

A cytogenetic study of five species in the genus *Osmunda*

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Abstract In the present study, observation was made on chromosome morphology and behavior during meiosis of spore mother cells (SMCs) for five species in the genus *Osmunda*: *O. angustifolia* Ching, *O. japonica* Thunb., *O. vachellii* Hook., *O. banksiifolia* (Presl) Kuhn., and *O. mildei* C. Chr. The chromosome number of root tip cells of the five species is uniformly $2n=44$. Chromosome pairing and synapsis were normal during meiosis and the common configurations at metaphase I were circular bivalents in *O. angustifolia*, *O. japonica*, *O. vachellii* and *O. banksiifolia*. Trivalents and univalents were occasionally observed in *O. banksiifolia*, while univalents at metaphase I, and chromosome bridges and fragments were observed at anaphase II in *O. angustifolia*. It is suggested that translocation and inversion are responsible for the phenomenon observed. No chromosome pairing and synapsis were observed in *O. mildei* from prophase I to metaphase I, and they resulted in abnormal chromosome behavior: more than 80% of the SMCs showing lagging chromosomes and unequal segregation of chromosomes. The spores produced were almost sterile because of abnormal chromosome constitution. Based on the departure from the normal homologous chromosome pairing and synapsis, it is suggested that *Osmunda mildei* might be an interspecific hybrid.

Key words chromosome behavior, meiosis, *Osmunda*.

Osmunda L. (Osmundaceae) is an ancient fern genus that originated in the Triassic (Foster & Gifford, 1974). It comprises 15 species, of which eight are distributed in China (Li et al., 2003). Shenzhen is situated in southern subtropical region at $22^{\circ}27'$ – $22^{\circ}52'$ N and $113^{\circ}46'$ – $114^{\circ}37'$ E. Five species are recorded in this area: *O. angustifolia* Ching, *O. japonica* Thunb., *O. vachellii* Hook., *O. mildei* C. Chr. and *O. banksiifolia* (Presl) Kuhn. Among them, *O. japonica* and *O. vachellii* are widely distributed, while the other three occur only in special habitats. *Osmunda mildei*, a rare species, was discovered in Hong Kong more than 100 years ago, but was mis-identified as *O. bipinnata* L. by Hooker in 1857. However, Christensen (1904) described it as a new species, *O. mildei* C. Chr. (Li et al., 2003). It was believed to be extinct, because it had not been found in its type locality and neighboring areas until we found it on three sites in Shenzhen among shrubs. It occurred at an altitude of 200–400 m, and the total number of individuals found was no more than 10. At the same time, *O. mildei* was collected again near its type locality. A new site with only one individual was reported in Mt. Qiyun, Jiangxi Province where *O. mildei*, *O. japonica* and *O. vachellii* occurred together.

Since Guignard (1899) reported $2n=44$ for *O.*

regalis (Löve et al., 1977), the chromosome numbers or karyomorphology of 15 taxa have been recorded, all with $2n=44$ (He et al., 2006). The chromosome configuration of SMCs at metaphase I and the chromosome number of somatic cells have been used for identification of natural hybrids and artificial monoploids, and for investigation of basic chromosome numbers of homosporous ferns and the origin of ancient polyploids (Klekowskj & Baker, 1966; Klekowskj, 1973; Löve et al., 1977; Gastony & Darrow, 1983; Kawakami et al., 2003). The $2n=44$ of *O. japonica* and *O. banksiifolia* was reported from Japan (Hirabayashi, 1963; Tatuno & Yoshida, 1966, 1967; Mitui, 1968; Takei, 1988) and karyomorphology and chromosome pairing of artificial monoploids were also investigated (Takei, 1988; Kawakami et al., 1999; Kawakami et al., 2003). All these studies were focused on the chromosome configuration of diakinesis/metaphase I to analyze chromosome pairing and further to determine the chromosome number, without studying the chromosome pairing behavior at prophase I. Recently we studied the chromosome behavior of *Osmunda* at meiosis and found that no pairing of homologous chromosomes occurred at metaphase I in *O. mildei*. It was different from the abnormal meiosis of three types of SMCs in ferns, and was also different from that of SMCs of artificial monoploids of *Osmunda* (Kawakami et al., 2003). Was it because synapsis of homologous chromosomes disappeared in

