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Chromosome Behavior During Meiosis and Development of Spore Mother Cells in the Chinese Quillwort *Isoetes sinensis* T. C. Palmer (Isoetaceae)

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ABSTRACT.—Chromosome behavior during meiosis of the tetraploid *Isoetes sinensis* was observed to be similar to that of basic diploid *Isoetes* species. This observation was consistent with the previous report that polyploid species of *Isoetes* are allopolyploids. Meiosis was generally similar in both megaspore and microspore mother cells, but differences were observed. Due to the smaller size of the microspore mother cells, during prophase I, chromosomes are not as dispersed as those in megaspore mother cells. Cytomixis was observed in all stages of meiosis in microspore mother cells, but it was not seen at any stage of meiosis in megaspore mother cells. Cytomixis, lagging chromosomes, chromosome bridges, chromosome fragments, and micronuclei, observed during meiosis in *I. sinensis*, can affect sexual reproduction, but it does not appear to be a major cause of population decline in this species. The major cause of decline is habitat degradation from human disturbance. The few remaining populations of the Chinese quillwort are fragmented and the numbers of individuals contained in these populations dwindle as a result of habitat degradation. Conservation efforts are needed to save *I. sinensis* from extinction.

The Isoetaceae is an ancient family of heterosporous lycopsids. It includes one cosmopolitan genus, *Isoetes*, which contains from 130 to 350 living species (Jermy, 1990; Hickey *et al.*, 2003). Species range in habit from evergreen aquatics to ephemeral terrestrials (Taylor and Hickey, 1992). Chromosome counts of *Isoetes* range from $2n = 2x = 20$, 22 to $2n = 12x = 132$ (Manton 1950; Löve and Löve, 1977; Kott and Britton, 1980; Hickey, 1984; Bhu and Goswami, 1990; Takamiya *et al.*, 1994). For the four Chinese species, Liu *et al.* (2002) reported $2n = 22$ counts for *Isoetes hypsophila* Handel-Mazetti, *I. taiwanensis* DeVol and *I. yunguiensis* Q. F. Wang & W. C. Taylor and $2n = 44$ for *Isoetes sinensis* T. C. Palmer. He *et al.* (2002) also reported a $2n = 44$ count for *I. sinensis*.

Chromosome behavior and pairing during meiosis are related to the phylogeny and ecology of plant populations. Synapsis, crossing over, and segregation of chromosomes during meiosis affect the evolution and adaptation of taxa. In this regard, heterosporous lycopods such as *Isoetes* have evolved a unique reproductive system worthy of study. There have been few studies of chromosome behavior during meiosis in spore mother cells of *Isoetes* except for chromosome figures in metaphase I for several Indian species by Bhu and Goswami (1990) and the Japanese taxa by Takamiya *et al.* (1996).

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Therefore, the goals of this study were to: 1) describe chromosome behavior during sporogenesis in the endangered species *I. sinensis*, 2) compare meiotic behaviors in microspore and megaspore mother cells and 3) determine if there are cytological irregularities during meiosis in *I. sinensis* that could affect its reproduction.

Materials And Methods

Samples were collected from two native *Isoetes sinensis* populations, one in Songyang County, Zhejiang Province that included two subpopulations 800 m apart and another in Xiuning County, Anhui Province. Collections were made in July 2002 and the living plants were cultivated in ponds in the Wuhan Botanical Garden, Chinese Academy of Sciences. The voucher specimens were deposited in the Wuhan Botanical Garden Herbarium, Chinese Academy of Sciences (HIB). To obtain somatic chromosome figures, young leaves from the shoot apex of six plants were pretreated in 0.1% colchicine for 3 hours before fixing. To obtain meiocytes in the proper stage for meiotic analysis, young intact sporangia were harvested from 20 different plants between 20 April and 20 May 2003. Both somatic and meiotic samples were fixed in Farmer's Solution (3:1 absolute ethyl alcohol: glacial acetic acid) for 30 minutes and then placed in refrigerated 70% alcohol for at least 30 minutes. After washing with distilled water 3 times, the sporangia were transferred to a 30 mmol/L (pH 4.5) citric acid buffer containing a mixed enzyme solution of 1.5% cellulase "Onozuka" Rs (Yakult Honsha Co. Ltd, Tokyo, Japan) and 1.5% pectolyase Y23 (Sigma P3026) at 34°C for one hour. The digested tissue was then washed 3 times in distilled water for a total of 30 minutes and transferred to fresh Farmer's Solution for 30 minutes. Sporangia were opened and meiocytes dispersed in 2–3 drops of Carnoy's fluid on a microscope slide. The slide was gently flamed until dry and then stained with Giemsa at pH 7.1 for 30 minutes. Each slide was washed for several seconds and examined for figures using a Leitz compound microscope.

