SCIENCE CHINA
Life Sciences

FROM CAS & CAE MEMBERS
•RESEARCH PAPER•

September 2018 Vol.61 No.9: 1011–1023
https://doi.org/10.1007/s11427-018-9305-7

Mating patterns and pollen dispersal in a Japanese larch (*Larix kaempferi*) clonal seed orchard: a case study

Xingbin Chen[1,2,3†], Xiaomei Sun[1,2†], Leiming Dong[1,2] & Shougong Zhang[1,2*]

[1]*State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing 100091, China;*
[2]*Key Laboratory of Tree Breeding and Cultivation of State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China;*
[3]*Jiangxi Provincial Key Lab for Plant Biotechnology, Jiangxi Academy of Forestry, Nanchang 330013, China*

Received February 1, 2018; accepted March 21, 2018; published online June 4, 2018

Pollination dynamics highly determines the genetic quality of seed orchard crops. However, there is less research about the effect of mating patterns on seed productivity of orchard crops. So far, clonal seed orchards have been producing genetically improved seedlings used for most Japanese larch (*Larix kaempferi* (Lamb.) Carr.) plantations in China. In the present study, a total of 17 highly variable simple sequence repeat (SSR) markers were used for genotyping a progeny trial population consisting of 647 open-pollinated progenies germinated from seeds which were collected from 63 maternal clones with 140 potential paternal clones in a Japanese larch clonal seed orchard in China. Paternity analysis was used in the present case study in order to evaluate the level of paternal gametic contribution, estimate pollen contamination and selfing rates, and investigate pollination patterns, pollen dispersal patterns and the impact of mating patterns on seed productivity of orchard crops. We observed 93.7% of the success rate of the parental assignment, unequal paternal gametic contribution (0–12.4%) with 6.3% of the progenies derived from pollen contamination or unsampled pollen donors, and absence of evidence for selfing. We also found that pollination rate highly depended on the distance between pollen donors and maternal parents, the majority of the identified crossing (65.7%) occurred between clones within a 150-m radius, and large variations in growth performance existed among the paternal half-siblings. Progeny growth performance (diameter at breast (DBH) and height (HGT)) was measured at Age-20 in order to investigate the impact of mating patterns on timber production of orchard crops. As either the paternal or maternal, two clones (i.e., clones Z38 and Z62) were identified to have produced progenies with higher average stem volume breeding values than that of all of the progenies. Specifically, the genetic gains for volume were 3.53% for the two clones as paternal parents, and 8.26% as the maternal parents at Age-20. Thus, both elite clones were ideal candidates for the construction of next-generation clonal seed orchards due to their synchronous reproductive phenology with greater crossing rate and higher genetic gain. These results improved the pedigree information to provide solid evidence of mating patterns for future design and effective management of seed orchards and for the development of viable long-term breeding strategies for other coniferous species.

***Larix kaempferi*, simple sequence repeats (SSRs), paternity analysis, pollen contamination, pollen dispersal, growth performance**

Citation: Chen, X., Sun, X., Dong, L., and Zhang, S. (2018). Mating patterns and pollen dispersal in a Japanese larch (*Larix kaempferi*) clonal seed orchard: a case study. Sci China Life Sci 61, 1011–1023. https://doi.org/10.1007/s11427-018-9305-7

†Contributed equally to this work
*Corresponding author (email: shougong.zhang@caf.ac.cn)

INTRODUCTION

Japanese larch (*Larix kaempferi* (Lamb.) Carr.) is monoecious, mainly wind-pollinated and outbreeding in addition to slight inbreeding due to the absence of self-incompatibility (Nishimura and Setoguchi, 2011). It is native to the mountainous regions of central Honshu Island in Japan, and was introduced into China at the end of the 19th century (Isoda and Watanabe, 2006; Sun et al., 2008). Since 1960s, clonal seed orchards of Japanese larch have been established in various regions in China by using superior clones as scions. These clonal seed orchards have been producing genetically improved seedlings, which had been used for most Japanese larch plantations in the sub-tropical alpine zones in China and Northeast China (Wang et al., 2000). Thus, this exotic species has become one of the most important plantation species in China due to its fast juvenile growth, adaptability to various environmental conditions, and exceptional wood properties (Lai et al., 2014). The plantation area of Japanese larch has been over 0.3 million hectares in China, and increases at the speed of 30,000 hectares per year.

The mating patterns and the levels of genetic diversity in the clonal seed orchards highly determine the plantations adaptability, biotic and abiotic resistance, and sustainability (Grattapaglia et al., 2014). The genetic makeup of seed orchard crops is determined by the degrees of deviation from panmixia, which is highly affected by the mating patterns, reproductive output and phenology, gene flow, and self-fertilization of the seed orchard crops (Codesido et al., 2005; Torimaru et al.,2013). The degree of deviation from random mating also determines the difference between the expected vs. realized genetic gain of seed orchards crops (Funda et al., 2015). Synchronization of flowering phenology, equality of female and male strobili production, and equal compatibility could promote random mating. However, an uneven parental gametic contribution might lead to substantial co-ancestry among seed lots and subsequently reduce the genetic diversity and the offspring fitness. It was demonstrated that the skewed mating in clonal seed orchards is mainly caused by inter-individual variation in male and female fecundity (Nielsen and Hansen, 2012), differential reproductive success (Funda et al., 2015), flowering asynchrony (Li et al., 2011), and pollen competitive ability (Nikkanen et al., 2000). Genetic contamination by the air-borne pollen from unselected trees outside the orchards generally reduces potential genetic gain, while self-fertilization causes inbreeding depression, which is usually associated with high seeding mortality (Stoehr and El-Kassaby, 1997). Therefore, a thorough assessment of the extent of pollen-mediated gene flow, selfing, parental gametic contribution, and pollen dispersal patterns is needed for the proper design and management of clonal seed orchards (Moriguchi et al., 2010).

Allozymes had been used to study the outcrossing rates in a *L. sibirica* and *L. decidua* seed orchard (Burczyk et al., 1997). The disadvantage of allozyme analysis in such studies is that they do not allow pollen donors to be identified due to the limited number of allozyme loci available. However, microsatellites (simple sequence repeats or SSRs) are highly polymorphic, co-dominant, and considered to be selectively neutral genetic markers (Silva et al., 2013). They have been used extensively for investigating the mating systems, pollen dispersal patterns, and pollen pool composition of forest trees such as *Pinus sylvestris* (Robledo-Arnuncio and Gil, 2005), *Juglans mandshurica* (Bai et al., 2007), *Acacia saligna* (Millar et al., 2008), *Castanea crenata* (Hasegawa et al., 2009) and *Phoenix canariensis* (Saro et al., 2014). SSRs have also been used to investigate the pollen contamination rate and paternal contributions of the *L. kaempferi*, *L. occidentalis* and *L. olgensis* seed orchards (Funda et al., 2008; Hansen, 2008; Wei et al., 2015). However, their genotyped offspring derived from germinating embryos and empirical phenotype data from the seedlings could not be or were not used for the analysis of the impact of mating patterns on timber production of orchard crops and the selection of desired clones. Moreover, the improvement of offspring's timber production is the ultimate goal of development of seed orchards; meanwhile, selecting desired clones could provide materials for the advanced seed orchard. So far, little is known about the impact of mating patterns of Japanese larch clonal seed orchard on seed productivity, which severely limits the further improvement of seed orchard yield and the construction of advanced generation clonal seed orchards.

