Genetics

Genetic diversity and paternal analysis of open-pollinated progenies of *Larix olgensis* seed orchard

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280 open pollinated progenies (seeds) and their parents, 49 paternal trees and 7 maternal trees that were planted in an open-pollinated seed orchard of *Larix olgensis* (Henry) at Qingshan Forest Farm, Heilongjiang Province，P. R. China, were studied by simple sequence repeats (SSR) markers. The genetic diversity between the parents and progenies, paternal pollen contribution to the progenies, the effect of crown-facing sides to pollination and pollen disperse distance were investigated. We found that the genetic diversity difference between the progenies and parents were not significant. For example, the average fixed indices of parental and progeny population were 0.0071 and 0.0063 respectively. In addition, no obvious fixed mating patterns were found in the seed orchard though the pollen contribution of each paternal parent was different, implying that some maternal parents could only be pollinated by few paternal trees. Interestingly, two alleles present only in five progenies that accounted for 3.09% of all progenies were not from any 49 paternal parents, suggesting there was invasion of foreign pollen in seed orchard. We also found the main effective pollen dispersal distance of *L. olgensis* varied from15 to 95 m. However, we did not observe significant correlation between effective pollen dispersal distance and male reproductive fitness in the seed orchard. Moreover, we found that the crown-facing sides of maternal trees had some influence on the paternal constitution of progenies, which, however, was not at significant level. Our study provided valuable information for more effective management of seed orchard and viable long-time breeding strategies to ameliorate *L. olgensis* or other coniferous species.
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Genetic diversity | *Larix olgensis (Henry)* | microsatellites (SSR) | paternal analysis | seed orchard

Larix olgensis (Henry) is one of the most important forest trees species in Northeast China with several laudable characteristics and traits, which include but are not limited to fast growth, wide distribution, favored adaptability, and exceptional wood properties. Currently plantations of *L. olgensis* are usually established with genetically improved seeds yielded from various breeding programs including seed orchards where elite trees were planted together to bear high quality genetic improved seeds. In such a scenario, the plantation productivity, adaptability, resistance and sustainability (Lexer et al, 2000) are exclusively determined by the genetic basis and diversity of the seeds yielded from the seed orchard. Therefore, the study of the genetic diversity of seeds and mating partnerships become an important aspect of seed orchard. The paternal parents pass their genetic materials and information to the progeny population through pollen whose quantity and dispersal patterns (male reproductive contribution rate) are determining factor in influencing the seed quality and genetic diversity within the seed orchard(Hamrick et al, 2000; Zhang et al, 2000). Conversely, we can better understand the pollen viability, spreading and pollination within the seed orchard by examining the open-pollinated seeds, which can provide scientific basis for designing optimal clone numbers and combinations as well as monitoring seed genetic diversity (Moriguchi et al, 2005; Zhang et al,2008). As coniferous species are wind-pollinated, paternal identification is approximately equivalent to pollen source identification (He & Ge, 2000). Although fluorescent staining(Levin & Kerster, 1974; Waser et al, 1996) and radioactive isotope labeling method (Schlising & Turpin, 1971) have been widely adopted in previous researches, and they are well-suited for qualitatively describing gene flow, they suffer from the defect in characterizing the effective spread pattern of pollen. Development of DNA molecular markers (e.g. RAPD, AFLP, SSR, etc.) provides a new avenue for quantitative analysis of paternal tree population. For example, microsatellite (SSR) markers have been successfully used for paternal analysis in multiple species that include but are not limited to *Abies alba*(Ziegenhagen et al, 1998), *Quercus macrocarpa* (Dow & Ashley, 1996; Dow & Ashley, 1998), *Pinus densiflora*(Lian et al, 2001), *Oleaeu ropaea* (De la Rosa et al, 2004) and *P. massoniana*(Ai et al, 2006). However, little is known about the mating system and genetics diversity of the progenies in *L. olgensis* seed orchard, and no similar studies in *L. olgensis* have been reported.

