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Microsporogenesis and meiotic behavior in nine species of the genus *Pinus*

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Abstract The meiotic behavior of 10 taxa (nine species and one variety) of the genus *Pinus* was investigated using pollen mother cells (PMCs) to reveal the differentiation among karyotypes. Chromosome spreads were prepared by conventional squashing. The meiotic index and the average configuration were higher, whereas the frequency of aberrance (chromosomal bridges, fragments, or micronuclei) was lower, in all 10 taxa compared with other gymnosperms. The meiotic index, average configuration, and frequency of irregularity were found to be uniform among the species. It was shown that the genomes of the *Pinus* species investigated were highly stable, confirming results of previous mitotic analyses in this genus. However, slight differentiation of homologous chromosomes among genomes was revealed by analysis of meiotic configurations in *Pinus nigra* var. *poiretiana*. Quadrivalents were observed in 9.31% of PMCs in this species. This is the first time that quadrivalents have been observed in gymnosperms.

Key words homologous chromosomes, irregularity, meioses, *Pinus*, pollen mother cell, quadrivalent.

Pinus L. is the largest genus of conifers and one of the most widespread tree genera throughout the Northern Hemisphere (Richardson, 1998). Recent classifications recognize two subdivisions of the genus *Pinus* (Richardson, 1998; Fu et al., 1999), namely subgen. *Pinus* (diploxylon) and subgen. *Strobus* (Lemmon) A. E. Murray (haploxylon).

Cytological studies suggest that *Pinus* is unusually conservative in its karyotypes (Darlington & Wylie, 1956; Hizume & Tanaka, 1979; Hizume, 1988) and all species of *Pinus* investigated are homoploid, with the basic number $x = 12$ (Darlington & Wylie, 1956; Hizume & Tanaka, 1979; Hizume, 1988; Li, 1995). It is difficult to identify different chromosomes within a genome or to compare the differentiation of homologous chromosomes among different genomes.

Ferguson (1904) and Lewis (1908) first observed the meiotic behavior of pine chromosomes, but found no irregularities. Later studies on meiotic behavior of the Pinaceae revealed the conservative nature of the pinaceous genomes. The frequency of chiasma is similar in different coniferous species (Sax, 1932, 1933; Sax & Sax, 1933). In addition, meiotic irregularities were similar in different coniferous species and were as frequent in the species as in their hybrids (Sax, 1960; Saylor & Smith, 1966). Thus, Sax concluded that structural changes in chromosomes were not important as an isolating mechanism in pine speciation. However, Hirayoshi et al. (1943) discovered univalents and a complicated quadrivalent in a putative hybrid (*P. densiflora* × *P. thunbergii*). Furthermore, Mergen et al. (1963) observed meiotic irregularities, such as lagging chromosomes, fragments, chromosome bridges, and micronuclei, in irradiated microspores of *P. echinata* Mill. and *P. taeda* L., although the meiotic behavior of most irradiated microspores was completely regular in the two species.

During the process of meiosis, homologous chromosomes partner up with one another in the shape of different bivalents. Studies of meiosis in more species may provide valuable information for the analysis of *Pinus* karyotypes (Hizume & Tanaka, 1979; Li, 1995).

In the present study, we observed the meiotic behavior of 10 taxa (nine species and one variety). The meiotic process in *P. bungeana* Zucc. ex Endl. (Pan et al., 1982), *P. banksiana* Lamb. (Sax & Sax, 1933; Salylor & Smith, 1966), *P. nigra* J. F. Arnold (Sax & Sax, 1933), *P. thunbergii* Parl. (Hirayoshi et al., 1943), *P. armandii* Franch. (Salylor & Smith, 1966), and *P. ponderosa* Dougl. (Salylor & Smith, 1966) has already been reported, whereas that in *P. koraiensis* Siebold & Zucc., *P. taiwanensis* Hayata, *P. echinata*, and *P. nigra* var. *poiretiana* Asch. & Graebn. is reported here for the first time.