The objectives of this study were to evaluate the mating patterns and the pollen dispersal and the impact of mating patterns on seed productivity in a Japanese larch seed orchard in China based on paternity analyses of a progeny trial population. Understanding of the parental reproductive success, selfing rate, level of pollen contamination, and the success rate of pollen augmentation is essential for the determination of both the genetic gain and diversity of the seed orchard crop. These parameters are also vital in the development of effective population management practices (such as bloom delay and supplemental mass pollination) in order to advance the Japanese larch clonal seed orchard management.

RESULTS AND DISCUSSION

Genetic diversity of the parental and progeny population

Genetic diversity was calculated in the 140 parental clones using the data collected from the 17 SSRs. The smallest number of alleles per locus (A_o) was 2 which was obtained from markers Q299 and H197, and the highest was 14 from

markers H140 and Q386 with a mean being 5.7 (Table 1). The observed heterozygosity (H_o) ranged from 0.214 (for marker H339) to 0.842 (for marker Q386) (Table 1). When the genotypes of a progeny and its maternal parent were identified, the average probability of paternal exclusion per locus was 0.302 while the average probability of paternal exclusion per multiple loci was above 0.999. The estimated frequency of null alleles was lower than 0.05 for most SSR markers (Table 1).

Genetic diversity was calculated in the 647 progenies derived from the 63 maternal clones using the data collected from the 17 SSRs. The number of alleles per locus (A_o) ranged from 2 (for marker H177) to 17 (for marker Q386) with a mean being 6.9 (Table 2). Compared to the parental population, the proportion of observed heterozygosity in the progenies displayed an increase, but not at a significant level (H_o=0.542 and H_e=0.525 vs. H_o=0.571 and H_e=0.557). Thus, the progeny population contained a higher number of alleles than their paternal population, suggesting relatively free genetic exchanges among the parental clones and possible introduction of novel alleles by pollen contamination (Kess and El-Kassaby, 2015). The average inbreeding coefficient (F) for the progeny population showed an excess of heterozygotes (-0.025), which was similar to that of the parental population (Tables 1 and 2). The estimated frequency of null alleles was lower than 0.05 for all of the 17 SSR markers (Table 2).

Paternity assignment

Gene flow from unknown pollen donors into clonal seed orchards raises concerns about the genetic integrity of progenies. Thus, it is important to investigate the extent of pollen contamination in a clonal seed orchard. The paternity analysis implicitly assigned 606 out of the 647 sampled progenies (93.7%) to 108 out of the 140 paternal clones (77.1%) in the clonal seed orchard. The unassigned 41 progenies (6.3%) did not match any of the 140 clones, and were considered to be derived from donors outside the orchard or unsampled, deceased clones (Table 3). The number of progenies assigned to paternal clone varied from 1 to 75, with an average of 5.61. The highest paternal contribution was 12.4% of the total assigned progenies (i.e., 606), and the top 10 paternal parents were collectively sired with 43.2% of the assigned progenies. Among the 108 identified paternal parents, each of 82 paternal clones (75.9%) sired two or more progenies. Paternal clones successfully mated with 1–42 maternal parents (Figure 1). The paternal contribution to the pollen pool indicated that 50% of the clones accounted for 91.7% of the fertilized eggs (Figure 2). The allelic frequencies in seed orchard crops sometimes deviate from their parental populations (Bilgen and Kaya, 2015; Sønstebø et al., 2018), even though balanced pollen contribution is important for producing seeds with elite parents' genetic superiority (Funda et al., 2016; Gonzaga et al., 2016).

Selfing in conifers causes inbreeding depression, high embryo abortion and seedling mortality (Moriguchi et al., 2005). Therefore, it is very important to assess and implement crop management practices that reduce self-fertilization in conifer seed orchards. The estimated outcrossing rate was 100%, indicating that selfing was absent in the analyzed progeny samples. This was consistent with previous reports that selfing was relatively low in *Larix* clonal seed orchards such as in orchards of *L. decidua* (Lewandowski et al., 1991), *L. sibirica* and *L. decidua* (Burczyk et al., 1997), *L. occidentalis* (Funda et al., 2008), *L. kaempferi* (Hansen, 2008), and *L. olgensis* (Wei et al., 2015). There was no specific self-incompatibility in *L. kaempferi* due to our other same age trail of 12×12 full-diallel mating design with selfing offspring (the paper under review). The absence of selfing in the present study could be attributed to our relatively large parental population (i.e., 152 clones) and the reported high abortion rate of *Larix* self-pollinated embryos (Burczyk et al., 1997; Slobodník, 2002; Slobodník and Guttenberger, 2005).

The χ^2 test for the goodness of fit of the null hypothesis that every clone and ramet made equal contributions to the seed production showed that the levels of paternal contributions significantly differed from each other (P<0.001). The paternal contribution per clone did not increase with the number of ramet per clone (i.e., clone size). Correlation between number of ramets and the paternal contribution per clone was not significant (P=0.209) with Pearson's correlation coefficients being 0.122. Notwithstanding, selections in both seed germinations and younger seedlings in nursery should be change genotype frequency of progeny. This indicates that many factors including reproductive energy and synchrony, and distance between parents could affect the actual reproductive success of the parental population (Schoen and Stewart, 1986; Roberds et al., 1991; Aronen et al., 2002; Moriguchi et al., 2005; Canchignia-Martínez et al., 2007).

Although paternity assignment does not permit any inference on the genetic information of unassigned fathers, our study was not problematic because the clones' number of unassigned progenies was low (23 out of 2108 ramets) and only the assigned progenies would be used as the selection candidates for the construction of next-generation clonal seed orchard. Multiple methods have been used for parentage assignment. CERVUS assigns progenies to their parent pairs based on the pair-wise likelihood comparison approach. The Bayesian approach could simultaneously estimate the parentage of sampled progenies and a wide range of population-level parameters, which could increase the power of parentage assignment while reducing bias in parameter estimation (Hadfield et al., 2006). The full-pedigree likelihood

Table 1 Diversity parameters for the 140 Japanese larch parental clones[a)]

Locus	A_o	H_o	H_e	F	PE-1P	PE-2P	PE-PP	PE-Id	f(null)
H46	4	0.676	0.602	–0.1294	0.180	0.310	0.454	0.755	–0.064
H52	5	0.338	0.338	–0.0052	0.059	0.185	0.317	0.541	–0.020
H140	14	0.659	0.613	–0.0804	0.228	0.413	0.622	0.823	–0.047
H177	3	0.507	0.482	–0.0567	0.115	0.186	0.281	0.618	–0.028
H197	2	0.400	0.338	–0.1879	0.057	0.140	0.224	0.503	–0.086
H217	3	0.643	0.603	–0.0724	0.180	0.318	0.465	0.765	–0.039
H233	4	0.696	0.594	–0.1752	0.180	0.331	0.491	0.773	–0.089
H299	6	0.779	0.708	–0.1032	0.292	0.464	0.647	0.865	–0.051
H339	4	0.214	0.217	0.0070	0.023	0.101	0.177	0.366	0.024
HL215	3	0.529	0.501	–0.0612	0.124	0.205	0.311	0.641	–0.035
HL391	5	0.223	0.248	0.0957	0.031	0.119	0.207	0.411	0.064
Q299	2	0.380	0.382	0.0022	0.072	0.154	0.241	0.544	–0.001
Q322	4	0.488	0.435	–0.1286	0.094	0.176	0.272	0.591	–0.063
Q375	4	0.410	0.493	0.1638	0.121	0.249	0.388	0.680	0.097
Q386	14	0.842	0.828	–0.0201	0.500	0.671	0.854	0.952	–0.014
Q397	12	0.736	0.808	0.0863	0.453	0.628	0.814	0.938	0.047
Y27	8	0.702	0.731	0.0358	0.313	0.485	0.664	0.878	–0.013
Average	5.7	0.542	0.525	–0.0382	0.178	0.302	0.437	0.685	0.017
Combined	–	–	–	–	0.973	0.999	0.999	0.999	–