Results

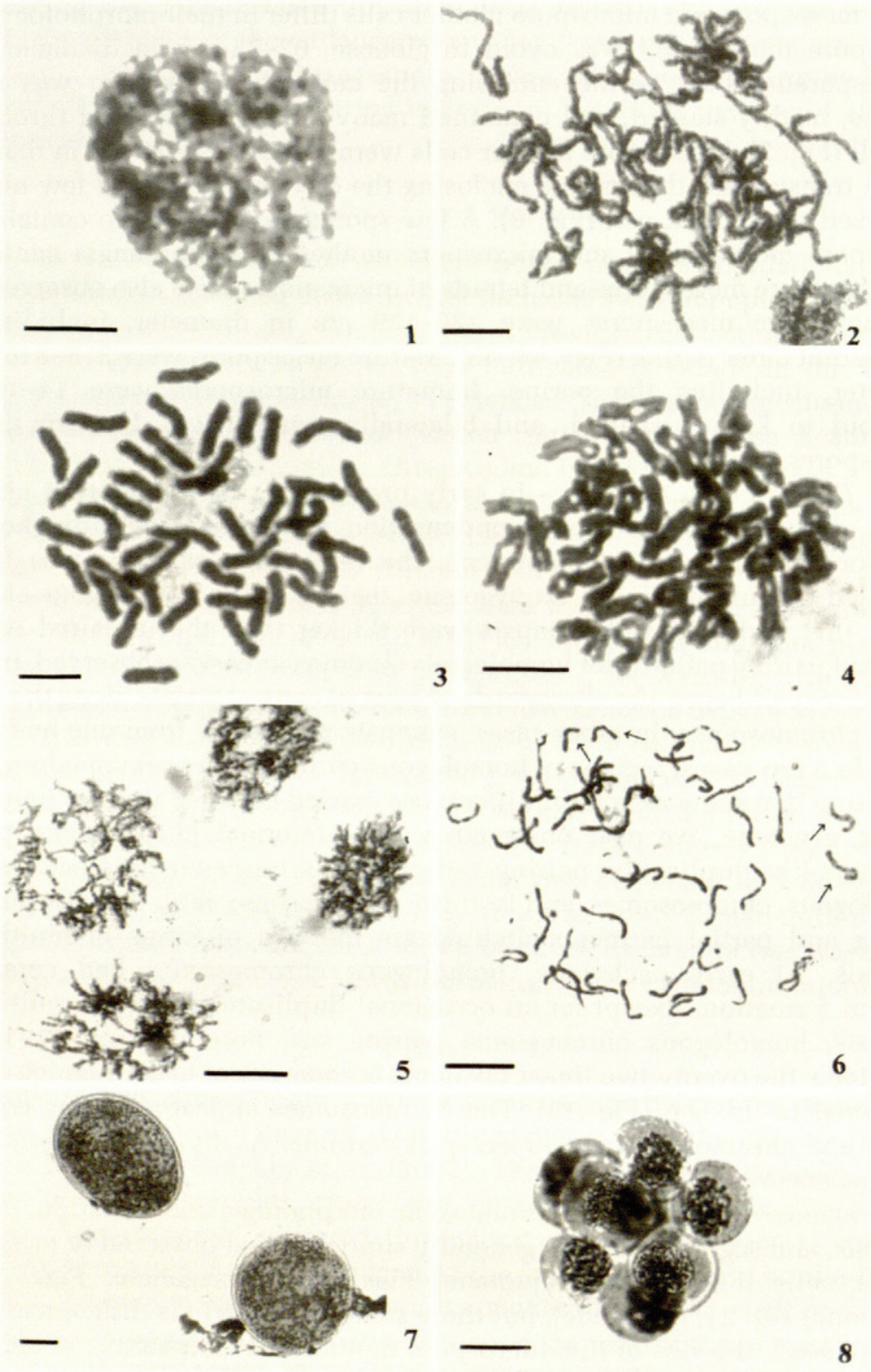
Somatic chromosome counts of *Isoetes sinensis* from both of the populations sampled were uniformly $2n = 44$. This is consistent with the previous counts by He *et al.* (2002) and Liu *et al.* (2002). The interphase nucleus in *Isoetes sinensis* is of the complex type, with heterochromatic fragments forming irregular chromocenters scattered throughout the nucleus (Fig. 1) and prophase chromosomes (Fig. 2) belonged to the interstitial type, i.e., the heterochromatic fragments and euchromatic fragments distributed with no obvious boundaries (Tanaka 1971, 1977). In metaphase, the ratio of the longest chromosome to the shortest was 1.35:1 and chromosome lengths ranged from 3.7–5.0 μm (Fig. 3). At anaphase, the centromere of each chromosome divided and the sister chromatids moved to opposite poles (Fig. 4). The mitotic divisions in a sporangium that proceeded meiosis were nearly synchronous, and the large and densely stained mitotic nuclei were obvious in the sporogenous tissue (Figs. 5–6).