In this study, genetic diversity, paternal contribution, the effect of crown-facing sides to pollen disperse distance and pollen contamination in *L. olgensis* seed orchard were studied based on 280 open-pollinated progenies, 49 paternal and 7 maternal trees with SSR markers. We investigated the genetic basis of progenies of open-pollinated seeds and analyzed the connection between genetic diversity and paternal composition, and contribution, and other factors that affected mating and seed quality. Our results provide valuable information and insights for producing genetics improved seeds and formulating new breeding plans and strategies for *L. olgensis* and other coniferous species.

Material and Method

Materials

Primary seed orchard of *L. olgensis* was established with preferentially selected artificial maternal trees in Qingshan Tree Farm, Linkou County, Heilongjiang Province in 1973. Seven zones (No. 1-7) were planted with grafting in 1974. The all 49 clones were planted by sequential dislocation to ensure the same clone trees with maximal distance and spacing of 5m × 10m.The seed orchard is surrounded by open lands or naturally regenerated secondary birch and oak forests, with a slope of 3-5 degree, soil thickness of 30-50cm and moderate soil fertility.

Sampling and handling

Seven maternal trees, No. 3, 12, 43, 21, 38, 26 and 9, were randomly selected from seven clones for collecting seeds from 7 zones as described above. For each tree, 5 cones were collected in the crown from the upper north and south sides respectively. The obtained seeds from the same side and same tree were mixed, and then 20 seeds were randomly selected and cultivated in the germination boxes, which were then translated into the illumination incubator with watering everyday and 25℃ temperature for germination. When the seedlings reached 5-6cm, the seed shells and residual endosperm were removed and the whole seedlings were preserved to extract DNA in the refrigerator at -20℃. For the 7 maternal trees and 49 candidate paternal trees (belonged to 49 clones) selected, tender needles of the one year old were respectively collected and frozen in refrigerator at -20℃ for DNA extraction.

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Table 1. Sequences of SSR primer sets

No.	Primer sequence（5'-3'）	Core sequence
S6	F) AGGATGCGTATGATATGCGC R) AACCATAACAAAAGCGGTCG	(CAT)7
S8	F) CAACTGACAAACAGCGTTTCA R) GCAGTGGTATCAACGCAGAG	(AT)5
S17	F) ATCATCCGCCTCTTCACATT R) CTCCTTCTGGTGTTGGTGGT	(GCA)5
S24	F) GAAGAACCCACAAACACAAG R) GGGCAAGAATTCAATGATAA	(AGC)8
S56	F) AGCGATCAAGCCGACAATAA R) AAACAAGGGTTCCAGCTCCT	(AT)5(CA)6
S49	F) CTATTTGAGTTAAGAAGGGAGTC R) CTGTGGGTAGCATCATC	(GT)15
S68	F) ACCCACGCCTTACAGATCAC R) AGCTTTCCTCTGGCCTTCTC	(TA)7(CAG)5(CTC)5
S71	F) CCTCCCAAAGCCTAAAGAAT R) CATACAAGGCCTTATCTTACAGAA	(TTG)7 (TTG)5
S37	F) TTGGATATTGCACCAAAAAGG R) GGGTACAATTCCTTGCTTTTCA	(TA)6(TG)6
S46	F) GGCAATTTGTAGCGGAAAGA R) AAGCTCCCATGGCATACTTG	(TA)6(GAG)5

F forward primer, R reverser primer

DNA extraction

The genomic DNA was extracted from 100 mg DNA materials of needles and seedlings by conventional CTAB method according to Shepherd *et al*(Shepherd et al, 2002). A total of 5 μl of DNA was electrophoresed on 1% agarose gel and stained with ethidium bromide and visualized under UV fluorescence. DNA quality was estimated based on band intensity of standard amounts of DNA.

Screening of SSR primers

In this study, SSR primers were firstly screened from those species where SSR had been implemented to study sibling relationships and kinships of different trees. 80 pairs of SSR primers from multiple species that include *Picea abies*, *Picea glauca*, *Cryptomeria japonica*, *Pinus taeda*, *Pinus strobus*, and *Larix kaempferi* were initially screened (Echt et al, 1999; Elsik & Williams, 2001; Elsik et al, 2000; Zhou et al, 2002). We screened these primers by using two genetic closely related clones to evaluate the polymorphism of SSR primers. If there was at least one different locus demonstrated by a pair of primers between the two closely related clone trees, this pair of primers was consider as polymorphism and was selected. After multiple times of screening, we eventually obtained 10 pairs of SSR primers that were considered to have high polymorphism (Table 1).