a) A_o, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F, inbreeding coefficient; PE-1P, average probability of paternity exclusion of a candidate parent given only the genotype of the progeny; PE-2P, average probability of paternity exclusion of a candidate parent given the genotype of the progeny and of one known parent; PE-PP, average exclusion probability for a candidate parent pair; PE-Id, average probability of identity exclusion of two unrelated individuals; PE-FS, average probability of identity exclusion of two full-sibs; f(null), estimate of null allele frequency (Summers and Amos, 1997).

Table 2 Diversity parameters for the 647 Japanese larch progenies[a)]

Locus	A_o	H_o	H_e	F	f(null)
H46	4	0.636	0.630	–0.0107	–0.0448
H52	6	0.451	0.421	–0.0727	–0.0486
H140	14	0.696	0.718	0.0303	–0.0353
H177	2	0.451	0.441	–0.0243	0.0120
H197	3	0.357	0.343	–0.0427	–0.0215
H217	6	0.612	0.608	–0.0063	–0.0110
H233	5	0.707	0.658	–0.0751	–0.0407
H299	7	0.781	0.728	–0.0734	–0.0377
H339	8	0.397	0.381	–0.0407	–0.0146
HL215	8	0.577	0.556	–0.0374	–0.0002
HL391	4	0.320	0.295	–0.0859	0.0146
Q299	3	0.404	0.437	0.0756	0.0486
Q322	4	0.427	0.403	–0.0600	–0.0185
Q375	4	0.505	0.486	–0.0406	–0.0048
Q386	17	0.860	0.843	–0.0202	–0.0292
Q397	14	0.859	0.850	–0.0109	–0.0391
Y27	8	0.667	0.676	0.0127	0.0065
Average	6.9	0.571	0.557	–0.0251	–

a) A_o, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F, inbreeding coefficient; f(null), estimate of null allele frequency given by the Individual Inbreeding Model-based estimator (Summers and Amos, 1997).

Table 3 Paternity analysis for the 647 Japanese larch progenies[a)]

Maternal clone	Number of progenies	Number of assigned progenies	Rate (%)	Number of assigned paternal clones	Assigned paternal diversity (%)	Outcrossing rate (%)
Z01	10	7	70	7	100	100
Z02	10	9	90	6	66.7	100
Z03	11	11	100	8	72.7	100
Z04	9	8	89	7	87.5	100
Z05	10	9	90	8	88.9	100
Z06	9	9	100	6	66.7	100
Z07	10	10	100	8	80	100
Z09	9	9	100	6	66.7	100
Z10	9	9	100	6	66.7	100
Z11	10	9	90	6	66.7	100
Z12	10	8	80	8	100	100
Z13	10	9	90	9	100	100
Z14	10	10	100	9	90	100
Z15	10	9	90	6	66.7	100
Z16	10	7	70	7	100	100
Z17	10	9	90	9	100	100
Z18	10	9	90	6	66.7	100
Z19	10	10	100	8	80	100
Z20	9	9	100	6	66.7	100
Z21	10	9	90	9	100	100
Z22	10	10	100	6	60	100
Z23	10	8	80	7	87.5	100
Z24	9	9	100	8	88.9	100
Z25	9	8	88.9	6	75	100
Z26	8	8	100	7	87.5	100
Z27	11	11	100	9	81.8	100
Z28	10	10	100	10	100	100
Z29	10	7	70	6	85.7	100
Z30	8	8	100	7	87.5	100
Z31	11	10	90.9	9	90	100
Z32	10	10	100	8	80	100
Z33	10	10	100	7	70	100
Z34	10	10	100	8	80	100
Z35	10	10	100	9	90	100
Z36	11	11	100	9	81.8	100
Z37	8	8	100	8	100	100
Z38	11	11	100	3	27.3	100
Z39	10	8	80	8	100	100
Z40	10	10	100	9	90	100
Z41	10	10	100	8	80	100
Z42	10	9	90	5	55.6	100
Z43	10	9	90	7	77.8	100
Z44	10	10	100	6	60	100
Z45	10	10	100	9	90	100
Z46	11	10	91	7	70	100

(To be continued on the next page)

(*Continued*)

Maternal clone	Number of progenies	Number of assigned progenies	Rate (%)	Number of assigned paternal clones	Assigned paternal diversity (%)	Outcrossing rate (%)
Z47	9	9	100	9	100	100
Z49	10	9	90	7	77.8	100
Z50	10	10	100	5	50	100
Z52	10	9	90	7	77.8	100
Z53	11	11	100	8	72.7	100
Z54	9	8	88.9	5	62.5	100
Z55	34	33	97.1	16	48.5	100
Z56	11	10	91	9	90	100
Z57	10	9	90	9	100	100
Z58	10	10	100	8	80	100
Z59	10	9	90	7	77.8	100
Z60	11	11	100	8	72.8	100
Z62	9	9	100	8	88.9	100
Z63	9	8	88.9	7	87.5	100
Z64	11	8	72.7	6	75	100
Z65	10	10	100	9	90	100
Z66	10	9	90	8	88.9	100
Z67	10	10	100	7	70	100
Total	647	606	–	108		–

a) Rate=Number of assigned progenies/Number of progenies; Assigned paternal diversity=Number of assigned paternal clones/Number of assigned progenies.

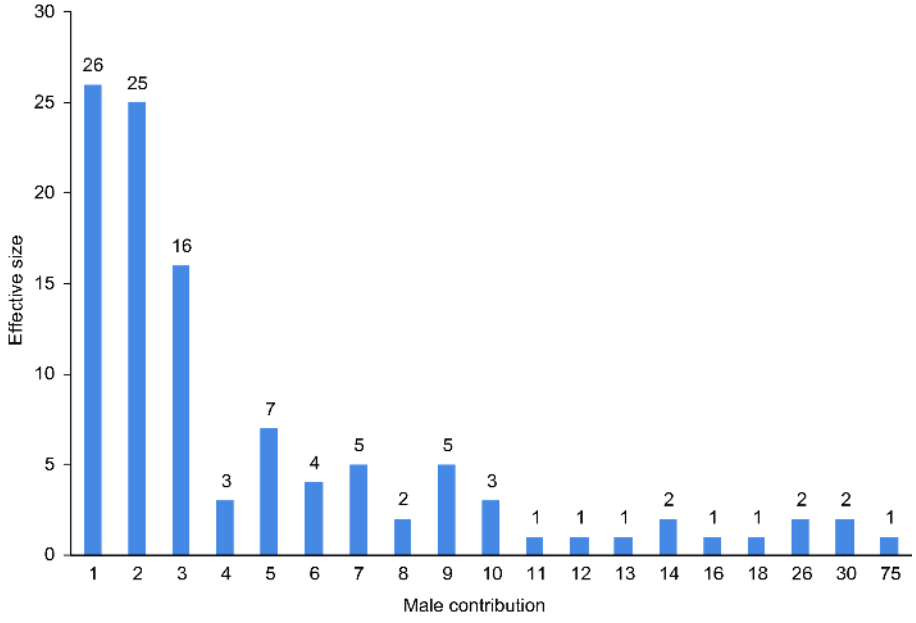

Figure 1 Distribution of paternal contributions of the 108 identified paternal clones in the Japanese larch clonal seed orchard.

method considers the likelihood of the entire pedigree structure and allows the simultaneous inference of parentage and sibship (Jones et al., 2010), which avoids the troublesome compatibility issues that could arise from pairwise methods. A combination of different methods would provide a better paternity assignment in clonal seed orchard studies.

