Temporal and Spatial Change of the Mating System Parameters in a Seed Orchard of *Pinus tabulaeformis* Carr.*

X. H. Shen[1], D. M. Zhang[2], Y. Li[1,] H. X. Zhang[3]

1 Beijing Forestry University, Beijing 100083. shenxh@bjfu.edu.cn

2 Institute of Shanghai Landscape Gardening Science，Shanghai 200232

3 Chinese Academy of Forestry, Beijing 100091

Abstract: It is more than 20 years, since we started to study flower characteristics and the mating system in the seed orchards of *Pinus tabulaeformis* Carr. A number of papers have been published in Chinese journals. Some results concerning variation of mating system parameters, pollen contamination and pollen dispersal with enzyme and SSR analysis are summarized in this paper.

Pinus tabulaeformis Carr. is an important tree species for Northern China due to its extensive distribution, tolerance in harsh sites, rather fast growth and high wood quality. Seed crops in seed orchards are stable, if insects are controlled. The high genetic quality and ample yield of seeds in the seed orchard is closely related with the mating pattern of seed orchards. In order to make a clear picture of the temporal and spatial change in the mating system parameters, namely outcrossing, inbreeding, selfing and contamination rates in the seed orchard, as well as pollen dispersal distance, field observation of flower characteristics with laboratory analysis was continuously carried out more than 20 years (Shen X. H. *et al*., 1985; Wang X. R. *et al.*, 1991). Some results with enzyme and SSR analysis are shown in this paper.

Location of seed orchard and progeny plantation

The seed orchard of *Pinus tabulaeformis* Carr. for field observation and seed sample collection, is located in Xingcheng County, Liaoning province, China at NL 40°43', EL 120°34'. It was built in 1974 on a mountain slope of 5°-15°, covering an area of 20 ha. The seed orchard comprises 49 clones, with systematical design, spacing 5m×5m. In 1993 roguing was conducted and about 1/3 trees were removed. A stand about 50 ha of the same species locates at 3km away from the seed orchard. The progeny testing plantation derived from the seed orchard locates more than 3 km away from the orchard.

Seed samples collection

Open-pollination seeds were collected from the seed orchard for 7 years, namely in 1984,

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1987, 1993, 1996, 2000 and 2005, 2006. For study of three different populations seed samples were taken from the seed orchard, above-mentioned stand and progeny testing plantation. 5-8 cones were taken from each sample trees, while 8-10 seeds from them were used for enzyme analyses and 69 to 92 seeds for each of two clones for SSR analysis at 12 primer pairs.

Allozyme and Enzyme System

Horizontal starch-gel electrophoresis, including 10 enzyme loci was applied for both embryos and endosperm analysis with 10 loci as genetic markers. There were 8 kinds of enzyme.

- ACP（Acid phosphatase, E.C.3.1.3.2）,
- LAP（Leucine-amino-peptidase , E.C.3.4.11.1）
- GOT（Aspartate aminotransferase, E.C.2.6.1.1）
- PGM（Phosphoglucomutase, E.C.5.4.2.2）
- MDH（Malate dehydrogenase , E.C.1.1.1.37）
- ADH（Alcoholdehydrogenase, E.C.1.1.1.1）
- SKD（Shikimate dehydrogenase , E.C.1.1.1.25）
- MNR（Menadione reductse , E.C.1.6.99.2

Each of the first six enzyme possesses one locus, while the remaining two - two loci for every one (Zhan C. X. & Li Y., 1999).