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Table 1 Source of materials

Taxon	Locality	Voucher
Subgen. *Strobus*		
P. bungeana Zucc. ex Endl.	Beijing	H. S. Deng 06137
P. koraiensis Siebold & Zucc.	Beijing	H. S. Deng 06171
P. armandii Franch.	Beijing	H. S. Deng 06143
Subgen. *Pinus*		
P. banksiana Lamb.	Beijing	H. S. Deng 06136
P. taiwanensis Hayata	Beijing	H. S. Deng 06134
P. ponderosa Dougl.	Beijing	H. S. Deng 06141
P. echinata Mill.	Beijing	H. S. Deng 06135
P. nigra J. F. Arnold	Beijing	H. S. Deng 06138
P. nigra var. *poiretiana* Asch. & Graebn.	Beijing	H. S. Deng 06142
P. thunbergii Parl.	Beijing	H. S. Deng 06132

All voucher specimens are stored in the herbarium of the Department of Biochemistry, Liuzhou Teachers College (Liuzhou, China).

1 Material and methods

Microspores of the 10 taxa were collected from trees cultivated in the Beijing Botanical Garden, Chinese Academy of Sciences (CAS), between April and May 2002. Voucher specimens were deposited in the herbarium of the Department of Biochemistry, Liuzhou Teachers College, Liuzhou, China (Table 1). Microspores were fixed in Carnoy's fixative (alcohol : glacial acetic acid 3 : 1, v/v) for 24 h and then transferred to 75% alcohol and stored at −20°C. Meiotic preparations were made according to the methods of Chen et al. (1979). First, microspores were immersed in 1 mol/L HCl for 1 h, soaked in double-distilled water for 30 min, then in 45% glacial acetic acid for 10 min, and then squashed on slides. Chromosomes were colored with a 1 : 20 Giemsa staining solution (pH = 7.0). Chromosome slides were observed and identified under an optical microscope (BH-2; Olympus, Japan). Microphotographs were taken under a Leica (Wetzlar, Germany; model DMRBA) microscope and adjusted using Photoshop 7.0.1 (Adobe, USA) to enhance the contrast.

Statistical data were analyzed using Excel 2000 (Microsoft, USA) and SPSS 10 (SPSS, Chicago, IL, USA).

2 Results and discussion

2.1 Differentiation of homologous chromosomes and conserved features of meiosis

Prophase I was further divided into five substages, namely leptotene, zygotene, pachytene, diplotene, and diakinesis. The chiasmata formed at diplotene were clearly visible (Fig. 2) at diakinesis. To ensure comparability, the number of chiasmata was counted during early diakinesis in all 10 taxa and was found to range from 2.29 in *P. banksiana* to 2.49 in *P. armandi*. The average number of chiasmata across the 10 taxa was 2.40 (Table 1), confirming results of previous studies (Sax, 1932, 1933, 1960; Sax & Sax, 1933).

