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Observation on meiotic behavior in three *Mahonia* species, with special reference to the intergeneric relationship of *Mahonia* and *Berberis*

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Abstract — Cytological characters in meiosis of three *Mahonia* species-*Mahonia fortunei, M. bealei* and *M. polydonta* were investigated using the method of wall degradation and hypotonic treatment for chromosomal preparations. Chromosome numbers of the three species were all 2n = 2x = 28; those of *M. fortunei* and *M. polydonta* represent the first report. The three species at metaphase I possessed the same configuration of 14II, however, the secondary association was also observed. During anaphase I, 1.5% of the cells of *M. fortunei* had lagging chromosomes and chromosome bridges. During anaphase II, lagging chromosomes, chromosome bridges and asynchronism in second division were observed, with abnormality rate in *M. fortunei* being about 9.5%. The rate of fertile pollen grains of the three species amounted to more than 90%, which was consistent with the cell abnormality rate at anaphase II. The results indicate that, overall, meiotic process of the three species is normal, which is in line with the high pollen fertility rate and high seed yield of the three species. Our results support the view that *Mahonia* and *Berberis* represent a sister group in phylogenetic evolution, and that they should be treated as two distinct genera.

Key words: abnormality rate of cells, *Berberis,* chromosome configuration, *Mahonia*, meiotic behavior, phylogeny, pollen fertility rate.

INTRODUCTION

Berberidaceae (Ranunculales) is a relatively primitive angiosperm group in the phylogenetic evolution (Hoot *et al.* 1999). Within this family, *Mahonia* is the second largest genus, containing approximately 60 species distributed in East Asia, South-east Asia, North and Central America and the western part of South America. In China there are 35 species of *Mahonia,* occuring mainly in Sichuan, Yunnan, Guizhou and southeast Tibet (Ying 2001).

Members of *Mahonia* are, with little exception, evergreen shrubs, rarely small trees. They are well known for their medicinal value; all parts of the plants can be used to refrigerate, strengthen physique and detoxicate. Extracts from the root and stem can also be used in printing and dyeing, and the plants may be grown for decoration in the courtyard. Therefore, they are economically important.

Cytological studies on *Mahonia* species have been limited to chromosome counts (Xu *et al.*1992**;** Zou and Jiang 1989; Hong 1990), that established the diploid and basic chromosome number as 2n = 2x = 28. No work on chromosome configuration and behavior in meiosis of *Mahonia* species has been reported. Meiosis is the precondition for sexual reproduction, and meiotic chromosome behavior, such as synapsis, exchange, and separation, have been utilized to address questions concerning genetics and reproductive biology. The purpose of the present research is to investigate chromosome configuration and behavior, and to determine pollen fertility in three species of *Mahonia*, as well as to enrich our database on chromosome number in the genus *Mahonia,* and to provide a baseline reference for furthering cytogenetic and phylogenetic studies within this genus.

MATERIALS AND METHODS

In this study we examined three species of *Mahonia*, namely, *Mahonia fortunei* (Lindl.) Fedde,

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M. bealei (Fort.) Carr. and *M. polydonta* Fedde. Pickled materials were collected from the Berberidaceae repository at the Wuhan Botanical Garden. Voucher herbarium specimens were prepared and were deposited in the herbarium of the Wuhan Botanical Garden, Chinese Academy of Sciences (HIB), Wuhan, P. R. China.

For the period of late August, 2004, to early September, 2005, the young flower buds were collected. Subsequently, young buds were dissected and the tiny, immature anthers were removed and fixed in Farmer's Solution (3:1 absolute ethyl alcohol: glacial acetic acid) for 30 minutes. The meiotic process was observed through the method of cell-wall degradation using a hypotonic treatment, followed by Giemsa staining (Zhu 1982). Pollen grains stained using aceto-orcein were examined to determine their fertility.

RESULTS

Cytological characters of the three species based on the pickled materials examined revealed no marked differences between species in the meiotic process and behavior. Therefore, a generalized and integrated description and analysis of the meiotic process in the three species are presented.