Primers and SSR-PCR reaction system

Selected SSR primer pair sequences

Locus	Primer-sequences	Repeat motif	Bands
RPTest11	F)AGGATGCCTATGATATGCGC R)AACCATAACAAAAGCGGTCG	(CAT)7	5
PtTX2123	F)GAAGAACCCACAAACACAAG R)GGGCAAGAATTCAATGATAA	(AGC)8	7
PtTX4001	F)CTATTTGAGTTAAGAAGGGAGTC R)CTGTGGGTAGCATCATC	(GT)15	8
PtTX3116	F)GCTTCTCCATTAACTAATTCTA R)TCAAAATTGTTCGTAAAACCTC	(GTT)10	10
PtTX4011	F)GGTAACATTGGGAAAACACTCA R)TTAACCATCTATGCCAATCACTT	(GT)20	7
RPS160	F)ACTAAGAACTCTCCCTCTCACC R)TCATTGTTCCCCAAATCAT	(ACAG)3AGGC (ACAG)3	8
Cjgssr124	F)AAAATGGGTCATGTCATGT R)CATTCTCCATCTCACTACCTAT	(GT)36	8
PR203	F)TGGGACCCCATATTCTGATG R) CATTCCACTAGTTCTCTCGCAC	(GA)14	10
PR4.6	F)GAAAAAAAGGCAAAAAAAAGGAG R) ACCCAAGGCTACATAACTCG	(CA)21(TA)6	7
PR011	F)TGAGGAATCCATTGACATGC R)TGATCCGTGTGATCATCTTATG	(CT)21(CA)8	8
RPTest1	F)GATCGTTATTCCTCCTGCCA R)TTCGATATCCTCCCTGCTTG	(ATA)7	6
PtTX2146	F)CCTGGGGATTTGGATTGGGTATTTG R)ATATTTTCCTTGCCCCTTCCAGACA	(GCT)21	7

12 primer pairs with abundant polymorphism bands were selected and the SSR-PCR

reaction system was established. In 15μL PCR reaction: 30 ng DNA, 0.25 mmol / L Mg2+, 0.2 mmol/L dNTP, 250nmol/L Primer (F), 250 nmol/L Primer (R), 0.375U Taq polymerase are the best. Detected by 6% polyacrylamide gel, the length of amplification products was 100 - 250 bp, the number of alleles per locus was varied from 5 to 10 (Zhang D. M. *et al.*, 2007).

Data analysis and programs applied

Inbreeding, selfing rate of both single loci and multilocus are estimated with Ritland mix mating systematic model MLT (Ritland, 1990). Pollen contamination rates in the seed orchard were predicted with GENFLOW, written by Adams et al. (Adams, W. T. and J. Burczyk. 1993.). For paternity analysis CERVUS, written by Marshall was applied (Marshall, 1998; http://helios.bto.ed.ac.uk/evolgen).

Results

1 Mating system parameters

The variations of mating system parameters in the seed orchard over time, space and for different clones in the seed orchard were studied. Seed samples were taken in 1984, initial bearing age; 1993, fruitful bearing stage before rouging; 1996, after rouging; 2000, bearing age. Result indicates that the multilocus rates of outcrossing (t_m) has no much difference in 1984 and 1993, which is 0.975 and 0.962 respectively. There is a little difference in selfing coefficient, 0.025 and 0.038 respectively. In 1996 and 2000, outcrossing rates contain some difference, 0.795 and 0.801 correspondingly; the selfing rates are 0.205 and 0.119 respectively. Seed orchard was rouged in 1993, mating parameters differs markedly before and after the operation, the rates of the outcrossing decreases from 0.975 in 1993 to 0.795 in 1996, while the selfing coefficient increases from 0.038 in 1993 to 0.205 in 1996 (Zhang D. M. *et al.*,2001b; 2004).

Table 1 Mating system parameter in the seed orchard for different years

	1984	1993	1996	2000	mean
Multilocus rates of out crossing t_m	0.975(0.039)	0.962(0.019)	0.795(0.056)	0.801(0.046)	0.883
Difference of outcrossing rate t_m-t_s	0.076(0.028)	0.141(0.035)	0.341(0.052)	0.239(0.040)	0.199
Multilocus selfing rate 1-t_m	0.025(0.000)	0.038(0.019)	0.205(0.056)	0.199(0.046)	0.117

Note: Numbers in brackets are estimated standard errors, the same below.

Distribution of clones with different rates of outcrossing

The distribution of clones with different rates of outcrossing at different stages of the seed orchard is shown in Figure 1. There were 17 flowering clones in 1984, for 11.8% clones the outcrossing rate ranged 0.4 - 0.6, while the rates higher than 0.8 account for 82.4%. In 1993, there was no clone with the rates lower than 0.6 for total 34 clones in the seed orchard before rouging, while the rates higher than 0.8 accounted for 88.2%. Clones with the rates of 0.2 - 0.4 and 0.4 -0.6 respectively accounted for 8.3%, 6.7% and 20.8%, 13.3% in 1996 and 2000. Clones with the rates over 0.8 accounted for 54.2% and 40% in 1996 and 2000 as well. It shows that the thinning management apparently affects the mating parameters in the seed orchard (Zhang D. M. *et al*., 2000).

