Pulkkinen (1994) point out that larger-sized orchards are less affected by non-orchard pollen contamination. In a previous study, we reported that pollen production in this *P. patula* orchard was 2.5 times higher than in natural stands (Muñoz-Gutiérrez et al. 2017). This suggests orchard pollen production can offset the impact of pollen flow from nearby stands. Several authors have argued that phenological variation and the degree of reproductive synchronization among seed orchard clones are the most important factors affecting the risk of pollen contamination (El-Kassaby et al. 1984; Reynolds and El-Kassaby 1990). When male and female bud phenology are not well synchronized in the orchard, the adverse effects of external pollen can be substantial (Harju and Nikkanen 1996). Furthermore, these effects can be particularly pronounced because female strobili are usually receptive before pollen dispersal occurs (Pulkkinen 1994).

The effects of elevation on pollen dispersal in natural stands

In the natural stands, a negative linear relationship with elevation was found for M_end and M_length, but not for M_onset (Fig. 1). These results agree with a previous report (Muñoz-Gutiérrez et al. 2017), where no linear trend was found for the onset of pollen dispersal along the elevational transect, but ending of pollen dispersal was delayed as elevation increased. Thus, direct measurements of bud phenology allowed us to detect similar elevational trends on the phenology of pollen dispersal (i.e., the same as those detected with pollen traps).

Trees that disperse pollen earlier generally have a longer dispersal period (Codesido et al. 2005). However, we did not find this relationship in the natural stands sampled along the elevational gradient. In our study, the joint effect of other factors, such as age and size of trees, may have obscured this relationship. At the higher elevations, trees were 16 to 20 years old and were smaller (DBH = 15 to 18 cm) (Muñoz-Gutiérrez et al. 2017). Despite these confounding effects, we observed a trend towards a shorter pollen dispersal period as elevation increased, similar to that reported for *Pseudotsuga menziesii* (Silen 1963), *Pinus radiata* (Griffin 1980) and *Pinus roxburghii* Sarg. (Khanduri 2012; Mohan et al. 2012).

Although M_onset was not associated with elevation, M_length was shorter at higher elevations. In *Pseudotsuga menziesii*, onset occurred later and the duration of pollen dispersal decreased linearly with elevation (Silen 1963). Despite the differences between these studies, the risk of pollen contamination decreases as the difference in elevation between the orchard and natural stands increases. For example, in our study, the overlap between the two decreased 2–4 days from the lower to the higher elevation stands.

Genetic variation in reproductive phenology of seed orchard clones

The LS-mean values of F_length and M_length varied about 3 days across years, but F_length was 9 d longer than M_length in both years (Table 4). These differences primarily result from an earlier F_onset. These results are similar to those reported for *Pinus radiata* (Codesido et al. 2005), *P. nigra* Arn. (Lario et al. 2001) and *P. sylvestris* (Burczyk and Chalupka 1997). Pulkkinen (1994) mentioned that "metandry" is fairly common in *Pinus* species; that is, female strobili usually become receptive before male strobili on the same tree are ready to release pollen. However, in our study, most of the earliness and longer length of the receptivity period is due to the criterion used to define F_onset (i.e., when phenological stage 2 was reached, assuming 20% receptivity). If we define F_onset as the date when phenological stage 3 is reached, only 23 and 13% of the clones in 2014 and 2015, respectively, exhibit the metandry effect, and F_length reduces to 15.2 and 14.5 days, respectively.

We found positive genetic ($r_g = 0.67$) and phenotypic ($r_p = 0.60$) correlations between F_onset and M_onset. These results indicate that clones with earlier female receptivity also start releasing pollen earlier. These results are similar to those found for *Pinus taeda* L. seed orchards (Askew 1988), where female receptivity and the onset of pollen dispersal were positively correlated ($r_p = 0.45$). Nikkanen (2001) also found that female receptivity and pollen release periods were positively correlated in *Picea abies* (L.) H. Karst. clones (e.g., r_p varied from 0.26 to 0.53 in different years). In *Pinus nigra*, Matziris (1994) found a weak correlation between the onset of receptivity and pollen dispersal ($r_p = 0.35$) in a single year, indicating that only a small portion (10%) of the variation in the onset of pollen dispersal was attributed to the date of female receptivity. Although the correlations between these phenological traits in *P. patula* was only moderate, our results indicate there is a risk of selfing that could negatively affect the seed crop.

We found a large amount of genetic variation in reproductive bud phenology, similar to that found in other conifers, such as *Pinus radiata* (Codesido et al. 2005), *Pseudotsuga menziesii* (El-Kassaby and Askew 1991) and *Picea sitchensis* (Bong.) Carr. (El-Kassaby and Reynolds 1990). However, in *P. patula*, we found no genetic variation in F_length. In addition, the moderate to high values of H_c^2 for most phenology traits show that they are under moderate to strong genetic control. Furthermore, the low values of σ_{rc}^2 and the high values of r_B indicate that phenological differences among clones are relatively stable between years.