Prophase - During the prophase stage, chromatins condensed into chromonemas, gradually becoming shorter and thicker toward the initiation of synapsis. At leptotene, despite the fact that the chromosomes had reproduced, they appeared like a single thread and scattered in a web pattern (Fig. 1).

When cells entered the zygotene stage (Figs. 2-3), homologous chromosomes began to pair. In some homologous chromosomes pairing proceeded at several sites along the whole length simultaneously. In others, synapsis proceeded gradually from one end to the other, stepwise. After synapsis, paired regions of the homologous chromosomes became obviously thicker than the unpaired regions.

At pachytene stage, chromosomes became even much thicker and shorter (Figs. 4, 16-17, and 28), but were still long and tangled with each other, so that it was difficult to distinguish the shape and structure of each bivalent. At early pachytene, the chromosomes were located on one side of the nucleus, while some bivalents contain regions without synapsis at the interstitial or terminal portions.

At diplotene stage (Figs. 5-6, 18 and 29-30), most bivalents presented a cruciform configuration, which were consistent with previous reports, namely, that *Mahonia* species had a symmetric karyotype (Zou and Jiang 1989). A few bivalents appeared parallel due to the advanced disappearance of synapsis, while v-form bivalents also appeared.

Finally, at diakinesis (Figs. 7-8, 19, 31 and 32), chromosomes became further condensed and appeared like short sticks. Bivalents appeared as in a cruciform configuration or presented a "V" form, due to the fact that chiasmata had moved to the end of the chromosome arms. In some cases, parallel bivalents without chiasmata were also present, due to the advanced disappearance of synapsis.

Metaphase I - During metaphase I (Figs. 9-10, 20-22, 33), the chromosomes appeared as 14 normal paired bivalents, which were mostly claviform, rarely globular. Furthermore, secondary association of two bivalents was also observed in some microspore mother cells. The segregation of bivalents was not synchronous; some segregated earlier, while others later.

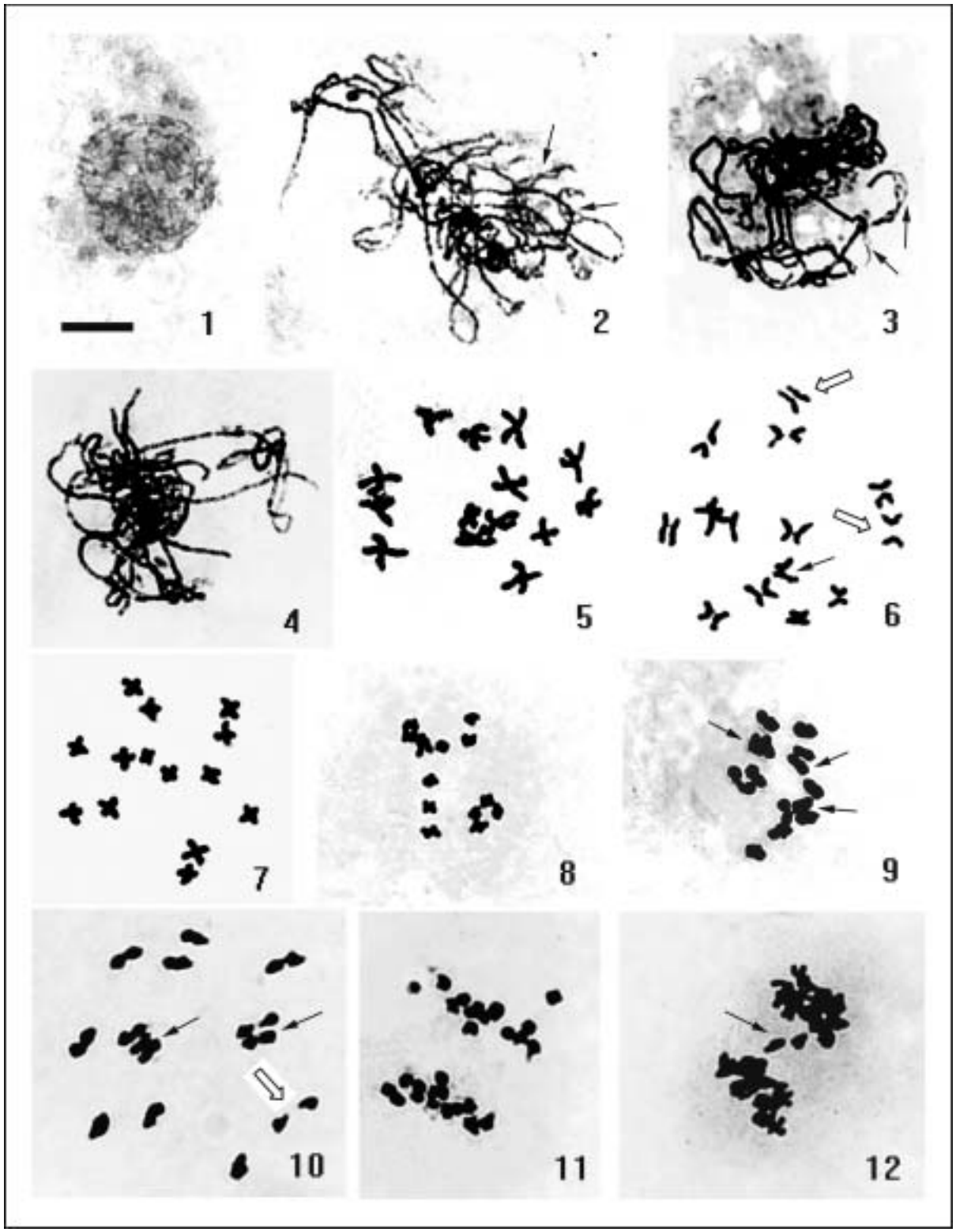
Anaphase I and anaphase II - At anaphase I (Figs. 11-12, 23-25, 34), after the 28 chromosomes had moved to opposite poles, 14 chromosomes could be seen clearly at each pole and the haploid chromosome number was clearly observed to be n = 14. Lagging chromosomes and chromosome bridges were also observed. Among 200 pollen mother cells of *M. fortunei* examined, only three cells were seen to be abnormal, with an abnormality rate of 1.5%.