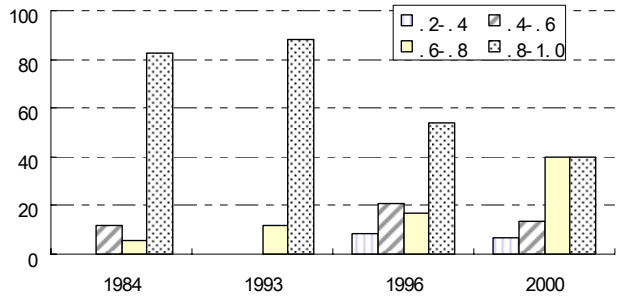

Figure 1 Distribution of clones with different outcrossing rates for four years

Outcrossing rates for three layers of crowns

Seed samples collected in 1987 were analyzed for this purpose. In general the outcrossing rates for seeds from upper crown are slightly higher than those from middle and lower positions (see Tables 2 and 3), although they are slightly various for different ramets-clones (Zhang D. M. *et al.*, 2004).

Table 2 Outcrossing & selfing rates for different layers of crown on an average

	Top	Middle	Low	Mean
Rates of multilocus outcrossing	0.910(.098)	0.909 (.098)	0.900 (.049)	0.906
Difference of the rates of outcrossing	0.160(.057)	0.044 (.057)	0.040 (.053)	0.081
Multilocus Selfing rate	0.090(.098)	0.091 (.098)	0.100 (.049)	0.094

Table 3 The outcrossing rates for three layers of five ramets

Clone	Upper			Middle			Lower		
4#	1.16*	(0.11)	n=46	2.00*	(0.00)	n=47	1.03*	(0.19)	n=71
5#	0.83	(0.06)	n=55	0.96	(0.09)	n=51	1.02*	(0.08)	n=48
19#				0.70	(0.09)	n=49	0.63	(0.18)	n=52
31#	0.96	(0.09)	n=53	1.27*	(0.13)	n=50	0.55	(0.12)	n=52
43#	1.12*	(0.06)	n=50	0.99	(0.07)	n=51	0.87	(0.09)	n=51

Note: According to the program stipulation, figures lager one are treated as one; n shows number of seeds analyzed.

2 Pollen contaminations

Based on comparing enzyme allozyme of clones with the seeds produced the seed orchard, foreign bands were discovered on locus *got2* allele *a, lap1 a, lap2 a* in 1984, 1983, 1996 and 2000; while *pgm1 e* - in 1993 and 1996 (See Table 4). It is obvious that the seed orchard was contaminated by the surrounding plantation (Zhang D. M. *et al.*, 2000).

Table 4 Foreign bands discovered in Seeds produced in the seed orchard

Locus	Allele	Clones	Gene frequency				Locus	Allele	Clones	Gene frequency			
			1984	1993	1996	2000				1984	1993	1996	2000
Got1	a	0.031	0.025	0.042	0.112	0.050	*Acp1*	a	0.255	0.055	0.381	0.191	0.128
	b	0.949	0.907	0.942	0.826	0.899		b	0.459	0.286	0.314	0.506	0.725
	c	0.020	0.068	0.017	0.062	0.050		c	0.286	0.658	0.217	0.237	0.147
Got2	a	0.000	0.014	0.017	0.158	0.050		d	0.000	0.000	0.053	0.000	0.000
	b	0.969	0.970	0.958	0.736	0.945		e	0.000	0.000	0.000	0.000	0.000
	c	0.031	0.016	0.025	0.106	0.005		f	0.000	0.000	0.036	0.000	0.000
Lap1	a	0.000	0.010	0.006	0.115	0.014	*Skd1*	a	0.020	0.161	0.107	0.248	0.115
	b	0.980	0.977	0.936	0.722	0.968		b	0.724	0.698	0.862	0.540	0.725
	c	0.020	0.013	0.036	0.152	0.018		c	0.255	0.141	0.030	0.211	0.161
	d	0.000	0.000	0.000	0.000	0.000	*Pgm1*	a	0.082	0.051	0.097	0.025	0.119
	e	0.000	0.000	0.000	0.000	0.000		b	0.357	0.327	0.300	0.220	0.468
	f	0.000	0.000	0.019	0.000	0.000		c	0.439	0.474	0.481	0.522	0.353
Lap2	a	0.000	0.018	0.045	0.152	0.037	*Adh1*	d	0.122	0.148	0.119	0.202	0.060
	b	0.980	0.980	0.877	0.761	0.927		e	0.000	0.000	0.003	0.031	0.000
	c	0.020	0.002	0.045	0.087	0.037		a	0.041	0.125	0.106	0.031	0.156
	d	0.020	0.002	0.045	0.087	0.037		b	0.673	0.770	0.883	0.866	0.784
	e	0.000	0.000	0.000	0.000	0.000		c	0.286	0.105	0.011	0.102	0.060
	f	0.000	0.000	0.034	0.000	0.000							