The megaspore and microspore mother cells differ in their morphology. Each megaspore mother cell was ovoid to globose, 62–75 μm in diameter, with a transparent, thick perine enclosing the cell. The cytoplasm was dense, viscous, readily stained, and contained many nucleoli dispersed throughout the cell (Fig. 7). Microspore mother cells were globose, 10–12 μm in diameter, with a transparent, thin perine enclosing the cell and contained few nucleoli dispersed throughout cell (Fig. 8). A few sporangia appeared to contain both megaspore mother cells and microspore mother cells. Sporangia containing both immature megaspores and tetrads of microspores were also observed (Fig. 45). Immature megaspores were 126–129 μm in diameter, including the transparent outer perine (Figs. 44–45). Mature megaspores were 313–316 μm in diameter, including the perine. Immature microspores were 14–16 μm, ellipsoid to kidney shaped, and bilaterally symmetrical. Uniform mature microspores were 26–28 μm.

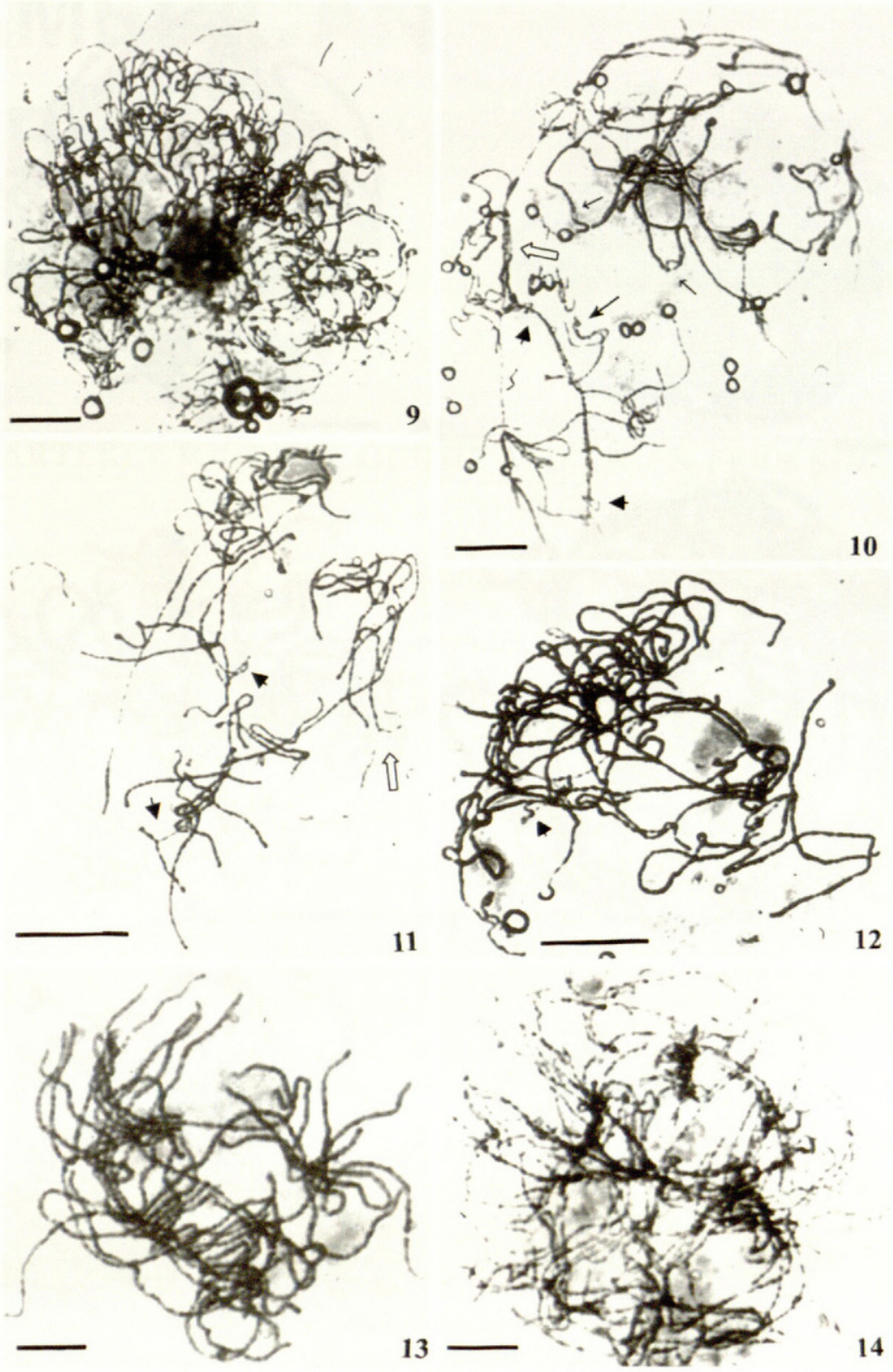
The First Meiotic Division.—In early prophase I, the filamentous chromosomes, derived from chromatin condensation, were dispersed throughout the megaspore mother cell. At leptotene, the thread-like chromosomes formed a tangled reticulum (Fig. 9). At zygotene, regions of the homologous chromosomes that had achieved synapsis were thicker than the unpaired regions. Different pairing patterns for homologous chromosomes were observed. In some cases, synapsis began simultaneously at several contact points along the length of the chromosomes. In other