At anaphase II (Figs. 13-15, 26-27, 35-36), when the centromere of each chromosome split, the chromatids segregated and became clearly visible as n = x = 14. The homologous chromosomes in the opposite poles re-divided asynchronously, because chromosomes at one pole began to divide, while those at the other did not; this resulted in the formation of triads. Lagging chromosomes and chromosome bridges were also observed. Overall, 9.5% pollen mother cells of *M. fortunei* were abnormal.

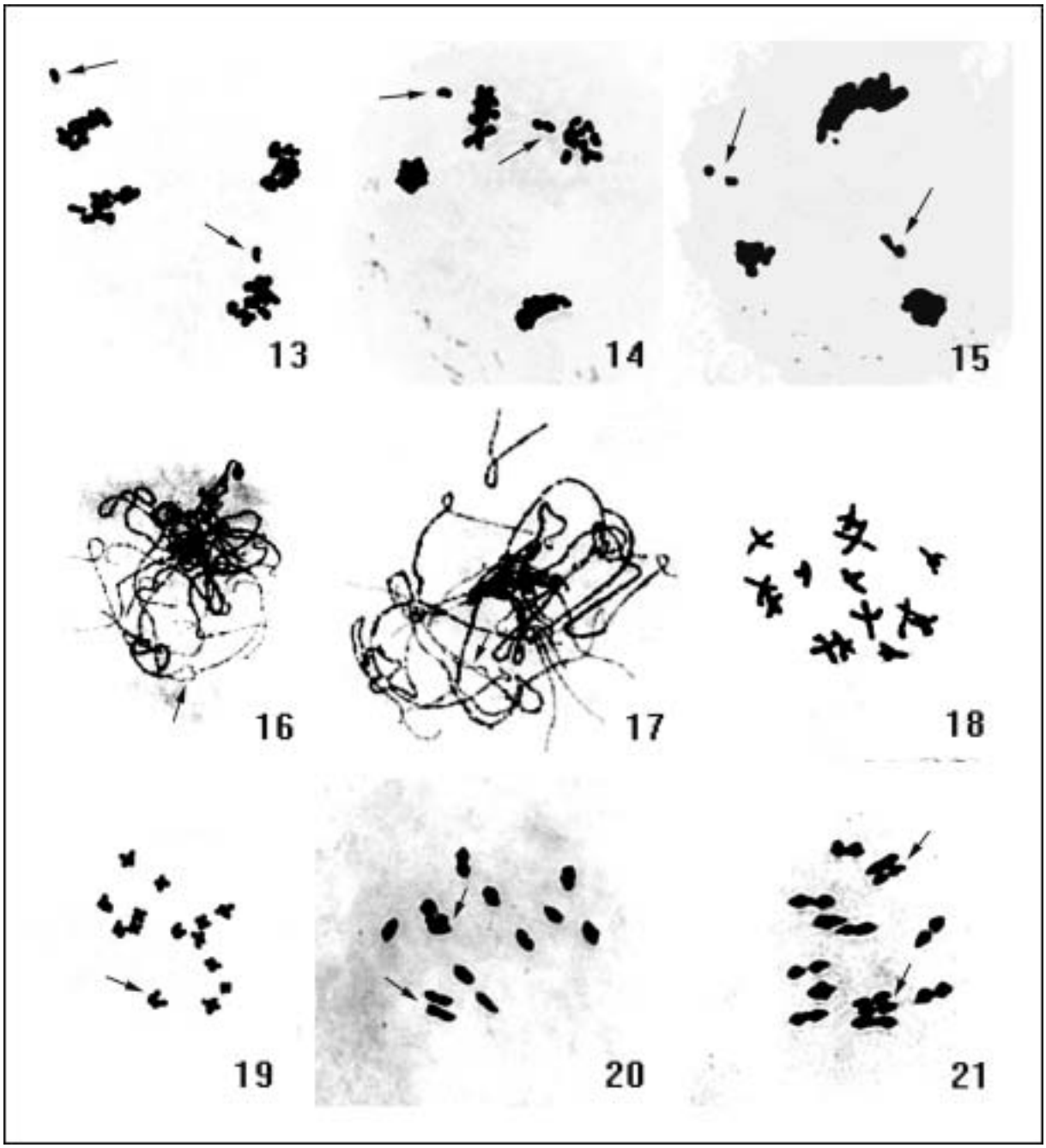
Examination of the pollen grains in the three species of *Mahonia* indicated that overall pollen fertility rate was higher than 90%.

DISCUSSION AND CONCLUSIONS

Previous cytological studies on *Mahonia* reported chromosome counts in four species of the