Based on the analysis of 8 enzyme loci for 49 clones and seeds collected from the ramets in the seed orchard in 1984、1993、1996 and 2000, the observed contamination rates are 0.326, 0.450, 0.532 and 0.385, while the estimated rates - 0.354、0.492、0.583 and 0.418 respectively. An average contamination rate is 0.462 (See Table 5). The contamination rate in 1996 is higher than that of 1993. It may be caused by rouging carried in 1993 (Zhang D. M. *et al.*, 2004).

Table 5 Estimates of pollen contamination in the seed orchard

Year	Seed analyzed	Observed rate	Correct coefficient	Estimated rate
1984	182	0.326	0.922	0.354
1993	149	0.450	0.914	0.492
1996	154	0.532	0.914	0.583
2000	109	0.385	0.922	0.418
Mean				0.462

To examine the contamination rates in different positions of crown, seed samples was taken from one ramet. The result is shown in Table 6. The rate in low layer is much higher than those in top and mid, but all the rates are much lower in comparison with the average rates in Table 5.

Table 6 Pollen contamination rate in different positions of crown in the seed orchard

Layer of Crown	Seed analyzed	Observed rate	Correct coefficient	Estimated rate
Top	186	0.108	0.891	0.121
Mid	255	0.123	0.891	0.138
Low	211	0.243	0.891	0.273
Mean		0.158	0.089	0.177

3 Pollen dispersal and pollination

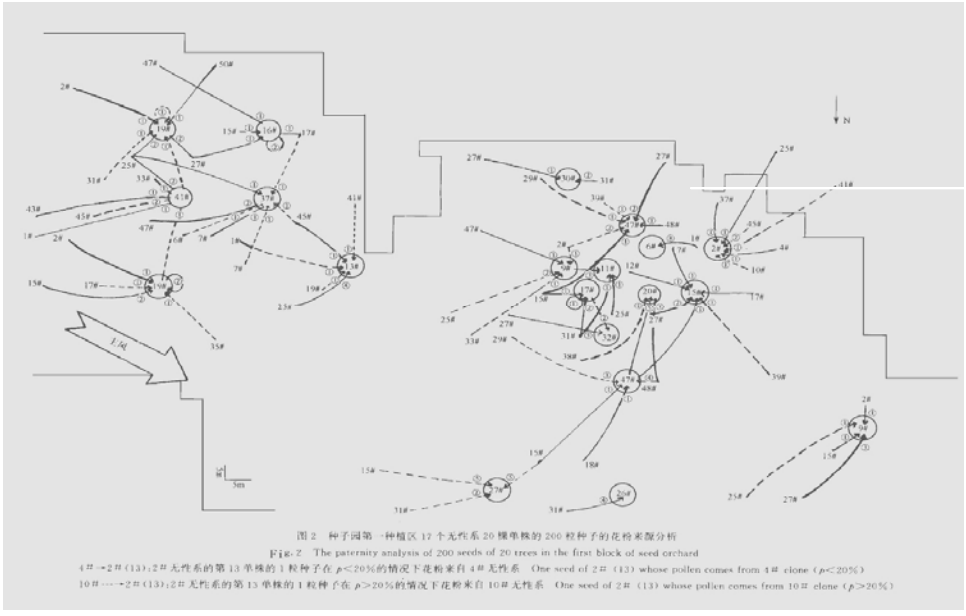

Figure 2 The paternity analysis of 200 seeds in the seed orchard with enzyme

The effective dispersal distance of pollen was investigated using enzyme analysis. 89 seed samples from the seed orchard were examined for pollen-father source in detailed (Zhang D. M. *et al.*, 2001a). Figure 2 might give some ideas of the pollen dispersal and pollination incident in the seed orchard. 17.8% pollen-father comes within a radius of 7 m from the neighboring seed tree; 24.4%