cases, synapsis proceeded from one end to the other. In a few cases, regions of homologous chromosomes remained unpaired indicating that some structural differences existed between the two homologs. During zygotene, we also observed a few abnormal chromosome pairing behaviors like duplication pairing and partial partner switching between two homologous chromosomes and a third chromosome (Fig. 10). Duplication pairing and partial partner switching are the two of forms of multivalent synapsis. At early pachytene, homologous chromosomes had completed bivalent formation. Except for an occasional duplicate pairing or multivalent synapsis, homologous chromosome pairing was normal (Figs. 11–12). At pachytene, the twenty-two linear bivalents became easier to distinguish as they continued to thicken (Fig. 13). The chromosomes appeared nearly equal in length and chromomeres were clearly discernible. At diplotene, chromosome chiasma occurred (Fig. 14).

During microsporogenesis, chromosome morphology, condensation, pairing, synapsis, and segregation were generally similar to that observed in megaspore mother cells (Figs. 15–16 leptotene; Figs. 17–18, zygotene; Figs. 19–20, pachytene; Fig. 21, diplotene), but there some were obvious differences. First, at prophase I, the size of the microspore mother cell constrains the spread of condensing chromosomes and hence chromosomes are more conjested than those in the megaspore mother cell (cf. Figs. 9–14 with Figs. 15–20). Second, cytomixis occurred throughout microsporogenesis (Figs. 22–26), whereas it was absent during megasporogenesis.