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advance or no homologous chromosomes existed at all? Or was it a special meiosis like mitosis? A systematic study on cytology of *Osmunda* was carried out to explore why no pairing of homologous chromosomes occurred in SMCs of *O. mildei*. The chromosome behavior at mitotic prophase and meiotic prophase I was observed, and the studies on chromosome morphology and behavior at mitosis have been published (He et al., 2006). The goals of this study are: (1) to observe the behavior of homologous chromosomes at leptotene to diakinesis in SMCs, and (2) to reveal differences between the species in chromosome behavior and morphology by studying the chromosome configuration during meiosis in SMCs.

1 Material and Methods

Samples of the five *Osmunda* species were all collected from Shenzhen (Table 1). To obtain SMCs at a proper stage for meiotic analysis, materials were collected and observed in the first ten days of April. The meiotic process was observed through the method of cell-wall degradation using a hypotonic treatment, followed by Giemsa staining (He et al., 2006). Each slide was washed for several seconds and examined using a Leitz compound microscope.

2 Results

2.1 *Osmunda vachellii*

The chromosome number of root tip cells at mitosis was $2n=44$ (Fig. 1). At prophase I slender twisted unineme chromosomes gradually became thick, straight and short (Fig. 2). At leptotene, chromatin condensed into unineme chromosomes, which formed a tangled reticulum with chromatides easily seen (Fig. 3). At zygotene, homologous chromosomes began to pair simultaneously at several points along the chromosome length (Fig. 4). At pachytene, 22 bivalents were not easily distinguished from each other in morphology and structure, but it was easy to observe the complex of bivalents due to the synapsis

of homologous chromosomes (Fig. 5). At diplotene, it was difficult to distinguish some bivalents although the chromosomes had thickened and shortened (Fig. 6). At diakinesis (Fig. 7) and metaphase I (Fig. 8), chiasmata were present. The average chromosome configuration was 22 II (Table 2). The leptotene-zygotene lasted for 48 hours. At anaphase I, when homologous chromosomes separated, 22 chromosomes appeared in the daughter cells (Fig. 9). At telophase, when chromosomes uncoiled into chromatin, nucleoli and nuclear membranes reappeared, and dyads formed. After the first meiotic division, cell walls formed, and cytokinesis was of a successive type. At prophase II, chromatin in the daughter nuclei of each dyad condensed and coiled into chromosomes again. At metaphase II, 22 chromatids could be easily seen (Fig. 10). During telophase II, when chromosomes uncoiled into chromatin, nucleoli and nuclear membranes reappeared, and a tetragonal configuration of tetrads formed.

2.2 *Osmunda banksiifolia*

The chromosome number for root tip cells of *O. banksiifolia* was $2n=44$ (Fig. 11). The chromosome configuration at early prophase I (Fig. 12), diakinesis and metaphase I indicated that the pairing and synapsis of homologous chromosomes were normal (Figs. 13, 14). The chromosome behavior was similar to that of *O. vachellii*. During diakinesis and metaphase I, bivalents were dominant, while trivalents were occasionally observed (Fig. 14), indicating that translocations occurred between non-homologous chromosomes. The average chromosome configuration was $0.05I+21.77II+0.14III$ (Table 2), and $n=22$ was clearly shown at anaphase I (Fig. 15) and II (Fig. 16).

2.3 *Osmunda japonica*

The chromosome number for root tip cells of *O. japonica* was $2n=44$ (Fig. 17). The chromosome behavior during meiosis was similar to that of *O. vachellii*. At zygotene (Fig. 18), pairing between homologous chromosomes began simultaneously at several points along the chromosome length, and the centromeres were obvious. At diplotene, repulsion occurred between homologues of the bivalents (Fig.