Amplification, electrophoresis and silver staining of SSR_PCR

The 20μL PCR System included: 2μL of 15ng • μL-1 template DNA; 2μL 10 × buffer solution; 2μL 2 mmol • L-1dNTPs solution; 2μL 2.5 mmol • L-1 MgCl2 solution; 10μmol • L-1 primers, 0.5U of Taq (TaKaRa); and 10.9μL double distilled water. The conditions for PCR amplification are: first 5 min denaturation at 94^{o}C; then 35 cycles of amplification that used 40s at 94^{o}C, 30s at 52^{o}C, and 40s at 72^{o}C, followed by a 10 min extension at 72^{o}C. PCR products were stored at 4^{o}C. PCR products were mixed with an equal volume of loading buffer, denatured at 95^{o}Cfor 5 min, and then vertically electrophoresed on a 6% denatured polyacrylamide gel. SSR fragments were displayed with silver staining and photographed.

Data processing and statistical analysis

Bands were artificially read and binary data were documented according to mobility ratios. Band presence was recorded as 1, where as band-absence was recorded as 0, with all ambiguous and unidentified bands being excluded. POPGENE 1.32(Francis et al, 1999) was used to calculate genetic diversity of parents and progenies by analyzing the following parameters: effective number of alleles(Ne), observed heterozygosity(Ho), expected heterozygosity(He), Nei Gene diversity(h) and fixation index (F).

CERVUS (Version 2.0) software was applied to analyze the 49 paternal trees and 280 open-pollinated progenies. This software can deal with progenies analysis with unknown parents by the maximum likelihood distribution method. 10000 cyclic simulations were conducted based on the observed allelic frequencies, that is, on two confidence levels of 95% and 80%, the one with the highest likelihood ratio was assumed to be the 'real' paternal of the progenies(He & Ge, 2000; Marshall et al, 1998). In this way, we could infer the possible paternal parents of 280 progenies.

Results

SSR polymorphism and genetic diversity between parental and progeny populations

Using 10 pairs of SSR primers, we detected 68 alleles within the parental and progeny populations, with allele numbers varying from 2 to 10 and average allele number of 7.2422 (Table 2). Among these, locus 8 had the maximum number of alleles of 10, while locus 3 had the minimum number of alleles of 2. Effective number of alleles ranged from 7.7853 of locus 8 to 1.3149 of locus 5. Meanwhile, there was great difference between different loci in diversity parameters; for example, the Sharon diversity index (I) of locus 8 was as high as 3.8914, while for locus 5, it was only 0.1567.

Based on the SSR outcome, we calculated various genetics parameters that reflected genetic diversity and summarized them in Table 2. The average Ho, He, h and F of parental population were 0.3821, 0.5833, 0.6683 and 0.0071, respectively. Among 7 maternal parents, No.26 seed tree had the maximum genetic parameters, with He being equal to 0.7542 and minimum F being equal to 0.0029. No.43 seed tree had the relatively lower heterozygosity, its He and F values were equal to 0.4249 and 0.0112, respectively. To the progeny population, the average Ho, He, h and F were 0.4543, 0.5137, 0.4875 and 0.0063, respectively, which are comparable to those of the parental population. The above results demonstrated the progeny populations of 7 maternal trees were products of an approximately random mating. Moreover, there was no significant difference of genetic parameters between the 7 maternal trees and 280 progenies, which also indicated that the genetic diversity among the progeny population of *L. olgensis* did not decline.