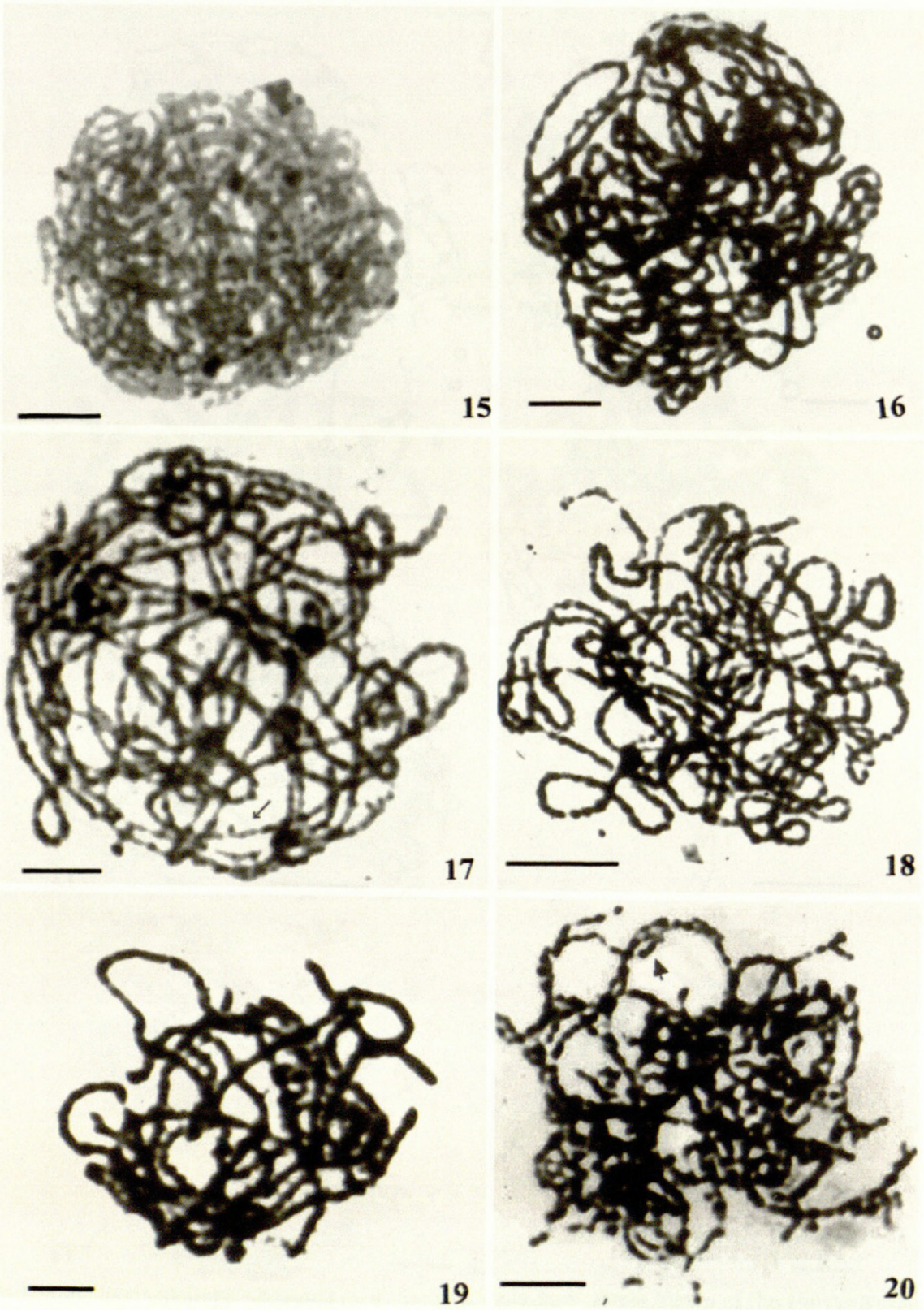
During diakinesis and metaphase I, bivalents were observed in megaspore mother cells and microspore mother cells (Figs. 27–28). Univalents or



FIGS. 1–8. Mitosis in *Isoetes sinensis*. 1–4. Somatic cells from young leaves of the shoot apex of *Isoetes sinensis*. 1. Interphase, showing heterochromatic fragments forming irregular chromocenters scattered throughout the nucleus. 2. Late prophase, showing chromosomes of interstitial type. 3. Metaphase. 4. Early anaphase, with centromere of each chromosome divided. 5–6. Chromosome figures of mitosis preceding meiosis. 5. Prophase (microspores), showing nearly synchronous mitotic divisions in a sporangium preceeding meiosis. 6. Early metaphase (microspores), showing homologous chromosomes (arrows). 7. Megaspore mother cells. 8. Microspore mother cells. Scale bars in Figs. 1 & 3 = 5 μm; Figs. 2, 4, 6 & 8 = 10 μm; Figs. 5, 7 = 20 μm.



FIGS. 9–14. Megasporogenesis in *Isoetes sinensis*. 9. Leptotene, showing the thread-like chromosomes. 10. Zygotene, showing a partial partner switching (hollow arrow), acrosyndesis, i.e. chromosomes pairing end-to-end (long arrow), unpaired region (short arrow), and slight structural differences in homologous chromosome (arrow heads). 11–12. Early pachytene. 11. Multivalent synapsis (hollow arrow) and partial duplicate pairing (arrow heads). 12. Partial duplicate pairing (arrow heads). 13. Pachytene, showing the linear bivalents. 14. Diplotene, showing chiasma. Scale bars in Figs. 10 & 14 = 5 μm; Figs. 9 & 11–13 = 10 μm.