Table 1 The origin of materials investigated

Species	Locality	Voucher
<i>Osmunda banksiifolia</i> (C. Presl.) Kuhn	Mt. Wutong, Shenzhen, China (深圳梧桐山)	Z. C. Chen & B. Yan (陈珍传, 闫斌) 011815
<i>O. vachellii</i> Hook.	Mt. Wutong, Shenzhen, China (深圳梧桐山)	Z. C. Chen & B. Yan (陈珍传, 闫斌) 011819
<i>O. angustifolia</i> Ching	Meishajia, Shenzhen, China (深圳梅沙尖)	B. Yan & G. D. Wang (闫斌, 王国栋) 050012
<i>O. japonica</i> Thunb.	Mt. Wutong, Shenzhen, China (深圳梧桐山)	Z. C. Chen & B. Yan (陈珍传, 闫斌) 010905
<i>O. mildei</i> C. Chr.	Mt. Tianxin, Shenzhen, China (深圳田心山)	Z. C. Chen & B. Yan (陈珍传, 闫斌) 0383

Table 2 The chromosome configuration at M1 in spore-mother cell of five *Osmunda* species

Species	Cell number	Chromosome configuration		
		I	II	III
<i>O. angustifolia</i>	22	0.18	21.91	
<i>O. japonica</i>	25		22.00	
<i>O. vachellii</i>	25		22.00	
<i>O. banksiifolia</i>	22	0.05	21.77	
<i>O. mildei</i>	24	43.83	0.08	

19), and pairing and synapsis were normal. At diakinesis and metaphase I, 22 circular bivalents were easily seen (Fig. 20), of which some were occasionally interlocked (Fig. 21). At anaphase I and anaphase II, the behaviour of chromosomes was normal, but micronuclei and chromosome fragments were occasionally observed (Fig. 22). At anaphase II, $n=22$ was easily seen (Fig. 23). Micronuclei, lagging chromosomes and chromosome fragments occurred in some SMCs, which might be associated with interlocked bivalents from prophase I to metaphase I.

2.4 *Osmunda angustifolia*

The chromosome number of somatic cells of *O. angustifolia* was $2n=44$ (Fig. 24). Chromosome paring and synapsis at leptotene, pachytene (Fig. 25), diplotene (Fig. 26), diakinesis and metaphase I were normal. At metaphase I, circular bivalents were dominant, but a small number of rod bivalents (Fig. 27) and univalents were infrequently observed, with the average chromosome configuration $0.18\text{I}+21.91\text{II}$ (Table 2). At anaphase I, when homologous chromosomes separated, micronuclei and chromosome fragments were occasionally observed (Fig. 28). At anaphase II, 22 chromosomes were clearly seen (Fig. 29), and chromosome bridges and fragments were occasionally found (Fig. 30). The spores with 1–3 micronuclei accounted for 27%, indicating abnormalities and abortion occurring during spore development of *O. angustifolia* (Figs. 31, 32).

2.5 *Osmunda mildei*

The chromosome number of root tip cells was $2n=44$ (Fig. 33). It took 48 hours for SMCs to finish prophase I and metaphase I, as in the other four species; the thread-like unineme chromosomes formed a reticulum (Fig. 34). No chromosome pairing and synapsis were observed (Figs. 35–39) during meiotic prophase I to metaphase I.