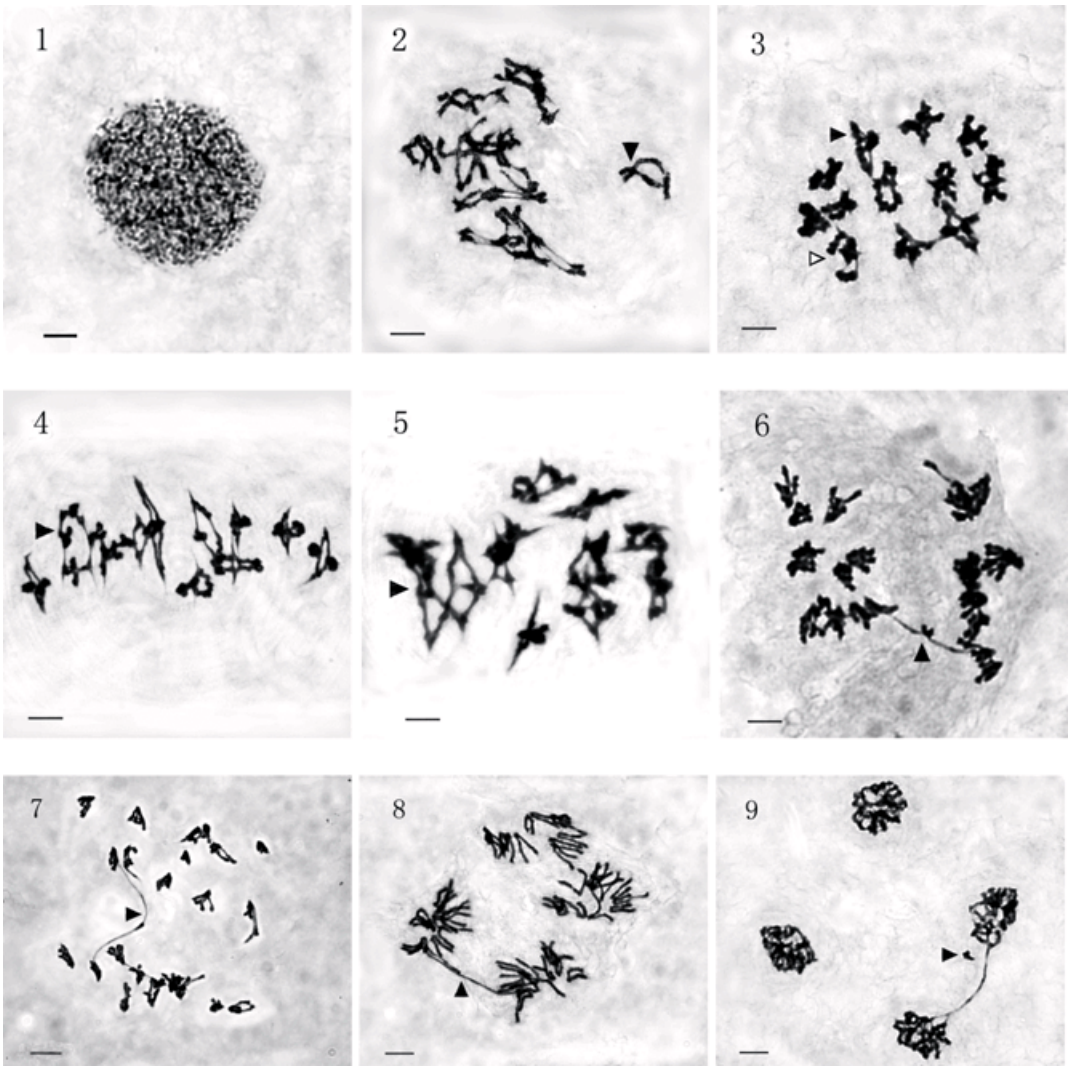
Three primary pairing patterns of homologous chromosomes were observed in pollen mother cells (PMCs; Figs. 3–5): (i) ring bivalents (II_0); (ii) rod bivalents (II_1); and (iii) univalents. Higher homologous chromosomes will frequently form a partnership as a result of the pairing of homologous arms, which then form a ring bivalent. Across all 10 taxa investigated in the present study, the lowest frequency of ring bivalents was found to be 11.44 (in *P. banksiana*) and the lowest meiotic index was found to be 95.37% (in *P. banksiana* and *P. ponderosa*; Table 2), values that are similar to and higher than, respectively, those found in most other gymnosperms.

The chromosome bridges at anaphase I arise mainly from two processes (Saylor & Smith, 1966): (i) nondisjunction of bivalents due to failure of chiasma terminization (Fig. 6); and (ii) crossing-over in the inversion regions (Figs. 7, 9). Sometimes these bridges will bring along chromosome fragments or micronuclei when they are broken. A higher frequency of meiotic aberrance (including chromosome bridges, fragments, or micronuclei) indicates strong differentiation among homologous chromosomes. In the first division of the 10 taxa investigated in the present study, the frequency of meiotic aberrance was found to range from 1.62% (*P. thunbergii*) to 7.09% (*P. armandi*) (Table 3), lower than that of other gymnosperms (Feng & Xu, 2002; He et al., 2004), which may imply a lower differentiation among homologous chromosomes in *Pinus*.

All these parameters investigated across the 10 taxa were demonstrated to be conserved features of meiosis in *Pinus*.