Table 2. Genetic diversity of parent and progeny population

Population		Average number Of alleles (A)	Average effective number of alleles (Ne)	Average observed Heterozygosity (Ho)	Average expected Heterozygosity (He)	Nei gene diversity (h)	Fixation index(F)
Parent	3	7.2812	3.0123	0.3876	0.6178	0.6781	0.0065
	12	8.4631	3.1461	0.4876	0.6978	0.7673	0.0032
	43	5.7601	2.4121	0.2679	0.4249	0.5458	0.0112
	21	5.9114	2.526	0.2756	0.4325	0.5934	0.0105
	38	7.3468	3.3416	0.3881	0.6296	0.6879	0.0063
	26	9.1203	3.7954	0.4925	0.7542	0.7896	0.0029
	9	6.8126	2.8926	0.3756	0.5264	0.6161	0.0087
Average of parent		7.2422	3.0180	0.3821	0.5833	0.6683	0.0071
Progeny		6.4156	2.3727	0.4543	0.5137	0.4875	0.0063

Table 3. Significance levels of paternal analysis and pollen contribution rates of paternity

Maternal trees (Clone No.)	Significance of level of paternity analysis			Pollen contribution rates					
				≥5%		4%~5%		≤4%	
	P<5 %	*P*<20 %	*P*<30 %	Number of clone	Percentage of contribution/ %	Number of clone	Percentage of contributio n/%	Number of clone	Percentage of contribution/ %
3	1	20	25	3	20.17	5	26.43	19	53.4
12	2	9	13	2	26.67	6	27.33	23	46
43	4	17	23	5	30.34	4	18.77	32	50.89
21	3	26	36	3	27.16	2	22.48	24	50.36
38	5	14	25	4	22.12	5	28.36	14	49.52
26	4	10	16	2	12.23	9	49.59	23	38.18
9	5	18	24	6	33.05	10	44.46	27	22.49
Total	24	114	162						

Paternal constitution

We set the parameter of CERVUS software to 10000 circles, candidate parental sampling ratio was 0.895, the matching genetic locus ratio was equal to 1.000 and the mismatching genetic locus ratio was 0.001. In this way, the paternal analysis of open-pollinated progenies showed only 24 progenies (8.57%) could be implicitly determined with their paternal parents at 95 % confidence level within the 280 progenies detected by 10 SSR primers. In order to improve the detection rate of the paternal parents, most previous studies relaxed the levels of significance (Ai et al, 2006, He & Ge, 2000; Marshall et al, 1998; Zhang et al, 2000). In this study, taking into account of the rate and accuracy of paternal detection, we used multiple significant levels. When confidence levels were set to 80-95% and >70%, 114 (40.71%) and 162 (57.85%) progenies were determined their paternal trees respectively. In the later case (Confidence > 70%), of 7 maternal trees, the paternal parents of 13 (No.9 seed tree)-37 (No.26 seed tree) progenies could be determined. On average, 23 progenies could be determined with their paternal parents for each maternal tree.

To further analyze the selfing rate of the seed orchard, the 7 maternal trees were also treated as paternal parents. Among the 162 progenies with determined paternal parents, only 3 were detected as selfing progenies. The selfing rate was of 1.85%, indicating quite low selfing in this seed orchard. This result also indicated that under the condition of open pollination, outcrossing was the predominant crossing manner for *L. olgensis* clonal seed orchard.

Paternal contribution

Our study found that all the 49 candidate paternal trees except No. 31 and No. 27 could produce progenies with the 7 maternal trees. On average, each paternal tree provided pollen for 3.18 maternal trees of selected 7 maternal trees. We found that the progeny numbers of paternal trees for different maternal trees vary from 9 12to 2921(P<30%), representing 18.37% and 59.18 % of all paternal candidates clones, with an average of 45.48%. Two paternal trees had progenies with all maternal trees, while 36 paternal trees had progenies with at least 2 maternal trees, and 25 paternal trees had progenies with at least 3 maternal trees. The rest 11 paternal trees only had progenies with 1 maternal tree. It was shown that there was no such situation that several paternal trees with the same origin tended to produce more progenies with particular maternal tree. In the other word, there was no fixed form of mating combinations.