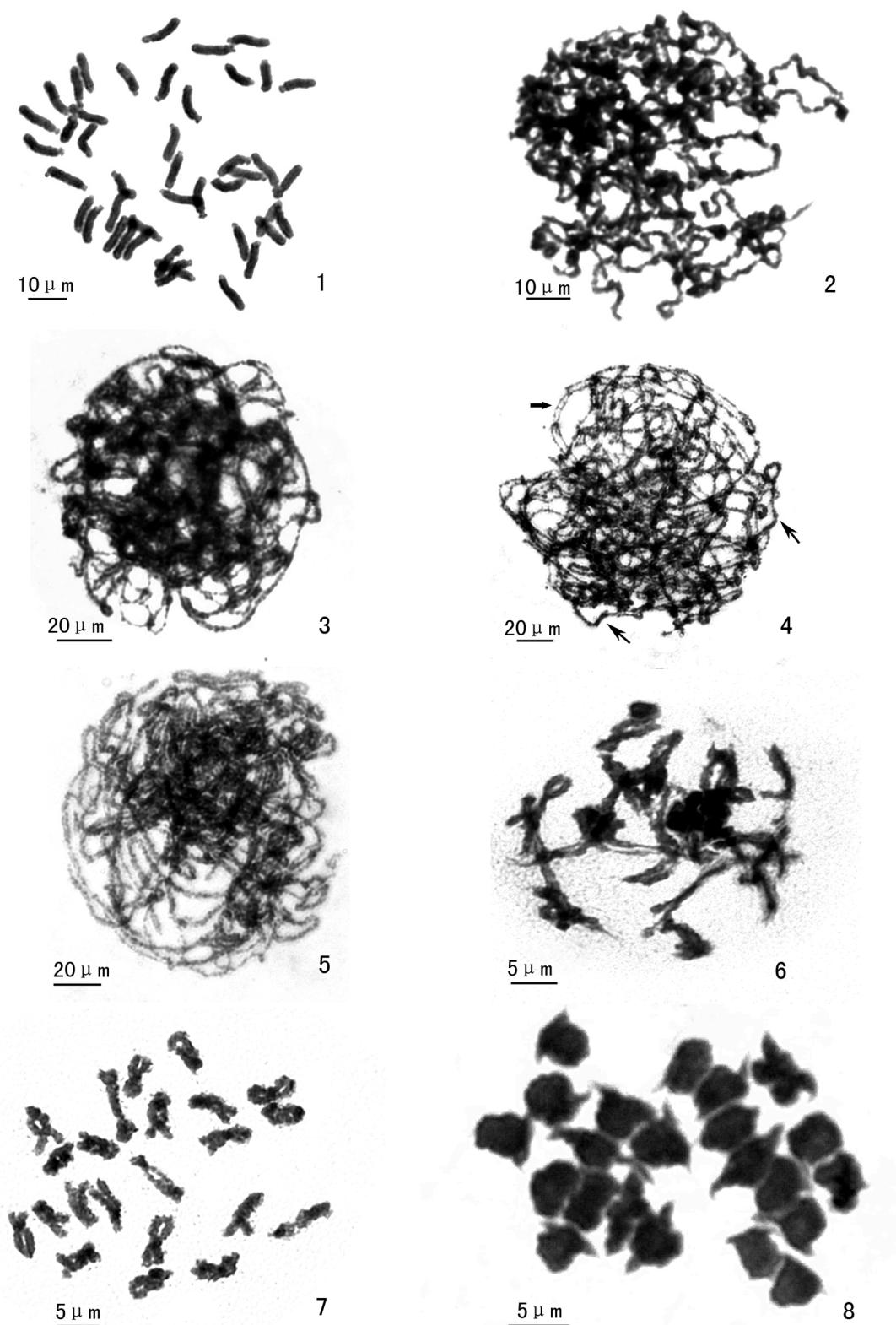
The chromosomes did not exhibit motions such as pairing, synapsis and repulsion. At metaphase I, there were usually 44 univalents, but occasionally one rod bivalent was observed (Fig. 39). The 44 univalents randomly moved to two poles, and many lagging

chromosomes, difficult to discern in number, appeared in 80% of the SMCs (Figs. 40–42). At anaphase I two daughter cells formed with unequal numbers of chromosomes, and some micronuclei were present at anaphase II. However, occasionally morphologically normal daughter cells were formed. When sister chromatids separated in meiosis II, more than 80% of cells had lagging chromosomes, chromosome bridges or fragments. Micronuclei were observed (Figs. 43, 44), and besides tetrads, polyads were also formed (Fig. 45). The spores were irregular in size and form (Fig. 46), and thus could not germinate under normal breeding conditions.