FIGS. 15–20. Microsporogenesis in *Isoetes sinensis*. 15. Leptotene. 16. Late Leptotene. 17–18. Zygotene, showing an unpaired chromosome region (arrow). 19–20. Pachytene. 19. Early Pachytene. 20. Later Pachytene, showing partial duplicate pairing (arrow head). Scale bars in Figs. 15–17 & 19–20 = 5 μm; Fig. 18 = 10 μm.

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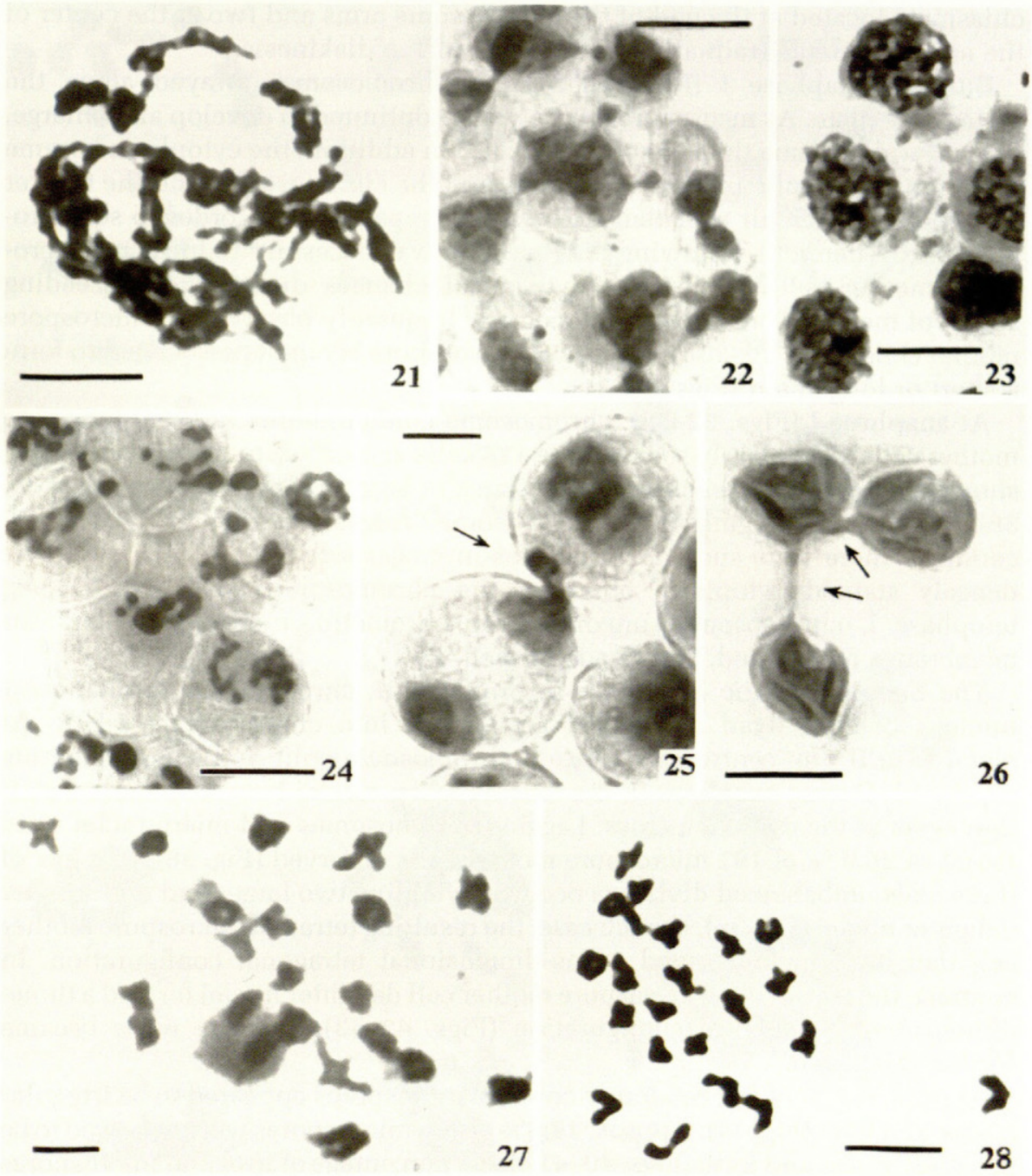