Pollination dispersal pattern

Information of pollen dispersal patterns in a clonal seed orchard is important for the design of new seed orchards and the development of effective management practices (Funda et al., 2008). The amplitude of average pollination distance

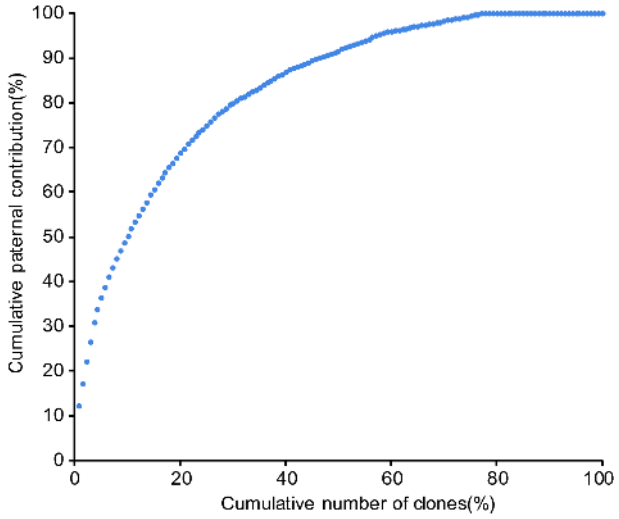

Figure 2 Relationship between cumulative number of paternal clones (%) and cumulative paternal contribution (%) in the Japanese larch clonal seed orchard. Numbers of paternal clones were cumulated sequentially from clones with the highest contribution.

among the studied maternal clones was 25.33–643.78 m (Table 4), indicating significant difference of average pollination distance. All of the maternal clones showed substantial variation in average pollination distance, with all mean coefficients of variation in average pollination distance being higher than 77.90. The distance between the maternal and paternal clones ranged within 740.00 m with an average of 149.22 m. About 65.7% of the crossing happened between clones within a 150.00 m radius, and only 7.9% of the crossing occurred between individuals with a distance larger than 400.00 m. This might partially result from the large, heavy and wing-less (non-saccate) pollen grains of Japanese larch (Doyle J, 1935; Slobodník, 2002). So, the average pollination distance for each Japanese larch maternal clone was dependent on its location in the clonal seed orchard and the affinity between paired gametes.

Since the maternal clone Z55 was located in the center of the clonal seed orchard and contained the largest number of progenies (i.e., 34 progenies) among the 63 maternal clones, the 34 progenies derived from the maternal clone Z55 were used for pollen dispersal pattern analysis. The paternity analysis of the maternal clone Z55 assigned 33 out of the 34 progenies to the seed orchard's paternal clones. The paternal clones at shorter distance from the maternal clone produced more progenies (Figure 3). Clone Z55 received pollen from donors at various distances from 8 to 233 m with 10 out of the 33 progenies (30.3%) being pollinated by the nearest clone (clone Z17 at the distance of 8 m). These two clones had synchronous reproductive phenology as observed in 2012–2015, which led to a greater crossing rate.

The impact of mating patterns on the timber production of orchard crops

Large variations in growth performance were observed among the paternal half-siblings. For example, the volume breeding values of the paternity ranged from –9.71 to 20.72 with the overall average individual volume breeding value of all of the 606 paternal half-siblings being 1.66 at Age-20. In addition, the progenies derived from the paternal clones with higher paternal contribution values had relatively worse growth performance. For example, the progenies derived from the paternal Z48 clone, which produced the highest number of progenies (i.e., 75 progenies), had the individual volume breeding value of 1.31 at Age-20, which were comparable to the overall average individual volume breeding values of all of the 606 paternal half-siblings at Age-20. There existed 37 paternal clones whose progenies had higher average individual breeding volumes than the overall average individual volume breeding value of all of the paternal half-siblings at Age-20. Six out of the 37 paternal clones had higher paternal contributions than the average paternal contribution of all of the 108 paternal clones, which were 5.61 progenies per clone at Age-20 (Table 5). Thus, the 6 clones could be used as pollen donors for controlled pollinations (CP), supplemental mass pollination (SMP), and controlled mass pollination (CMP) in future. As either the paternal or maternal material, clones Z38 and Z62 produced progenies with higher average volume breeding values than that of all of the progenies at Age-20. Specifically, the genetic gains for volume were 3.53% for the two clones as paternal parents, and 8.26%, as the maternal parents at Age-20. Thus, both clones were ideal candidates for use in the construction of next-generation clonal seed orchards.

Implications of paternity assignment for Japanese larch domestication

The paternity assignment is the technology based on DNA fingerprinting for maternally known but paternally unknown offspring and is mainly used in the fields of population and conservation genetics (Bai et al., 2007). Further, Yousry El-Kassaby and his colleagues proposed "Breeding without Breeding" (BwB) in 2007 with an attempt to circumvent artificial matings and field testing (El-Kassaby and Lindgren, 2007). The time interval of a breeding cycle can be shortened to 4 years and major part of the genetic response to selection achieved by traditional method was captured by BWB (El-kassaby and Lstibůrek, 2009; Hansen and McKinney, 2010). Meanwhile, the genetic diversity in parents will be passed on to the offspring. However, breeders should keep in mind the limitations of paternity assignment (or pedigree reconstruction) including BWB. The genotyping cost would exponentially grow along with the increasing number of parents in seed orchard or offspring sample size which might be over the budget of the program. Finally, paternity assignment and BWB are only suitable for the improvement