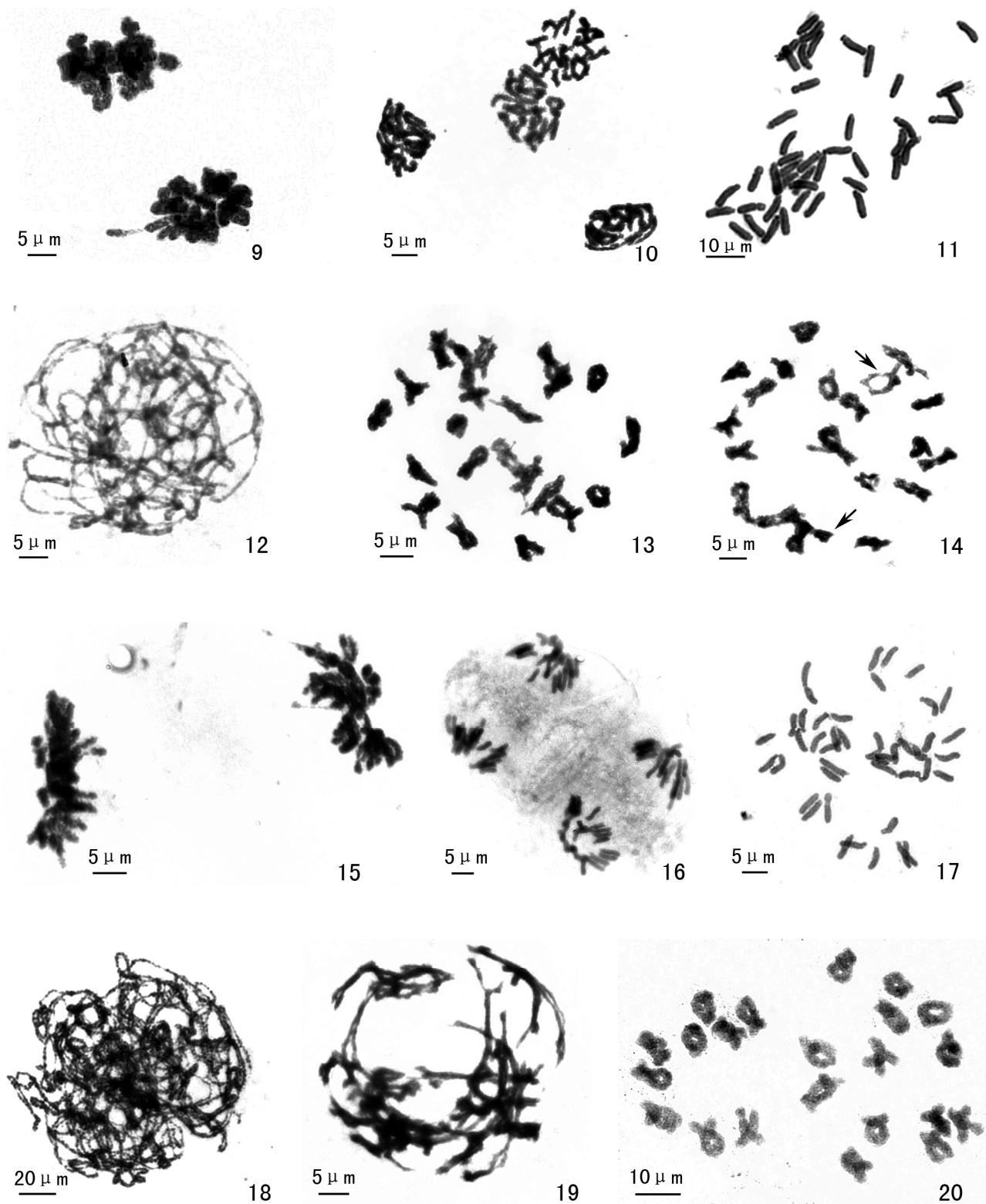
3 Discussion

The chromosome numbers of the five species were uniformly $2n=44$. This is consistent with the previous reports (Tatuno & Yoshida, 1966; Takei, 1988). Studies on chromosome morphology and behavior during meiosis of SMCs of four widespread species indicate that their meiosis was normal, except that trivalents were present in *O. banksiifolia*, possibly due to translocation. One or two bivalents sometimes completed synapsis in advance and subsequently became univalents in *O. angustifolia*, which suggests that some differences occur between the homologues. The suggestion is supported by the presence of chromosome bridges and fragments in anaphase II. In *O. mildei*, however, no chromosome pairing occurred from prophase I to metaphase I, which obviously departs from the synapsis and pairing of homologous chromosomes. It resulted in abnormal chromosome behavior: lagging chromosomes and unequal segregation of chromosomes in more than 80% of the SMCs. The resulted spores were almost sterile because of abnormal chromosome constitution.