FIGS. 21–28. Microsporogenesis and Megasporogenesis in *Isoetes sinensis*. 21. Late Diplotene. Usually the processes of microsporogenesis in *Isoetes sinensis* from Leptotene to Diplotene are similar to that observed in megasporogenesis. 22–26. Cytomixis of microspore mother cells in meiosis. 22. Cytomixis at Leptotene. 23–24. Cytomixis at Metaphase I. 25. Cytomixis at Telophase I (arrow). 26. Cytomixis at tetrad stage (arrows). 27. Diakinesis in megaspore mother cells. 28. Diakinesis in microspore mother cells. Scale bars in Figs. 27 = 5 μm; Figs. 21, 25 & 28 = 10 μm; Figs. 22–24 & 26 = 20 μm.

multivalents were not seen. In microspore mother cells, secondary synapsis was observed. The configurations formed by these synapses were dependent on the number and position of the chiasmata present in the bivalents. For example, Fig. 28 shows a rhombic bivalent configuration created by two

chiasmata located at the end of the chromosome arms and two at the center of the arms. Nucleoli gradually disappeared during diakinesis.

During metaphase I (Figs. 29–31), all chromosomes arrayed along the metaphase plate. As megaspore mother cells continued to develop and enlarge, the cell wall became thicker and more rigid. In addition, the cytoplasm became filled with metabolic products that obscured the chromosomes and the thicker wall made it difficult to flatten and spread preparations in order to see chromosomes. Therefore, following diakinesis, it was necessary to focus on microspore mother cells to describe cytological changes during the succeeding stages of meiosis. Pairing of bivalents was frequently observed in microspore mother cells (Figs. 29–30). Sometimes two or more bivalents associated to form a short or long chain (Figs. 30–31).

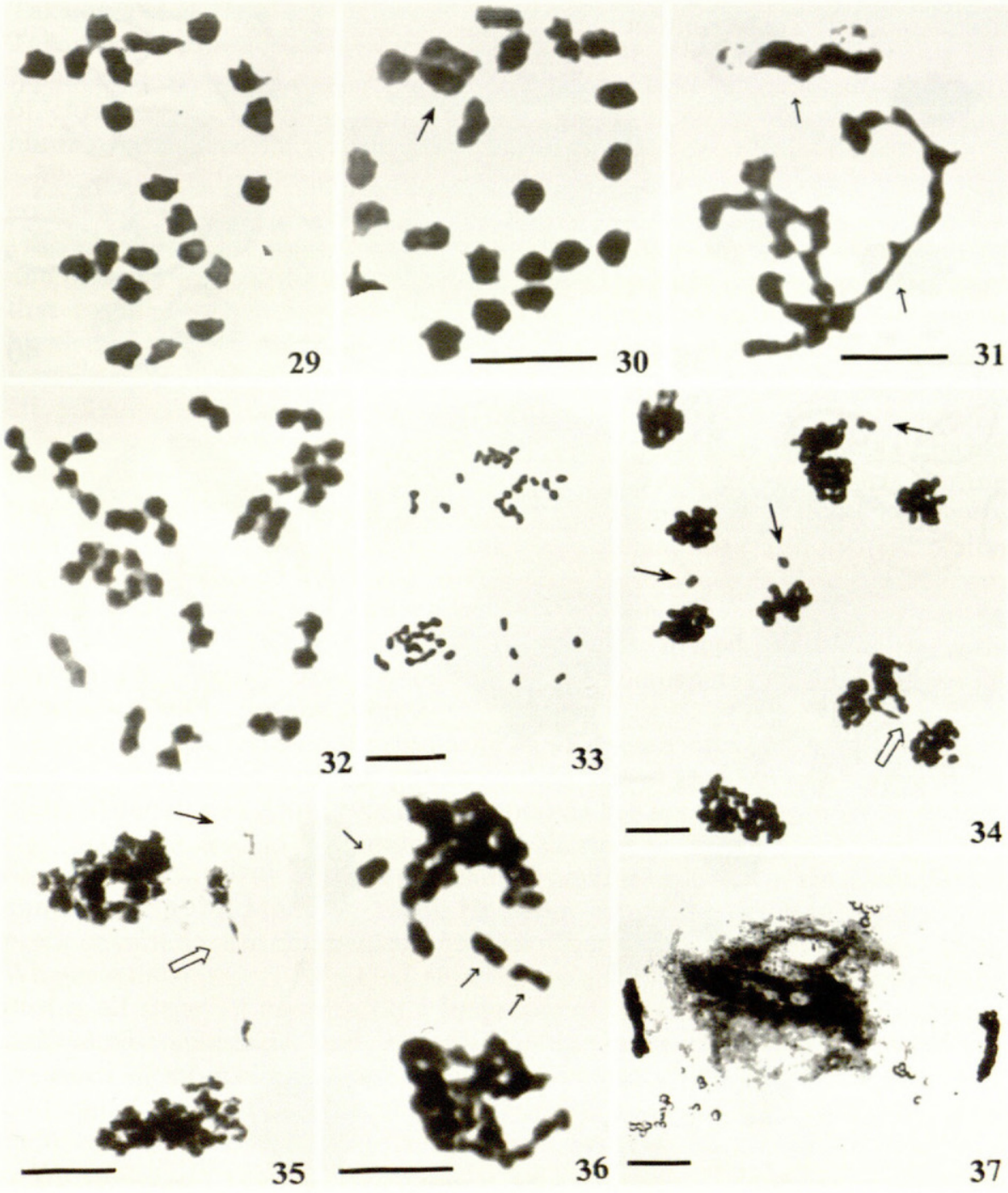
At anaphase I (Figs. 32–33), chromosome configurations in the microspore mother cells were clearly seen. Of the 118 cells evaluated in anaphase I, 24.4% showed abnormalities such as the presence of lagging chromosomes (Figs. 34, 36), chromosome fragments, or chromosome bridges (Figs. 34–35). It was uncertain if there were such abnormalities in megaspore mother cells since the densely stained cytoplasm obscured the chromosomes (Fig. 37). During telophase I, chromosomes uncoiled into chromatin, nucleoli and nuclear membranes reappeared, and dyads formed.