Table 4 Differences in average pollination distance among maternal clones

Maternal clone	Number of assigned paternal clones	Mean	Std. error	Amplitude	CV (%)
Z01	7	214.71	210.65	27.00–530.00	87.15
Z02	6	147.33	164.33	27.00–514.00	115.15
Z03	8	227.82	109.57	18.00–346.00	48.10
Z04	7	259.00	133.79	113.00–418.00	51.66
Z05	8	131.00	69.42	24.00–212.00	52.99
Z06	6	291.56	105.53	50.00–372.00	36.19
Z07	8	98.70	122.86	18.00–343.00	124.48
Z09	6	76.44	80.21	4.00–208.00	104.93
Z10	6	75.33	62.62	20.00–209.00	83.12
Z11	6	58.11	17.58	28.00–84.00	30.25
Z12	8	54.25	63.82	8.00–206.00	117.63
Z13	9	88.22	90.50	4.00–252.00	102.58
Z14	9	141.20	103.17	16.00–300.00	73.07
Z15	6	74.78	98.33	8.00–285.00	131.49
Z16	7	32.00	30.81	8.00–100.00	96.29
Z17	9	128.22	77.80	60.00–278.00	60.68
Z18	6	107.67	186.27	4.00–574.00	173.01
Z19	8	81.00	77.94	16.00–230.00	96.22
Z20	6	85.56	68.86	36.00–250.00	80.49
Z21	9	66.67	66.27	8.00–232.00	99.41
Z22	6	80.40	41.62	20.00–138.00	51.77
Z23	7	48.50	31.09	8.00–100.00	64.10
Z24	8	101.33	80.34	4.00–254.00	79.29
Z25	6	435.00	264.08	12.00–680.00	60.71
Z26	7	387.50	241.87	23.00–600.00	62.42
Z27	9	126.64	74.71	28.00–303.00	58.99
Z28	10	198.80	80.99	24.00–324.00	40.74
Z29	6	86.29	75.45	8.00–230.00	87.44
Z30	8	420.50	195.36	100.00–670.00	46.46
Z31	10	135.30	94.93	27.00–295.00	70.16
Z32	10	191.80	187.90	12.00–687.00	97.96
Z33	10	475.30	294.24	50.00–750.00	61.91
Z34	10	252.70	83.08	32.00–330.00	32.88
Z35	10	92.50	69.48	15.00–220.00	75.11
Z36	11	123.91	99.95	8.00–266.00	80.66
Z37	8	202.13	89.52	32.00–300.00	44.29
Z38	11	36.18	69.70	9.00–236.00	192.65
Z39	8	167.88	86.38	24.00–300.00	51.45
Z40	10	68.90	66.67	9.00–234.00	96.77
Z41	10	206.80	201.86	4.00–730.00	97.61
Z42	9	105.11	99.17	9.00–248.00	94.35
Z43	9	643.78	74.17	575.00–736.00	11.52
Z44	10	337.90	205.18	20.00–580.00	60.72
Z45	10	155.20	55.14	70.00–260.00	35.53
Z46	10	60.60	41.46	8.00–104.00	68.42

(To be continued on the next page)

(*Continued*)

Maternal clone	Number of assigned paternal clones	Mean	Std. error	Amplitude	CV (%)
Z47	9	25.33	13.56	8.00–40.00	53.54
Z49	9	119.44	90.20	15.00–236.00	75.52
Z50	10	127.40	208.12	9.00–648.00	163.36
Z52	9	99.33	110.32	24.00–350.00	111.06
Z53	11	54.55	56.97	8.00–180.00	104.44
Z54	8	53.50	33.51	24.00–102.00	62.64
Z55	33	66.52	70.57	8.00–233.00	106.10
Z56	10	76.70	63.73	8.00–207.00	83.09
Z57	9	116.89	121.28	8.00–400.00	103.75
Z58	10	114.70	69.93	16.00–254.00	60.97
Z59	9	231.78	127.40	96.00–446.00	54.97
Z60	11	115.36	100.54	22.00–400.00	46.46
Z62	8	127.56	88.46	24.00–258.00	97.96
Z63	8	105.88	89.87	12.00–274.00	61.91
Z64	10	135.13	119.22	8.00–328.00	32.88
Z65	9	81.80	53.35	24.00–188.00	75.11
Z66	10	57.67	55.10	8.00–164.00	80.66
Z67	11	110.60	93.66	8.00–296.00	44.29
Total		149.22			77.90

Table 5 The paternal contributions and the average individual volume breeding value of 6 paternal Japanese larch paternal clones at Age-20

Paternal clone	Number of ramets	Number of assigned progenies	The average individual breeding value of paternal half-siblings
Z76	10	26	23.46
Z17	20	30	22.52
Z34	63	13	7.80
Z43	31	30	3.80
Z38	75	10	5.86
Z62	52	6	3.30
Average of the 6 clones	41.83	19.17	11.12
Overall average of the 140 clones	15.1	5.61	

programs in tree species still in their primary stages. Open-pollinated matings should be superseded by more rigorous control-pollinated mating designs in advanced breeding generation due to their shortcomings such as the unbalanced paternal contribution just as the findings in this study.

The present case study provided valuable information about the paternal gametic contribution, pollen contamination and selfing rates, pollination patterns, pollen dispersal patterns, and the impact of mating patterns on seed productivity of orchard crops. Notwithstanding, selections in both seed germinations and younger seedlings in nursery should be change genotype frequency of progeny. We only exploited a relatively small amount of SSR markers (17) for parental assignment and could provide solid results with cheap genotyping effort and estimate the mating pattern of a real seed orchard for improving seed orchard construction and management accordingly. Since the present study was conducted in a Japanese larch clonal seed orchard in Northeast China, it might be an under representation of the natural variations of the species. Thus, a more comprehensive study is needed in different regions and different years in the future. In order to further reduce pollen contamination and improve parental balance, some precautions should be taken. First, the numbers of paternal clones in the Japanese larch clonal seed orchards should be about 60–80 since approximately 70 paternal clones contributed to >90% of the progenies. Second, the buffer zones should be more than 400 m in width around the Japanese larch clonal seed orch-