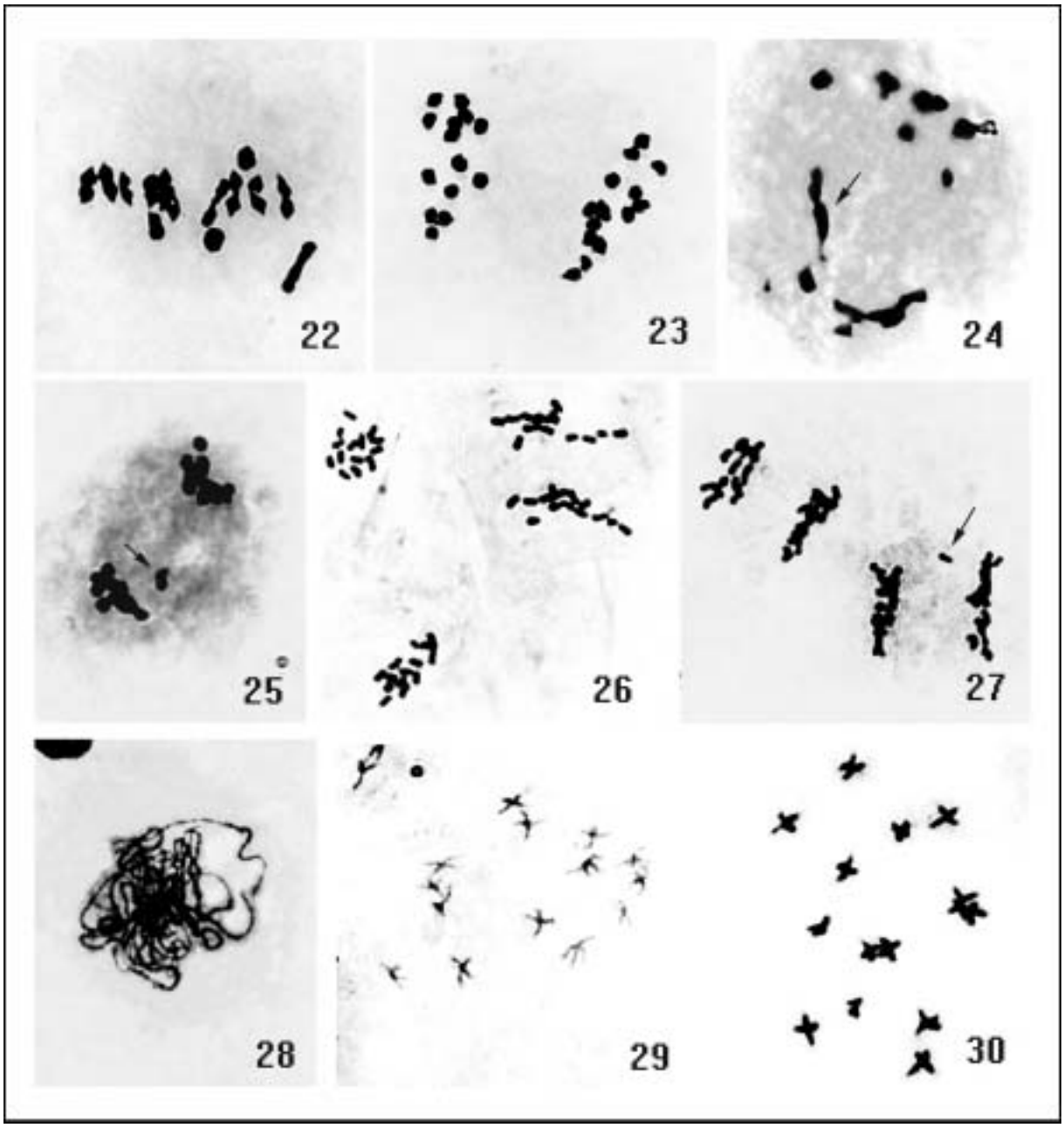


Figs. 1-12 — Chromosome figures of *M. fortunei* in meiosis. Fig. 1.At leptotene. Figs.2-3. At zygotene. Arrows show the paired and unpaired regions clearly distinguishable. Fig. 4. At pachytene. Figs.5-6. At diplotene. Fig. 6. Solid arrow shows a V-form bivalent and hollow arrows show the advanced disappearance of synapsis. Figs.7-8.At diakinesis. Figs.9-10. At metaphase I; solid arrows show secondary association of two bivalents, and hollow arrow shows the earlier segregation of bivalents. Figs. 11-12. At anaphase I. Fig. 12. Arrow shows a lagging chromosome.