The Second Meiotic Division.—In prophase II, chromatin in the daughter nucleus of each dyad condensed and coiled into chromosomes again. At metaphase II, the centromere of each chromosome split and the chromatids separated. During anaphase II, the four daughter nuclei became arranged as if they were at the ends of a cross. Lagging chromosomes and micronuclei were found in 26.9 % of 191 microspore mother cells observed (Fig. 38). In a few of these cells, unbalanced divisions occurred yielding two larger and two smaller daughter nuclei (Fig. 39). In such case, the resulting tetrad of microspore mother cell daughter nuclei formed a one-dimensional tetragonal configuration. In contrast, the tetrad of the megaspore mother cell daughter nuclei formed a three-dimensional tetrahedral configuration (Figs. 42–43), and the walls became further thickened.

Approx. 8.4 % of 152 randomly counted megaspores appeared to be irregular in size and form (Fig. 44). Approx. 14.8% of 266 microspores were observed to be irregular in size and form (Figs. 40–41). This percentage of irregular microspores was considerably lower than the 24.4% incidence of abnormal chromosomal behavior noted in microspore mother cells during anaphase I (Figs. 34–36).

Discussion

Seven species of *Isoetes* are currently recognized in East Asia (Liu *et al.*, 2002, Takamiya *et al.*, 1997). These include four basic diploid species ($2n = 22$) *I. asiatica* (Makino) Makino, *I. hypsophila*, *I. taiwanensis* and *I. yunguiensis* and three polyploid species *I. sinensis* ($2n = 44$), *I. japonica* A. Braun ($2n = 66$), and *I. pseudojaponica* M. Takamiya, Mitsu Watan. & K. Ono ($2n = 88$). Chung and Choi (1986), described *I. coreana* Y. H. Chung & H. K. Choi ($2n = 66$), but



FIGS. 29–37. Microsporogenesis and Megasporogenesis in *Isoetes sinensis*. 29–31. Metaphase I in microspore mother cells. 29. 22 bivalents. 30–31. Secondary synapsis (arrows). 32–37. Anaphase in microspore mother cells. 32. Early anaphase I, showing sister chromatids together only at their centromeres. 33–37. Anaphase I, 33. Chromosomes at poles. 34. Mother cells, showing a chromosome bridge (hollow arrow) and lagging chromosomes (arrows). 35. Chromosome bridge (hollow arrow) and chromosome fragment (arrow). 36. Lagging chromosomes (arrows). 37. Anaphase I in megaspore mother cells. Scale bars in Fig. 32 = 5 μm; Figs. 29–31, 33 & 35–37 = 10 μm; Fig. 34 = 20 μm.