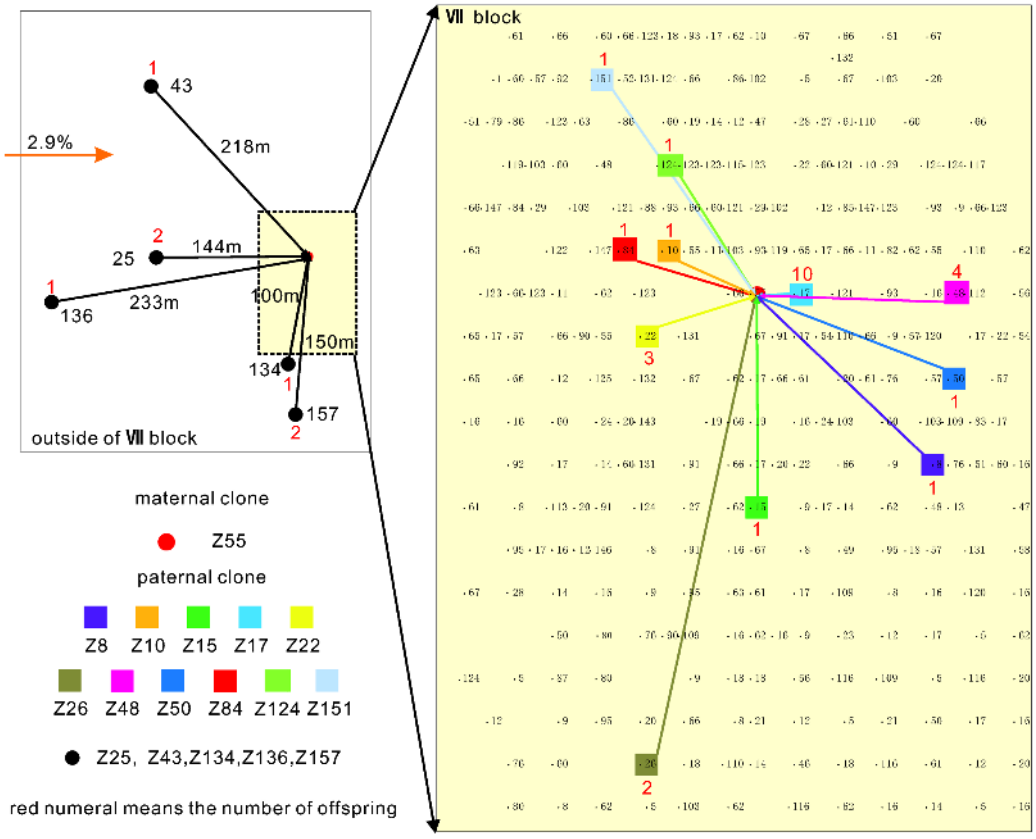

Figure 3 Distribution of the paternal parents of the 34 progenies of the maternal clone Z55, which was located in the center of the clonal seed orchard and contained the largest number of progenies among the 63 maternal clones. The paternity analysis of the maternal clone Z55 assigned 33 out of the 34 progenies to 11 paternal clones, i.e., clones Z8, Z10, Z15, Z17, Z22, Z26, Z48, Z50, Z84, Z124 and Z151. Clones Z8, Z10, Z15, Z17 and Z22 were located within the VII block and produced 26 offspring. Clones Z26, Z48, Z50, Z84, Z124 and Z151 were located outside of the VII block with their distance to Clone Z55 being indicated, and produced 7 offspring. Arrows with %, the percentage of progenies derived from pollen contamination. Tree distance=4 m. Red numerical numbers indicate the numbers of offspring.

ards. Third, during constructing advanced generation Japanese larch seed orchard, some new seed orchard designs could not be ignored for promoting assortative mating, and minimizing inbreeding (randomized complete block (White et al., 2007), minimum inbreeding design (MI) (Lstibůrek et al., 2015) and Optimum neighborhood seed orchard design (Chaloupková et al., 2016)). Fourth, the few elite clones (i.e., the clones Z38 and Z62) with both higher paternal contribution and breeding value as either the paternal or maternal identified by progeny test could be used for the establishment of next-generation Japanese larch clonal seed orchards. Fifth, imbalanced paternal contribution may be disadvantageous since the increased coancestry among progenies might lower genetic variation and lead to decreased fitness of progeny plantations. It is worthwhile to use some pollen management strategies such as cone induction (e.g., use of gibberellins to increase reproductive output), use of controlled pollination whenever possible, and supplemental mass pollination to increase the genetic quality of the produced seeds (Stoehr et al., 2006; Fernandes et al., 2008; Kaya and Isik, 2010).

This study evaluated the paternal contribution, pollen contamination and mating system, pollen dispersal distances and impact on seed productivity in a Japanese larch clonal seed orchard based on progeny testing experiment and SSR markers. The parental assignment, unequal paternal contribution, rate of uncertain paternal pollen donors, absence of selfing, lower pollen contamination and the distance between pollen donors and maternal parents were reported. The results improved the valuable pedigree information for designing and managing future advanced seed orchard of Japanese larch and for the development of viable long-term breeding strategies for other coniferous species.

MATERIALS AND METHODS

Seed orchard

This study was carried out in a 14.7 hm^2 Japanese larch clonal seed orchard at the DaGuJia National Larch Breeding Centre, Liaoning Province, China (long. 124°47′, lat. 42°22′ N, elev. 200–600 m). The clonal seed orchard was estab-

lished in 1965 by grafting with the 152 elite clones selected from the Japanese larch plantations in Northeast China. It consisted of 12 blocks with 4×4 m spacing. The clones were planted by sequential dislocation to ensure the same clone trees with minimum distance of 24 m. Based on the growth performance of the ramets, a selective thinning was conducted in 1984. In 1985, the orchard was composed of a total of 2,108 ramets belonging to 152 clones with 1–82 ramets per clone. This year, the mean space between ramets, mean bole height and crown width were about 6, 18 and 6 m, respectively. By 2013, the clonal seed orchard contained a total of 2,085 ramets belonging to 140 clones since natural mortality costed 12 clones containing 23 ramets.

Plant materials

In the fall of 1985, 73 out of the 152 clones were randomly selected and open-pollinated seeds were evenly collected from crown margin of one ramet of each of the 73 clones to scientifically estimate the distance of pollen dispersal. These seeds were pre-chilled and sown by clones in a commercial nursery in May 1986. A progeny trial was planted in 1988 and a randomized complete block design was used at 2×2 m spacing with 3–8 trees per plot and five replications per clone. Needle samples were collected in the summer of 2013 for genomic DNA extraction from 647 progenies representing 63 out of the 73 maternal clones with 8–11 progenies per maternal clone, including the 34 progenies of the maternal clone Z55 which was located in the center of the clonal seed orchard.

Genomic DNA extraction, PCR amplification and electrophoresis

Genomic DNA was extracted from all of the 140 parental clones in the clonal seed orchard and the 647 progenies in the progeny trial using the modified CTAB method (Yang et al., 2011). The 171 SSR markers published in Yang et al. (2011) and Chen et al. (2015) were tested, and 17 out of the 171 SSRs were selected for use in the present study (Supplementary material 1) since their PCR amplification gave clear, reproducible banding patterns. PCR reactions were conducted in a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) in a total volume of 15 μL, which contained 20 ng DNA, 1× PCR buffer, 2 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} dNTPs, 10 pmol each primer, and 0.5 Unit Taq DNA polymerase (TaKaRa, Dalian, China). The thermal program was: 4 min at 94°C, then 30 cycles of 45 s at 94°C, 45 s at 56°C and 45 s at 72°C, followed by 7 min at 72°C. The PCR products were analyzed using an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA) with the internal size standard GeneScan™ 500 ROX™ (Applied Biosystems, Foster City, CA, USA). Fragments were scored using the GeneMapper v.4.0 software (Applied Biosystems, Foster City, CA, USA).

Genetic diversity analyses

POPGENE 1.32 (Yeh and Boyle, 1997) was used to calculate genetic diversity of parents and progenies by analyzing the following parameters: observed number of alleles per locus (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F) with $F=(H_e-H_o)/H_e$. Average probabilities of parentage exclusion (PE) and identity (PI) were estimated for each microsatellite marker using the CERVUS 3.0 software (Kalinowski et al., 2007). PE corresponds to the single-locus probability of exclusion of an individual tree erroneously determined as the parent of a progeny. PI corresponds to the probability that two random individuals display the same genotype at the microsatellite markers. Single locus estimates were then used to estimate the combined multi-loci exclusion probabilities. An estimate of null allele frequency was also obtained using the CERVUS 3.0 software.

Paternity analysis

Paternity analysis was conducted using the CERVUS 3.0 software (Kalinowski et al., 2007). The critical LOD-score value was obtained from the simulation of paternity analysis. The paternity of a progeny was assigned with the potential paternal parents being 140 and the proportion of the potential paternal parents being 0.85, based on the observation of the best match between the observed and expected assignments in the present study (Bai et al., 2007). The minimum loci number was 9 and the mistyped genotyping was 0.01. The number of the potential paternal parents specified in the simulation was the approximate size of the paternal gene pool for the studied population, which should include both sampled and unsampled paternal parents. CERVUS assigned paternity to a particular paternal parent if the difference in the likelihoods of the most likely paternal parent and the second-most likely paternal parent exceeded a threshold that was estimated in the simulation. Paternity was assigned at 95% for the strict confidence level (CL) and 80% for the relaxed CL in 10,000 simulation cycles. Progenies that could not be assigned to any of the 140 paternal clones were considered to be a product of pollen contamination from trees outside the orchards or from the unsampled 23 ramets due to natural mortality. Risk of false positives was very small due to the low proportion of the unsampled parental ramets (i.e., 23 out of 2,108).