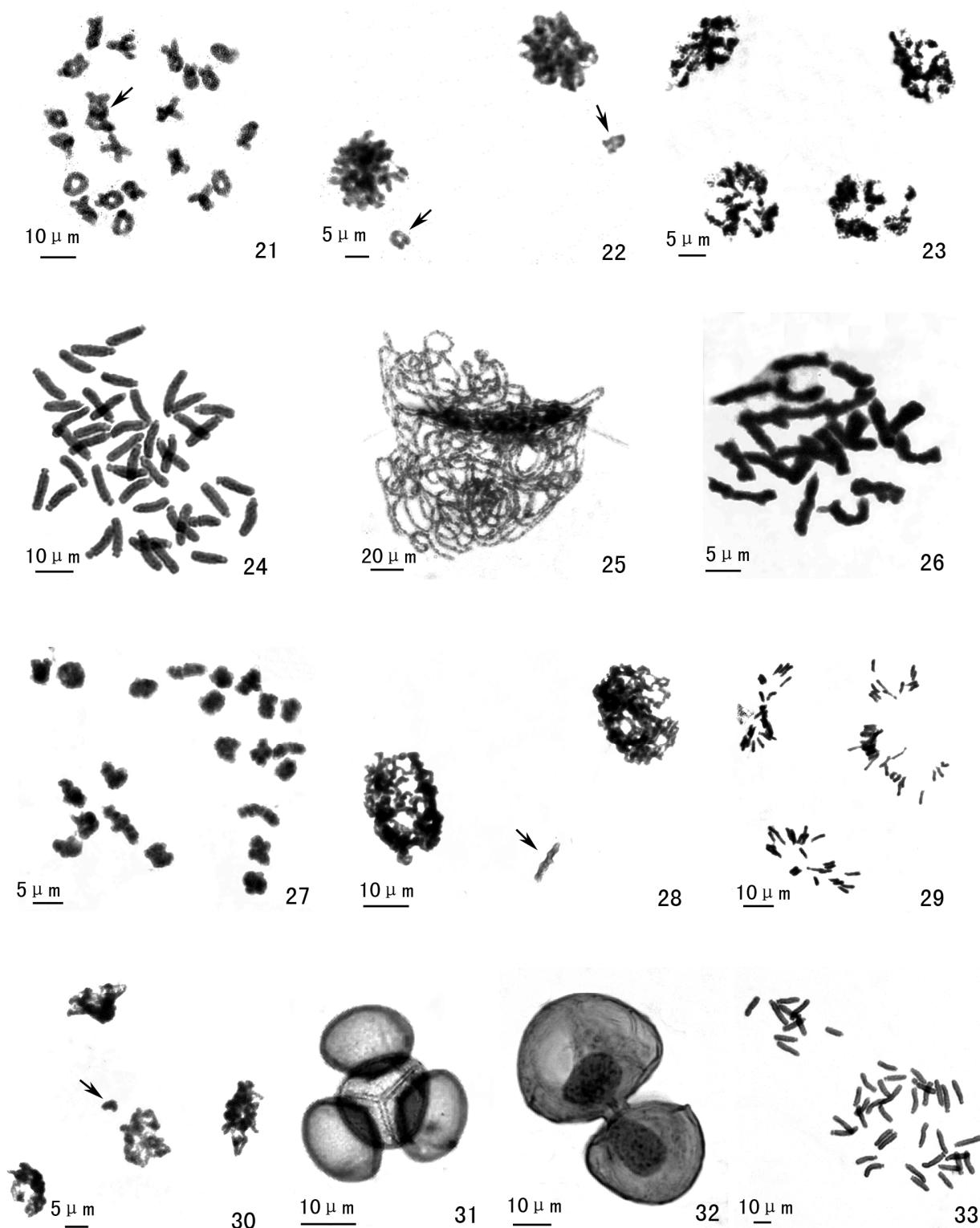
No chromosome pairing was observed in SMCs of *Asplenium aethiopicum*, where meiosis ceased when restitution nuclei formed. The SMCs later split into two unreduced spores (Braithwaite, 1964). The abnormal meiosis in *A. aethiopicum* is distinctly different from that of *O. mildei*. The observation on meiosis of SMCs of the artificial monoploid of *O. japonica* showed that meiosis took place in its SMCs, producing dyads. The meiosis was believed (Kawakami et al., 2003) to be same as that in SMCs of some ferns: the meiosis ceases in restitution nuclei, cytokinesis does not take place, while fertile dyads are produced in meiosis II (Braithwaite, 1964). In the study of Kawakami et al. (2003), tetrads were observed in some SMCs, but the chromosome behavior