In this study, M_onset and M_length showed stronger genetic control than the F_onset and F_length, contrary to what has been found in other species such as *Pinus radiata* (Griffin 1984; Codesido et al. 2005), *P. nigra* (Matziris 1994) and *Picea abies* (Nikkanen 2001). In those studies, phenological traits related to female receptivity had a similar or even stronger genetic control than phenological traits related to pollen dispersal. However, genetic control for F_end was slightly stronger than for M_end in *P. patula* clones, similar to the results found in *P. radiata* (Griffin 1984; Codesido et al. 2005). In the case of female receptivity, the greater genetic control of F_onset, relative to F_length, coincided with the results of Matziris (1994) in *P. nigra*. This author indicated that the date of reproductive bud flush was under greater genetic control than the duration of receptivity.

The negative phenotypic correlations of F_onset with F_length (−0.85) and M_onset with M_length (−0.90) indicate that clones with a longer female receptivity or pollen dispersal period begin earlier in the year. Thus, the risk of genetic contamination

in these clones is less, because pollen dispersal in natural stands begins later than in the orchard. Codesido et al. (2005) mentions that *P. radiata* clones with longer female receptivity or pollen dispersal had a greater possibility of transmitting their genes to orchard progeny (Boes et al. 1991).

Implications for genetic contamination in the seed orchard

Pollen contamination in seed orchards is a fairly common phenomenon documented for wind-pollinated coniferous species (El-Kassaby et al. 1989; Adams and Burczyk 2000; Slavov et al. 2005). However, the amount of genetic contamination detected has varied from as low as 1% in *Picea glauca* (Stewart 1994) to as high as 85% in *Pinus brutia* (Kaya et al. 2006), or 91% in *Pseudotsuga menziesii* (Adams and Birkes 1989). Several factors contribute to this wide variation in pollen contamination, and pollen dispersal phenology is one of them (Adams and Burczyk 2000). The broad overlap between reproductive phenology in the *P. patula* seed orchard and pollen dispersal in natural stands shows there is a risk of genetic contamination in the orchard. This would lower the genetic quality of the seed crop and genetic gains in plantations when using this germplasm. Other studies have shown that phenological isolation reduces the risk of pollen contamination, such as in *Pinus sylvestris* (Parantainen and Pulkkinen 2003); *Pseudotsuga menziesii* (El-Kassaby and Ritland 1986) and *Picea abies* (Pakkanen et al. 2000).

However, we show that the risk of pollen contamination varies from year to year because the pollen dispersal period shifts according to the weather. The greatest risk of genetic contamination comes from the neighboring stands situated at lower or similar altitude compared to the orchard. This is because the length of the dispersal period decreases as elevation increases and, therefore, the degree of overlap also decreases.

In addition, the strong genetic control and genetic stability of reproductive phenology indicates that risk of genetic contamination varies among seed orchard clones. For example, clones with early F_onset and F_end are less exposed to non-orchard pollen, reducing their possibility of contamination. Moreover, genetic variation of clones in M_onset, M_end and M_length causes different levels of phenological overlap between external pollen and single-clone pollen dispersal in the seed orchard. Thus, competition with external pollen varies among clones, and so their potential contribution of male gametes to the seed crop.

Even though clones with earlier female receptivity have a lower risk of genetic contamination by external pollen, they may have lower seed set because of low pollen production in the orchard early in the season. However, the broad genetic variation in female and male phenology in the orchard, and the modest correlations between male and female phenology promote cross-pollination. Management activities that foster earlier onset of reproductive phenology in the orchard (El-Kassaby and Reynolds 1990) would be beneficial to reduce the risk of genetic contamination by outside pollen.

Conclusions

The study showed that there were no differences between years in the onset of female receptivity and pollen dispersal in the seed orchard, but both periods were longer during the second year. In natural stands, the onset of pollen release showed a large difference between years. On the other hand, pollen dispersal in the seed orchard began earlier and

ended later than in nearby natural stands, resulting in a longer duration of pollen dispersal at the orchard in both years. Thus, a risk of genetic contamination of the orchard exists, but the risk might differ from year to year because the degree of overlap between pollen dispersal in natural stands and female receptivity in the orchard clones varies by year.

Genetic variation was found in reproductive phenology, and moderate genetic and phenotypic correlations were found between the onset of female receptivity and pollen release. This indicates that the timing of male and female phenology are correlated and there is a risk of selfing. The high genetic control and genetic stability of clones for most phenological traits indicate that the risk of genetic contamination varies among clones, and depends on how early they become receptive. Management activities to accelerate the phenological events inside the orchard or to increase the presence of pollen from the orchard to compete favorably with external pollen would be valuable.

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