To evaluate the contribution of each clone as a pollen donor, we performed χ^2 test of goodness-of-fit against the null hypothesis that the contribution of each clone was equal. The values for the observed contributions were the number

of the progenies of given paternity, as determined by the paternity analysis. In order to correct for the differences in the numbers of ramets between clones, the expected contribution value was estimated from the following equation: (Total number of progenies from each identified paternal clone)×[(Number of ramets for that clone)/(Total number of ramets)]. The total number of ramets was 2,108. In order to illustrate the relationship between the number of ramets and the paternal contribution per clone, Pearson product-moment correlation analysis was performed to examine whether the paternal contribution per clone increased with the number of ramets per clone (i.e., clone size).

The distance from the identified paternal parent to the maternal parent was determined by the location of the ramet closest to the maternal parent in order to roughly determine effective pollen dispersal distance.

To illustrate the impact of mating patterns on timber production of orchard crops, diameter at breast (DBH) and height (HGT) were measured for the 647 progenies in the fall of 2006 (Age-20). Individual tree volume (VOL; m^3) was calculated using the following tree volume formula (Sun and Yang, 2011):

$$\mathrm{VOL}=0.0000592372\times \mathrm{DBH}^{1.8655726}\times \mathrm{HGT}^{0.98098926}.$$

Breeding values were estimated by the best linear unbiased prediction (BLUP) method based on individual-tree linear mixed models:

$$y=X\beta+Z_1a+Z_2p+e,$$

where $\boldsymbol{y}$ is the vector of individual tree observations on each trait, β is a vector of fixed-effect estimates (block effect), a, p and e are vectors of random additive genetic effects, plot effects and residual effects, respectively. X and Z are incidence matrices for fixed and random model terms. The additive, plot and error effects were assumed uncorrelated with zero means and normally distributed as $a \sim N(0, A\sigma_a^2)$, $p \sim N(0, I\sigma_p^2)$ and $e \sim N(0, I\sigma_s^2)$, where I is an identity matrix and A was the additive relationship matrix.

SSR data and phenotypic data can be found in Supplementary material 1.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was supported by the Forestry Industry Research Special Funds for Public Welfare Projects (201504104). We gratefully thank Wusheng Liu for his guidance and thorough review of the manuscript.*

Aronen, T., Nikkanen, T., Harju, A., Tiimonen, H., and Häggman, H. (2002). Pollen competition and seed-siring success in *Picea abies*. TAG Theor Appl Genets 104, 638–642.

Bai, W.N., Zeng, Y.F., and Zhang, D.Y. (2007). Mating patterns and pollen dispersal in a heterodichogamous tree,*Juglans mandshurica* (Juglandaceae). New Phytologist 176, 699–707.

Bilgen, B.B., and Kaya, N. (2015). Chloroplast DNA variation and pollen contamination in a *Pinus brutia* Ten. clonal seed orchard: implication for progeny performance in plantations. Turk J Agric For 38, 540-549.

Burczyk, J., Nikkanen, T., and Lewandowski, A. (1997). Evidence of an unbalanced mating pattern in a seed orchard composed of two larch species. Silvae Genet 2, 176–180.

Canchignia-Martínez, H.F., Hernández-Delgado, S., González-Paz, M., Motte, E., and Mayek-Pérez, N. (2007). Genetic relationships among *Schizolobium parahybum* (Vell.) Blake (Leguminosae) ecotypes from Ecuador and other countries. Silvae Genet 5, 214–221.

Chen, X.B., Xie, Y.H., and Sun, X.M. (2015). Development and characterization of polymorphic genic-SSR markers in *Larix kaempferi*. Molecules 20, 6060–6067.

Chaloupková, K., Stejskal, J., El-Kassaby, Y.A., and Lstibůrek M. (2016). Optimum neighborhood seed orchard design. Tree Genet Genomes 12, 105.

Codesido, V., Merlo, E., and Fernández-López, J. (2005). Variation in reproductive phenology in a *Pinus radiata* D. Don seed orchard in northern Spain. Silvae Genet 1995, 4–5.

Doyle, J. (1935). Pollination in *Tsuga*, *Cedrus*, *Pseudotsuga*, and *Larix*. Sci Proc R Dublin Soc, 191–204.

El-Kassaby, Y.A., and Lindgren, D. (2007). Increasing the efficiency of breeding without breeding through phenotypic pre-selection in open pollinated progenies. In Joint Meeting of the South. For. Tree Improve. Conf. and the Western For. Genetics Association, Galveston, Texas. pp. 15–19.

El-Kassaby, Y.A., and Lstibůrek, M. (2009). Breeding without breeding. Genet Res 91, 111–120.

El-Kassaby, Y.A., Stoehr, M.U., Reid, D., Walsh, C.G., and Lee, T.E. (2007). Clonal-row versus random seed orchard designs: interior spruce mating system evaluation. Can J For Res 37, 690–696.

Fernandes, L., Rocheta, M., Cordeiro, J., Pereira, S., Gerber, S., Oliveira, M.M., and Ribeiro, M.M. (2008). Genetic variation, mating patterns and gene flow in a *Pinus pinaster* Aiton clonal seed orchard. Ann For Sci 65, 706–706.

Funda, T., Chen, C.C., Liewlaksaneeyanawin, C., Kenawy, A.M.A., and El-Kassaby, Y.A. (2008). Pedigree and mating system analyses in a western larch (*Larix occidentalis* Nutt.) experimental population. Ann For Sci 65, 705–705.

Funda, T., Wennström, U., Almqvist, C., Andersson Gull, B., and Wang, X. R. (2016). Mating dynamics of Scots pine in isolation tents. Tree Genets Genomes 12, 112.

Funda, T., Wennström, U., Almqvist, C., Almqvist, C., Torimaru, T., Gull, B.A., and Wang, X.R. (2015). Low rates of pollen contamination in a Scots pine seed orchard in Sweden: the exception or the norm? Scand J Forest Res 30, 573-586.

Gonzaga, J.M.S., Manoel, R.O., Sousa, A.C.B., Souza, A.P., Moraes, M.L. T., and Sebbenn, A.M. (2016). Pollen contamination and nonrandom mating in a *Eucalyptus camaldulensis* Dehnh seedling seed orchard. Silvae Genetica 65, https://doi.org/10.1515/sg-2016-0001.

Grattapaglia, D., do Amaral Diener, P.S., and dos Santos, G.A. (2014). Performance of microsatellites for parentage assignment following mass controlled pollination in a clonal seed orchard of loblolly pine (*Pinus taeda* L.). Tree Genets Genomes 10, 1631–1643.

Hadfield, J.D., Richardson, D.S., and Burke, T. (2006). Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. Mol Ecol 15, 3715–3730.

Hansen, O.K. (2008). Mating patterns, genetic composition and diversity levels in two seed orchards with few clones—Impact on planting crop. For Ecol Manage 256, 1167–1177.

Hansen, O.K., and McKinney, L.V. (2010). Establishment of a quasi-field trial in *Abies nordmanniana*—test of a new approach to forest tree breeding. Tree Genets Genomes 6, 345–355.