It could be seen from Table 3 that the numbers of paternal trees that contributed pollen exceeding 5% of the total progenies for the 7 maternal trees was 3, 2, 5, 3, 4, 2 and 6, accounting for 6.12%, 4.08%, 10.2%, 6.12%, 8.16%, 4.08% and 12.24%. The pollen contribution rates of these paternal trees were 20.17%, 26.67%, 30.34%, 27.16%, 22.12%, 12.23% and 33.05% respectively. The amount of parent trees with less than 4% pollen contribution rate to maternal parent were 19, 23, 32, 24, 14, 23 and 27, which accounted for 38.78% 46.94%, 65.3%, 48.98%, 28.57%, 46.93% and 55.1% of the total paternal trees respectively. The pollen contribution rates of these paternal trees to 7 maternal parents were 53.4%, 46.0%, 50.89%, 50.36%, 49.52%, 38.18% and 22.49% of the all pollens received. In addition, we found No.6 and No. 35 paternal trees could provide more effective pollen to all the maternal trees than others, with the average pollen contribution rates of these 2 paternal trees to 7 maternal trees reaching 14.73%, 26.67%, 14.28%, 21.78%, 12.53%, 12.23% and 12.69%.

Influence of pollen from outside the seed orchard

The study also revealed the invasion of foreign pollen from outside orchard. In the electrophoresis results of 10 pairs of SSR primers, we found 2 alleles that were only found in progenies but not in candidate parents. The 2 unique alleles were detected by 3 locus of SSR and found to be presented in5progenies that account for 3.09% of the total detected progenies. Given the situation where the width of buffer zone around this seed orchard is about 200 m,

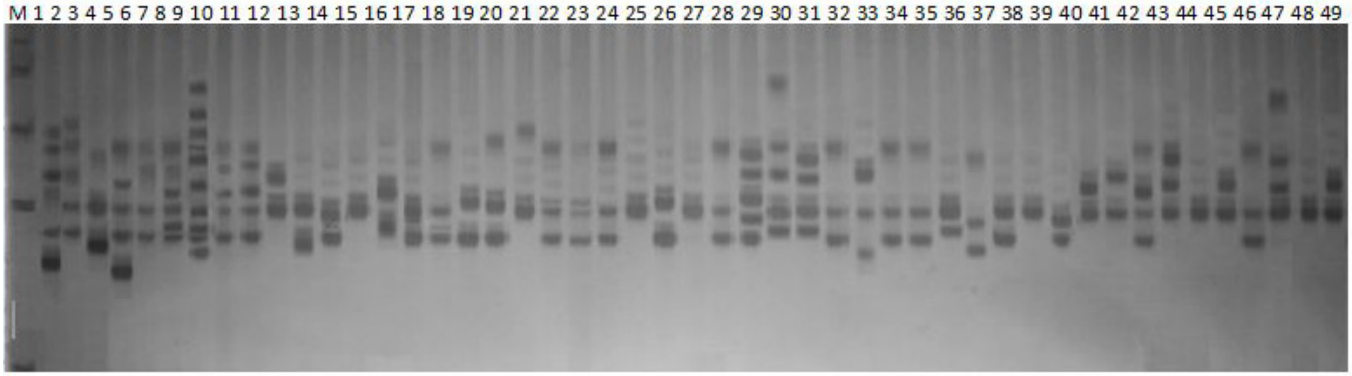

Figure 1. SSR products of 49 paternal trees amplified by No 8 primer. M:DL2000 Marker； 1-49: the 1-49 paternal trees

the chance of foreign pollen invading from natural population of outside of seed orchard would be very little in most circumstances. However, there were *L. olgensis* or *L. kaempferi* in nearby slope of adjacent mountain, and small amount of their pollen could intrude under the aid of strong gust wind during the time the pollen of *L.olgensis* were prevalent. Therefore, it is recommended to further widen the buffer zone or remove genetically related species outside of seed orchard, which is essential for ensuring the genetic quality of larch orchard seeds.