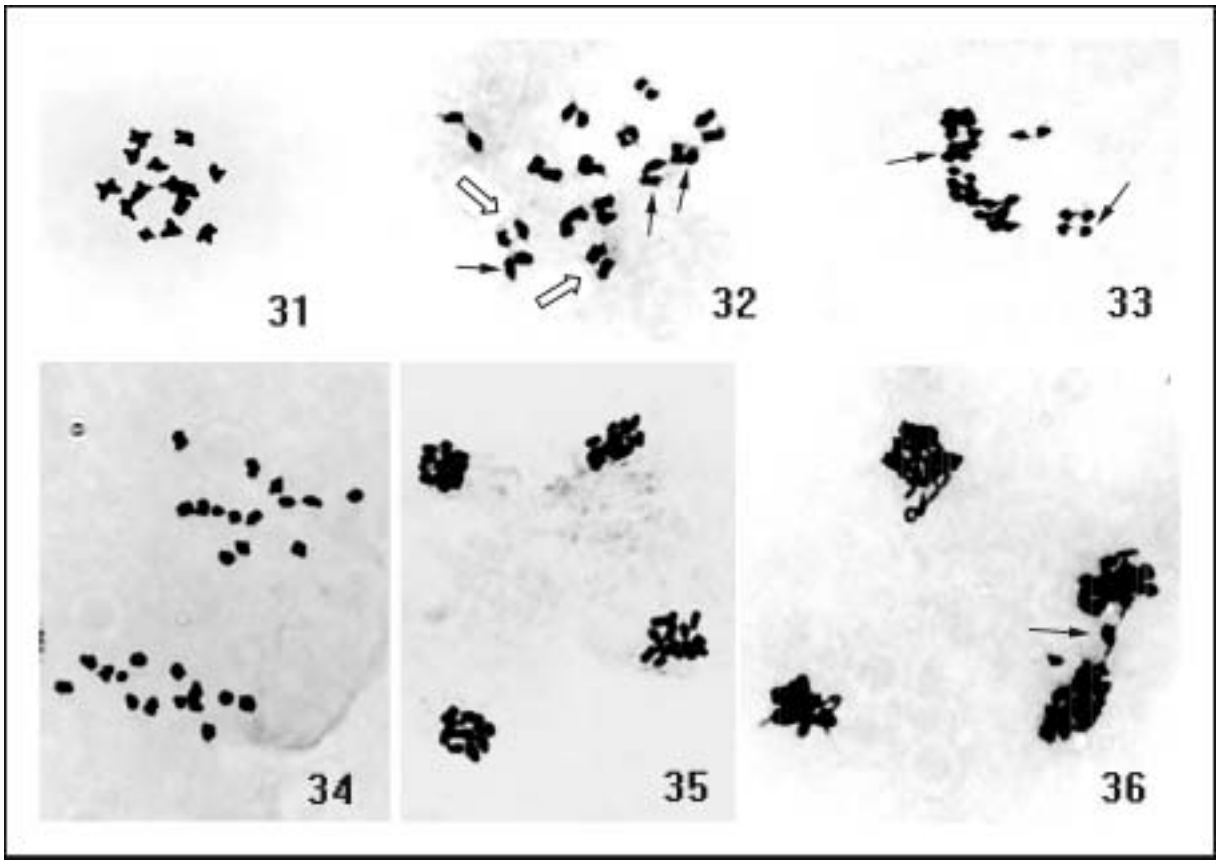
Figs. 13-21. At anaphase II. Arrows show lagging chromosomes. Figs. 14-15. Obvious asynchronization of the re-division of chromosomes. Figs. 16-27 — Chromosome figures of *M. bealei* in meiosis. Figs. 16-17. At pachytene; arrows show unpaired regions. Fig. 16. An early pachytene. Fig. 18. At diplotene. Fig. 19. At diakinesis; arrow shows a V-form bivalent. Figs. 20-22. At metaphase I. Figs. 20-21. Arrows show secondary association of two bivalents.

genus, including *M. bealei*, studied in the present research, and established the chromosome number of the four species to be 2n = 2x = 28 (Xu *et al.*1992; Zou and Jiang 1989). The karyotype of one of the species, *Mahonia leveilleana* Schneider belongs to 1A type, and its chromosomes lack a satellite (Zou and Jiang 1989). No reports on the meiotic process in *Mahonia* species had been published. In the present paper, we examined the meiotic chromosome configurations and behaviors in *M. fortunei, M. bealei* and *M. polydonta,* which showed that the three species all had normal diploid count of 2n = 2x = 28. This is the first report on the chromosome numbers for *M. fortu-*



Figs. 23-25. At anaphase I. Fig. 24. Arrow shows a chromosome bridge. Fig. 25. Arrow shows a lagging chromosome. Figs. 26-30. At anaphase II. Fig. 26. Obvious asynchronization of the re-division of chromosomes. Fig. 27. Arrow shows a lagging chromosome. Figs. 28-36 — Chromosomes of *M. polydonta* at meiosis. Fig. 28. At pachytene. Figs. 29-30. At diplotene. Scale bar =10 μm.