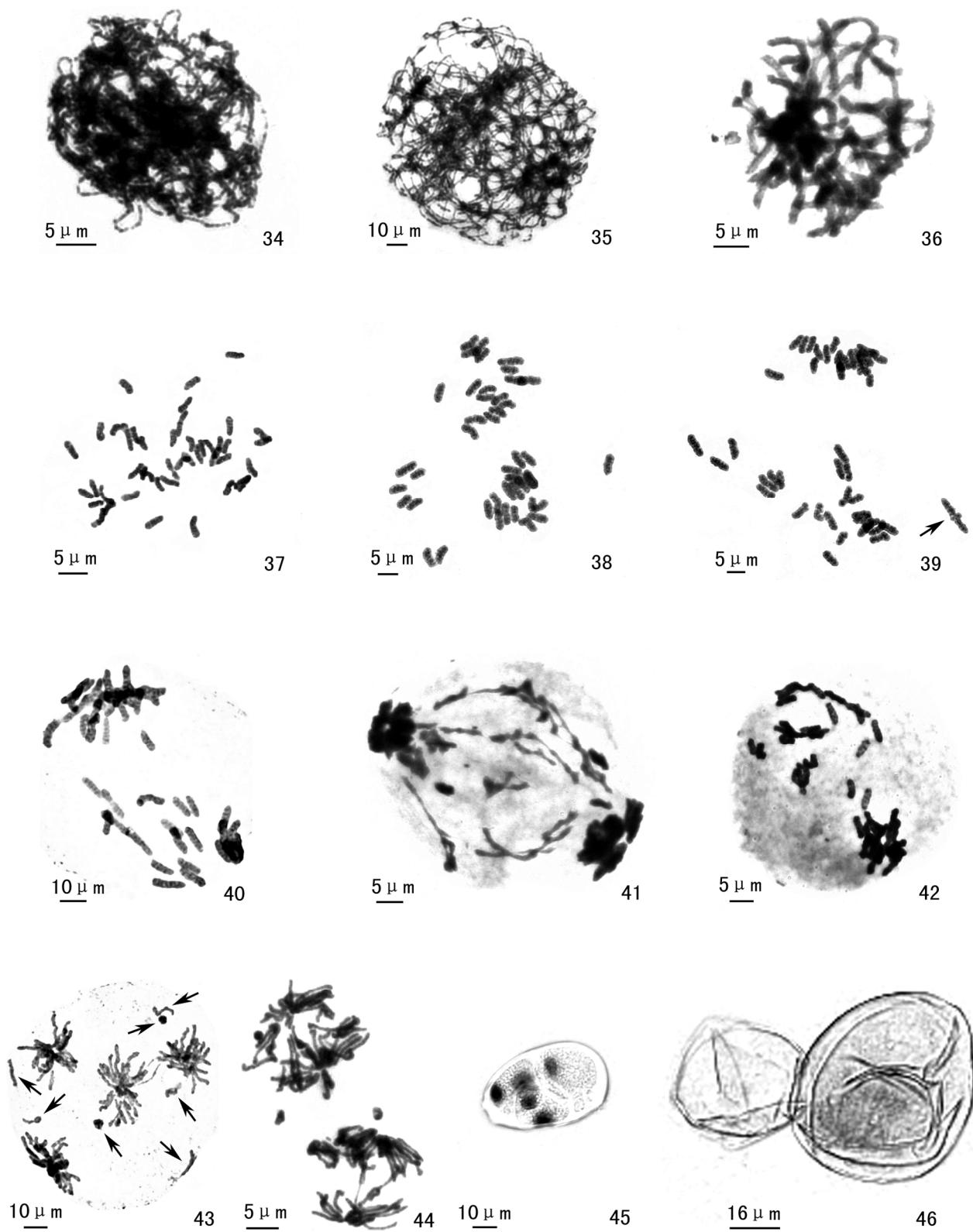
Figs. 1–8. Chromosomes of root tip cells and meiosis of SMCs in *Osmunda vachellii*. 1, 2. Mitosis of root tip cells. 3–8. Meiosis of SMCs. 3. Leptotene. 4. Zygote. Thick arrowhead indicates a bivalent which is not synapsis, and thin arrowheads indicate synaptic bivalents. 5. Pachytene. 6. Diplotene. 7. Diakinesis. 8. Metaphase I.