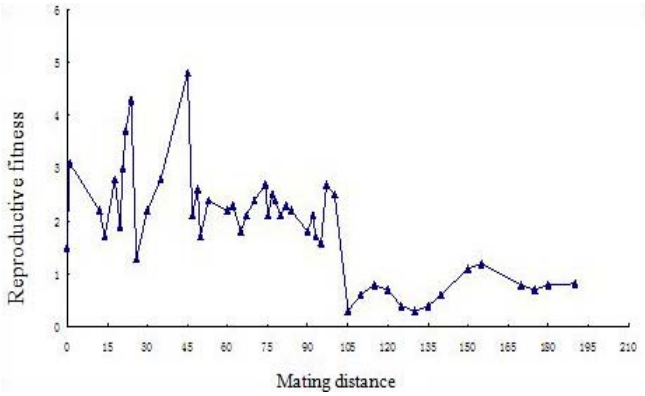

Figure 2 Relationships between reproductive fitness and average mating distance to maternal trees

Effective pollen dispersal distance and paternal reproductive fitness

The relationship between effective pollen dispersal distance and paternal reproductive fitness was analyzed. The result showed that as the pollen dispersal distance increase, the chance of successful mating became smaller and flow intensity of pollens also decreased gradually (Figure 2). However, there was no significant correlation between effective pollen dispersal distance and paternal reproductive fitness. Moreover, the maximal effective pollen dispersal distance of *L. olgensis* was as far as 180 m, but mainly effective distance range was between 0 to 95 m. 145 progenies were virtually produced within this distance ranges, accounting for 89.39% of progenies with identified paternal parents. Within the range of 95-180m, only 17 progenies account of 10.61% were produced. This further revealed a rudiment that maternal trees tend to accept more pollen from nearby paternal trees. Meanwhile, pollen dispersal distance of *L. olgensis* was mainly distributed within the range of 15-95 m (Figure 2). Though paternal trees were still able to provide pollen for maternal trees as the distance increased, the numbers of maternal trees capable of being successfully pollinated and subsequently producing seeds were dramatically reduced.

Paternal constitution and parent mating distance of progenies in the south and north facing sides of crown

The relationships between the paternal and maternal numbers and their mating distance of the crown south and crown north were studied (Table 4). The result showed the different sides of maternal crowns had some influence on the paternal constitution of progenies; however, the influence did not go beyond the significant level. The paternal trees of progenies in south- and north-facing sides of 7 maternal trees' crowns were basically identical at the same level, and the paternal constitution of progenies in the south and north sides of crowns showed no obvious discrepancy or similarity. Taking No.9 maternal tree as an example, the number of progenies of its south side of crown was nearly as same as that of the north crown side.. For the No.38 maternal tree, the number of paternal constitution was the almost same as that of the north side. For parents mating distance of progenies in the south and the north, the average mating distance, also termed as effective pollen dispersal distance, of south side of maternal crown was increased to some extent as compared with that of the north sides, especially for No.3 maternal tree, the average parent mating distance of its' south side was 20m more than that of the north side. In addition, the average parents mating distance of both its south and north sides were longer than the other 6 maternal trees, which may be arisen from its unique pollen structures and/or surrounding environment. It is located along the road in the first zone of the seed orchard. Therefore, more investigation is necessary to identify the possible reason.

Discussion

This study focused on the genetic diversity of 280 open-pollinated progenies from 49 paternal trees and 7 maternal trees of primary seed orchard of *L. olgensis* in Qingshan Tree Farm, Linkou County, Heilongjiang Province. P. R. China. Although the genetic diversity parameters were different between the parent and progeny populations, for example, the average Ho and h of parents were 0.3821 and 0.6683 and progenies were 0.4543 and 04875, respectively, the difference was not significant. Such result was to large degree similar to conclusion of nature *L. olgensis* population genetics diversity(Lai &Wang, 1997). Similar results have been also obtained in other species (Arus & Shields, 1983; Brown et al, 1990). However, the results from other species have indicated that progenies within the seed orchard could maintain a higher genetic diversity than natural populations (Robledo-Arnuncio & Gil, 2004, Lai &Wang, 1997). In addition, we found the average F was 0.0071 for parents and 0.0063 for progenies, indicating there was a weak phylogenic relationship among the parents in this seed orchard. However, the population of progenies were similar to the progenies of random-mating in nature stands.