Hasegawa, Y., Suyama, Y., and Seiwa, K. (2009). Pollen donor composition during the early phases of reproduction revealed by DNA genotyping of pollen grains and seeds of*Castanea crenata*. New Phytologist 182, 994–1002.

Isoda, K., and Watanabe, A. (2006). Isolation and characterization of microsatellite loci from *Larix kaempferi*. Mol Ecol Notes 6, 664–666.

Jones, A.G., Small, C.M., Paczolt, K.A., and Ratterman, N.L. (2010). A practical guide to methods of parentage analysis. Mol Ecol Resources 10, 6–30.
Kalinowski, S.T., Taper, M.L., and Marshall, T.C. (2007). Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol Ecol 16, 1099–1106.
Kess, T., and El-Kassaby, Y.A. (2015). Estimates of pollen contamination and selfing in a coastal Douglas-fir seed orchard. Scand J For Res 4, 266–275.
Kaya, N., and IsIk, K. (2010). Genetic identification of clones and the genetic structure of seed crops in a *Pinus brutia* seed orchard. Turk J Agric For 34, 127-134.
Lai, M., Sun, X., Chen, D., Xie, Y., and Zhang, S. (2014). Age-related trends in genetic parameters for *Larix kaempferi* and their implications for early selection. BMC Genet 15, S10.
Lewandowski, A., Burczyk, J., and Mejnartowicz, L. (1991). Genetic structure and the mating system in an old stand of Polish larch. Silvae Genet 2, 75–79.
Li, W., Wang, X., and Li, Y. (2011). Stability in and correlation between factors influencing genetic quality of seed lots in seed orchard of *Pinus tabuliformis* Carr. over a 12-year span. PLoS ONE 8, e23544.
Lstibůrek, M., Stejskal, J., Misevicius, A., Korecký, J., and El-Kassaby, Y. A. (2015). Expansion of the minimum-inbreeding seed orchard design to operational scale. Tree Genet Genomes 11, 1-8.
Millar, M.A., Byrne, M., Nuberg, I., and Sedgley, M. (2008). High outcrossing and random pollen dispersal in a planted stand of *Acacia saligna* subsp. saligna revealed by paternity analysis using microsatellites. Tree Genets Genomes 4, 367–377.
Moriguchi, Y., Tani, N., Itoo, S., Kanehira, F., Tanaka, K., Yomogida, H., Taira, H., and Tsumura, Y. (2005). Gene flow and mating system in five *Cryptomeria japonica* D. Don seed orchards as revealed by analysis of microsatellite markers. Tree Genet Genomes 4, 174–183.
Moriguchi, Y., Yamazaki, Y., Taira, H., and Tsumura, Y. (2010). Mating patterns in an indoor miniature *Cryptomeria japonica* seed orchard as revealed by microsatellite markers. New Fors 39, 261–273.
Nielsen, U.B., and Hansen, O.K. (2012). Genetic worth and diversity across 18 years in a Nordmann fir clonal seed orchard. Ann For Sci 69, 69–80.
Nikkanen, T., Aronen, T., Häggman, H., and Venäläinen, M. (2000). Variation in pollen viability among *Picea abies* genotypes—potential for unequal paternal success. TAG Theor Appl Genets 101, 511–518.
Nishimura, M., and Setoguchi, H. (2011). Homogeneous genetic structure and variation in tree architecture of *Larix kaempferi* along altitudinal gradients on Mt. Fuji. J Plant Res 124, 253–263.
Roberds, J.H., Friedman, S.T., and El-Kassaby, Y.A. (1991). Effective number of pollen parents in clonal seed orchards. Theoret Appl Genets 82, 313–320.
Robledo-Arnuncio, J.J., and Gil, L. (2005). Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. Heredity 1, 13–22.
Saro, I., Robledo-Arnuncio, J.J., González-Pérez, M.A., and Sosa, P.A. (2014). Patterns of pollen dispersal in a small population of the Canarian endemic palm (*Phoenix canariensis*). Heredity 113, 215–223.
Schoen, D.J., and Stewart, S.C. (1986). Variation in male reproductive investment and male reproductive success in white spruce. Evolution 40, 1109–1120.
Silva, P.I.T., Martins, A.M., Gouvea, E.G., Pessoa-Filho, M., and Ferreira, M.E. (2013). Development and validation of microsatellite markers for *Brachiaria ruziziensis* obtained by partial genome assembly of Illumina single-end reads. BMC Genomics 14, 17.
Slobodník, B. (2002). Pollination success and full seed percentage in European larch (*Larix decidua* MILL.). J For Sci 1, 271–280.
Slobodník, B., and Guttenberger, H. (2005). Zygotic embryogenesis and empty seed formation in European larch (*Larix decidua* Mill.). Ann For Sci 2, 129–134.
Sønstebø, J.H., Tollefsrud, M.M., Myking, T., Steffenrem, A., Nilsen, A.E., Edvardsen, Ø.M., Johnskås, O.R., and El-Kassaby, Y.A. (2018). Genetic diversity of Norway spruce (*Picea abies* (L.) Karst.) seed orchard crops: Effects of number of parents, seed year, and pollen contamination. For Ecol Manage 411, 132–141.
Stoehr, M., Mehl, H., Nicholson, G., Pieper, G., and Newton, C. (2006). Evaluating supplemental mass pollination efficacy in a lodgepole pine orchard in British Columbia using chloroplast DNA markers. New For 31, 83–90.
Stoehr, M.U., and El-Kassaby, Y.A. (1997). Levels of genetic diversity at different stages of the domestication cycle of interior spruce in British Columbia. Theoret Appl Genets 94, 83–90.
Summers, K., and Amos, W. (1997). Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog,*Dendrobates ventrimaculatus*. Behaval Ecol 8, 260–267.
Sun, X., Zhang, S., Zhou, D., and Wang, X. (2008). Phenological variation of *Larix* species and their intra-species and inter-species hybrid families and early selection (in Chinese). Sci Silvae Sin 1, 77–84.
Sun, X.M., and Yang, X.Y. (2011). Applications and analysis of methods for breeding value prediction in forest trees (in Chinese). J Beijing For Univ 2, 65–71.
Torimaru, T., Wennström, U., Andersson, B., Almqvist, C., and Wang, X.R. (2013). Reduction of pollen contamination in Scots pine seed orchard crop by tent isolation. Scandinavian J For Res 28, 715–723.
Wang, Y.C., Dong, X.G., Wang, X.S., and Ma, H. (2000). Study on seed production and fruiting law of seed orchard in *Larix kaempferi* (Lamb.) Carr. (in Chinese). Sci Silvae Sin 2, 53–59.
White, T.L., Adams, W.T., and Neale, D.B. (2007). Forest Genetics, 1st ed. CABI.
Wei, Z., Qu, Z., Hou, C., Liu, Y., Zhang, L., Yang, C., and Wei, H. (2015). Genetic diversity and paternal analysis of open-pollinated progenies of *Larix olgensis* seed orchard. J Nat Sci 1, e19.
Yang, X., Sun, X., and Zhang, S. (2011). Short note: Development of six EST-SSR markers in Larch. Silvae Genet 3–4, 161–163.
Yeh, F.C., and Boyle, J. (1997). POPGENE, the user-friendly shareware for population genetic analysis. Mol Biol Biotechnol, 724–731.

SUPPORTING INFORMATION

Supplementary material 1

The supporting information is available online at http://life.scichina.com and https://link.springer.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.