nei and *M. polydonta*. We also established that the chromosome configurations of all the three species consisted of 14II at metaphase stage. The rate of sterile mother cells of *M. fortunei* was below 10% at anaphase I and anaphase II, while the rates of pollen fertility in the three species was above 90%. Thus, the meiotic process and behavior of the pollen mother cells of the three *Mahonia* species investigated were basically normal. Because of the presence of saccate nectaries on the abaxial surface of the petals, individuals of *Mahonia* are able to achieve an effective pollination mechanism associated with entomophily. As a result, *Mahonia* species have successfully developed a high rate of reproduction and achieved a high proportion of mature seeds, both of which may be attributed to the normal meiotic behavior, low rate of pollen grain sterility, and efficient pollina-



Figs. 31-32. At diakinesis. Fig. 32. Solid arrows show V-form bivalent and hollow arrows show parallel bivalents without chiasmata. Fig. 33. At metaphase I; arrows show secondary association of two bivalents. Fig. 34. At anaphase I. Figs. 35-36. At anaphase II. Fig. 36. Arrow shows a chromosome bridge.

tion mechanism. Furthermore, *Mahonia* species also possess the capacity of tillering, namely, reproduction by lateral buds. All of these endowed *Mahonia* with a successful propagation methods, resulting in its present world-wide distribution.

Taxonomically, *Mahonia* is closely related to *Berberis*, and was once treated as a section of *Berberis* (DERMEN 1931), based on the results of cytological studies, which established similar chromosome numbers in the two taxa. Nevertheless, *Berberis* and *Mahonia* continued to be recognized as two separate genera by AHRENDT (1961), who undertook a taxonomic revision of these two taxa. Later, SINGH *et al.* (1974; 1978), after conducting studies on the architecture and epidermal characteristics of the leaves of members of the Berberidaceae, also affirmed that *Mahonia* and *Berberis* should be maintained as two different genera. Recent palynological and molecular studies provided further support that *Mahonia* and *Berberis* are two distinct genera, and established that they formed a sister group phylogenetically (ZHANG and WANG 1983; KIM and JANSEN 1994; WANG *et al.* 2001).

The basic chromosome numbers of *Berberis* and *Mahonia* have been established to be x = 14(HONG 1990). Only 6% of the 83 *Berberis* species examined showed a case of polyploidy, while the other 94% all showed a count of 2n = 2x = 28. BOTTINI *et al.* (1999) examined the meiotic metaphase of 13 *Berberis* species, in which he found that, except for two tetraploid species with 2n = 4x = 56, other species all had a diploid number of 2n = 2x = 28. He also found that the chromosome configurations at metaphase were 14II, and that there was secondary association of two bivalents in the *Berberis* species studied at meiotic metaphase. Furthermore, chromosome configurations and behaviors of the two genera at meiotic metaphase were very similar, which again indicated the close genetic relationship between them. In the present paper, the chromosome numbers of three species of *Mahonia* were shown to be 2n = 2x = 28, while also showing secondary association of two bivalents at metaphase. Thus, the results of

the present research are consistent with those of previous reports.

Mahonia and *Berberis* share many morphological characteristics, such as flowers tricycly, petals with saccate nectarines adaxially, stamens sensitive to touch, peltate stigma that is not connivent at the center, but covered by the epidermal hairs, pollen grains 4-6(-multi)-colpate, and seeds with the same ornamentation on the surface (FENG, 1998). Additionally, their cytokinesis is successive in microspore mother cells following meiosis.

Mahonia and *Berberis* are distinguished by the former having compound leaves and no acerose thorn on the branches, while the latter having simple leaves and acerose thorns on the branches. In regard to the chromosome numbers and meiotic characters, the two genera are very similar except that the *Mahonia* species that have been studied are all diploid, while 6% of *Berberis* species are polyploid. In conclusion, based on the results of the present research, we support the assertion that *Mahonia* and *Berberis* represent a sister group in phylogenetic evolution, and that they have probably evolved from a common ancestor, as demonstrated by their many common plesiomorphies**.** Taxonomically, we also support the view that *Mahonia* and *Berberis* should be treated as two distinct genera.

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