Figs. 9–20. Chromosomes of root tip cells and meiosis of SMCs in *Osmunda vachellii*, *O. banksiifolia*, and *O. japonica*. **9, 10.** Meiosis of SMCs in *O. vachellii*. **9.** Anaphase I. **10.** Anaphase II. **11–16.** *O. banksiifolia*. **11.** Mitosis of root tip cells. **12–16.** Meiosis of SMCs. **12.** Zygote. **13, 14.** Diakinesis. Arrowheads show trivalents. **15.** Anaphase I. **16.** Anaphase II. **17–20.** *O. japonica*. **17.** Mitosis of root tip cells. **18–20.** Meiosis of SMCs. **18.** Zygote. **19.** Early Diplotene. **20.** Diakinesis.



Figs. 21–33. Chromosomes of root tip cells and meiosis of SMCs in *Osmunda japonica*, *O. angustifolia*, and *O. mildei*. 21–23. Meiosis of SMCs in *O. japonica*. 21. Diakinesis. Arrowhead shows interlocking of two bivalents. 22. Anaphase I. Arrowheads show lagging chromosomes. 23. Anaphase II. 24–32. *O. angustifolia*. 24. Mitosis of root tip cells. 25–31. Meiosis of SMCs. 25. Pachytene. 26. Diplotene. 27. Early metaphase I. 28. Anaphase I. Arrowhead shows lagging chromosome. 29, 30. Anaphase II. Arrowhead shows lagging chromosome. 31. Tetrad. 32. Abnormal spores. 33. Mitosis of root tip cells in *O. mildei*.



Figs. 34–46. Meiosis of SMCs in *Osmunda mildei*. 34–36. Prophase I. 37–39. Metaphase I. Arrowhead indicates a rod bivalent. 40–42. Anaphase I. 43, 44. Anaphase II. Arrowhead shows lagging chromosomes. 45. Telophase II. 46. Sterile spores.

was not mentioned. The absence of chromosome pairing in SMCs of *O. mildei* at metaphase I is similar to that in the material investigated by Braithwaite (1964) and in SMCs of the artificial univalents of *O. japonica*, but the study of *O. japonica* failed to observe the prophase I. The abnormal cytogenetics is a character of interspecific hybrid F_1 , and therefore *O. mildei* is probably F_1 of a natural interspecific hybrid, which completes its life history in a particular habitat. The North American *Osmunda × ruggii* is a natural hybrid of *O. cinnamomea* and *O. regalis* (Tryon, 1940; Wagner 1974; Wagner & Wagner, 1978; Li & Haufler, 1994). *Osmunda lancea* var. *latipinnula* is a natural hybrid of *O. lancea* and *O. japonica* based on the analysis of karyotypes (Tatuno & Yoshida, 1967). Therefore, interspecific hybridization likely happens in this genus.

Osmunda mildei has sporadic distribution and coexists with *O. vachellii* and *O. japonica*. It was suggested in a previous study that *O. mildei* might be a hybrid of *O. angustifolia* and *O. japonica* based on analysis of karyotype and leaf morphology of the three taxa (He et al., 2006). The new locality of *O. mildei* in Mt. Qiyun, Jiangxi Province casts doubt on this conclusion since *O. angustifolia* is distributed only in southern subtropical region. Careful field explorations and comparative studies on morphology of the five species indicate that *O. mildei* might be a sterile interspecific hybrid between *O. japonica* and *O. vachellii*. The hypothesis is supported by allozyme analysis and chloroplast DNA sequences (unpublished data). If *O. mildei* is an interspecific hybrid, it is another example of homoploid hybrids. The origin of *O. mildei* as an interspecific hybrid may explain why few individuals were reported in the field.

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