The paternal analysis of 280 open-pollinated progenies from 7 maternal trees within the seed orchard showed that the pollen sources of 135 progenies could be confirmed in 80 % confidence level, namely, the paternal trees of 48.21% progenies could be determined, Such percentage was much lower than the those reported in some coniferous species like *Pinus koraiensis* (Feng et al, 2010), *Pinus sylvestris*(Robledo-Arnuncio & Gil, 2004) *and Populus nigra*(Vanden Broeck et al, 2004). In these species, the paternal parents of nearly 100% progenies were identified. Nevertheless, our results were consistent with 46-54% level reported in *Pinus massoniana*(Ai et al, 2006), *Pinus tabulaeformis*

Table 4. Paternal constitution and parents mating distance of progenies in the south- and north-facing sides of crown

Items	Maternal trees						
	3	12	43	21	38	26	9
Male numbers in the south side of crown	9	6	10	4	11	12	16
Male numbers in the north side of crown	10	7	8	5	11	14	13
Average mating distance in the south side of crown (m)	115	59	132	78	69	51	83
Average mating distance in the north side of crown (m)	95	62	124	61	73	49	75
Assigning paternity progenies in the south side of crown	17	14	12	18	16	17	8
Assigning paternity progenies in the north side of crown	11	18	13	14	15	20	5

(Zhang et al, 2009) and *Eucalyptus grandis*(Jones et al, 2008). The number of paternal parents was considered as the most important factor in paternal analyzing, and it should be generally less than 100(He & Ge, 2000). In this study, the number of male parents was only 49, which suggests the number of male parent might not the primary reason for the relatively lower percentage of paternal determining. Moreover, the marker types, the numbers of multiple polymorphic loci, and the polymorphism levels as well as the reproduction characteristics of the plants themselves may also have great impact on the results of paternal analyzing to some extent(Lian et al, 2001).

In this study, the 47 trees of 49 candidate male parents produced progenies with 7 female parents. The paternal constitution of progenies from 7 maternal trees varied from 18.37% to 59.18%, with an average being equal to 45.48%. Such a result suggested there were no paternal trees originated from the same site tended to produce more progenies with a particular maternal parent, indicating there were no predominant fixed mating combinations in the orchard. However, the contribution of pollen from different paternal trees to maternal trees was different. There were 2 trees supplying more effective pollen to female trees, whose pollen contribution to 7 female trees reached 14.73%、26.67%、14.28%、21.78%、12.53%、12.23% and 12.69%. Tan *et al* (2011) also found few male parents supplied more pollen in *P.massoniana* orchard. The results showed some female trees could only received pollens from few male trees to some extent, and the clones of this seed orchard was needed to be selected in future.

The effective pollen spreading pattern showed the main spreading distance of *L. olgensis* orchard was from 0 to 95m. The 95 m effective spreading distance of *L. olgensis* is longer than 45 m reported in *P. koraiensis*(Feng et al, 2010) and 35 m in *Liriodendron chinense (Yaguang & Huogen, 2008)* respectively, but is comparable to 100 m in *P. massoniana* (Tan et al, 2011). In addition, the longest detectable pollen spreading distance in *L. olgensis* was 180 m, which is approximately the same as 192 m reported in *P.massoniana* (Tan et al, 2011). . The longer spreading distance of *L. olgensis* pollen was possibly caused by the optimal pollen grain weight, and unique structure as well as gust wind. There were 145 progenies within 0-95m pollen spreading range, which occupied 89.39% of progenies population whose male parent could be determined. There were 17 progenies within 95-180 m pollen spreading range, accounting for 10.61% of progenies population. The results further demonstrated the female parents would preferably accept the pollen from the nearest trees. In addition, there was no significant correlation between the pollen effective spreading distance and paternal propagate fitness by analyzing their correlation.

Though the pollution of pollen from outside was found, the percent of foreign pollen contamination was only 3.09%, which was lower than 4.06% (Tan et al, 2011) and 9.44%(Ai et al, 2006) that were reported in *P. massoniana*. It was reported earlier that the capacity to identify the pollen pollution increases when there are fewer numbers of orchard clones and more polymorph loci(Zhang et al, 2000), implying that it is an important means to reduce foreign pollen contamination by keeping more clones in the seed orchard to increase the quantity of the pollen from elite paternal trees during flowering time.

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