2.2 Quadrivalents with interhomolog translocation

Quadrivalents are infrequent in gymnosperms. Hirayoshi et al. (1943) observed a complicated quadrivalent in a putative hybrid. In the present study, quadrivalents with interhomolog translocation were observed in 9.31% of PMCs of *P. nigra* var. *poiretiana*. Translocation quadrivalents (Figs. 4, 5) were observed in 9.31% of PMCs of *P. nigra* var. *poiretiana*. This is the first time that quadrivalents have been observed in gymnosperms. At metaphase I, the quadrivalents were in the shape of a ring or a figure 8, but no chain was observed. The fragments exchanged in the quadrivalents should be large in size. There may be little differentiation between either the parts exchanged or the remaining parts that are not exchanged. Therefore, the pairing patterns of homologous chromosomes with translocation were either



Figs. 1–9. Behavior of meiotic chromosomes. **1**. Leptotene, *P. taiwanensis*. **2**. Diplotene, *P. echinata*. The arrowhead indicates a chiasma. **3**. Diakinesis, *P. bungeana*. The black arrowhead indicates the ring bivalent, whereas the open arrowhead indicates the rod bivalent. **4**. Metaphase I, *P. nigra* var *poiretiana.* The arrowhead indicates an "O" quadrivalent. **5**. Metaphase I, *P. nigra* var *poiretiana*. The arrowhead indicates a figure 8 quadrivalent. **6**. Anaphase I, *P. taiwanensis*. The arrowhead indicates a bridge arising from failure of chiasma terminization. **7**. Anaphase I, *P. thunbergii*. The arrowhead indicates an inversion bridge. **8**. Anaphase II, *P. bungeana*. The arrowhead indicates a bridge arising from the second division. **9**. Telophase II, *P. banksiana*. The arrowhead indicates a fragment and bridge arising from the first division. Scale bars = 10 μm.

Table 2 Average configuration and meiotic index

Taxon	Average configuration				Meiotic index	No. cells
	IV	II_0	II_1	I		
Subgen. *Strobus*						
P. bungeana		11.6956	0.2656	0.038800	97.46	1263
P. koraiensis		11.9493	0.0507	0	99.58	533
P. armandi		11.9723	0.0230	0.000719	99.77	2783
Subgen. *Pinus*						
P. banksiana		11.4449	0.4964	0.058760	95.37	970
P. taiwanensis		11.8612	0.1341	0.004153	98.85	1204
P. ponderosa		11.8047	0.1891	0.006219	98.37	804
P. echinata		11.9565	0.4352	0	99.64	563
P. nigra var *poiretiana*	0.1863	11.7092	0.1013	0.003268	99.13	612
P. nigra		11.8601	0.1393	0.000603	98.83	1658
P. thunbergii		11.8255	0.1712	0.003231	98.55	617

I, univalent; II_0, ring bivalent; II_1, rod bivalent; IV, quadrivalent.

Table 3 Frequency of meiotic aberrance*

Taxon	First division		Second division	
	No. cells	Average	No. cells	%
Subgen. *Strobus*				
P. bungeana	293	6.8254	601	0.8319
P. koraiensis	304	5.1473	986	0.9128
P. armandi	301	7.0898	1638	0.9768
Subgen. *Pinus*				
P. banksiana	345	4.2849	508	0.3937
P. taiwanensis	527	3.5172	480	0.2083
P. ponderosa	513	3.9068	1121	0.1784
P. echinata	289	2.1156	1339	0.1494
P. nigra var. *poiretiana*	643	4.8729	1376	0.3634
P. nigra	306	3.6868	456	0.4386
P. thunbergii	888	1.6209	1521	0.1972

*Meiotic aberrance includes bridges, fragments, and/or micronuclei.

two bivalents or one quadrivalent. Offspring nuclei that arise from alternate segregation of the figure 8 quadrivalent develop normally, whereas offspring nuclei that arise from adjacent segregation of the ring quadrivalent, which contain deletions and duplication of DNA sequences, are abnormal (Hong, 1990).

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