approaches within 10 - 20 m; 55% pollen arrives within a radius of 20 - 30 m. The effective dispersal distance of pollen is less than 30 m. In addition, it demonstrates that pollen-father sources of 8 seeds for each ramet are quite diverse. For example, pollen sources for clone No 6 (ramet No 14) completely derived from clone No 1 at reliability > 0.95; 50% pollen for clone No 26 (25) came from clone No 31; 62.5% pollen for clone No 20 (1) – clone No 47, while for most of ramets, namely clones No 2 (13)、No 9 (6)、No13 (33)、No 16 (44)、No 17 (4)、No 37 (46) and No 41 (45) pollen-father derived diversely. In some cases pollen-father of 8 analyzed seeds originated from 6 clones.

The results with SSR analysis of open-pollinated seeds for two clones No 11 and 24 is shown in Figure 3. 11.1% -12.8% of pollen-father comes within a radius of 10 m from the seed tree; 37.0% - 40.4% within 10 - 20 m; while 17.2%- 22.2% comes within a radius of 20 -30 m from the seed tree. The outcome is all most the same as enzyme analysis.

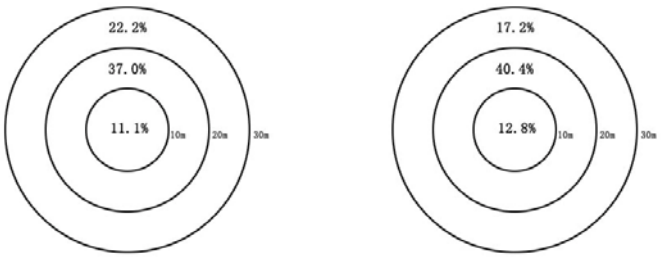

Figure 3 The paternity analysis for two clones (left – No 11; right – 24) with SSR

4 The mating system parameters for three different populations

Natural stand, seed orchard and Progeny testing plantation were examined. The estimated outcrossing rates of single-loci (ts) and multi-locus (tm), inbreeding and selfing rates are 0.638, 0.821, 0.742; 0.864, 0.962, 0.953; 0.226, 0.141, 0.211 and 0.136, 0.038, 0.047 correspondingly. There are considerable differences in ts and tm among the populations. It indicates that selfing rate is much high in the natural stand, while the inbreeding rate in the stand and progeny plantation populations is higher in comparison with the seed orchard (Zhang D. M. *et al.*, 2000).

Table 7 The mating system parameters for three populations

	Natural stands	Seed orchard	Progeny plantation	Mean
ts	0.638(0.049)	0.821(0.044)	0.742 (0.023)	0.734(0.039)
tm	0.864(0.048)	0.962(0.019)	0.953 (0.021)	0.926(0.029)

Tm-ts	0.226(0.042)	0.141(0.035)	0.211(0.019)	0.193(0.032)
1-tm	0.136(0.048)	0.038(0.019)	0.047(0.021)	0.740(0.029)
F	0.259(0.062)	0.211(0.083)	0.477 (0.029)	0.316(0.058)

Conclusion

Modern technology provides an opportunity to gain an insight into understanding essential, but invisible fact happened in seed orchards (El-Kassaby,Y. A. & K. Ritland, 1986; .El-Kassaby Y. A. et al., 1989; Harju A, Muona O. 1989; El-Kassaby Y. A. & S. Reynolds, 1990) and our investigation affords some idea about temporal and spatial variation on outcrossing, selfing, inbreeding and contamination rates as well as on pollen dispersal distance in a seed orchard of *Pinus tabulaeformis* carr.. All these data are theoretical importance for sustainable, healthy development of seed orchards, although they are not sufficient and not accurate enough as expected. To fully solve the facing problem we have long way to go. Inaccurate biology analysis and statistical methods used today should be improved and the study of mating system in combination with field observation is vital.

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