ACTA BIOLOGICA CRACOVIENSIA Series Botanica 45/2: 175–182, 2003

MICROTUBULE PATTERNS AND ORGANELLES DURING MICROSPOROGENESIS IN APOMICTIC *CHONDRILLA JUNCEA* L.

MARIA KOŚCIŃSKA-PAJĄK[1*] AND JÓZEF BEDNARA[2]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, ul. Grodzka 52, 31-044 Cracow, Poland*
[2]*Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University, ul. Akademicka 19, 20-033 Lublin, Poland*

Received April 6, 2003; revision accepted July 10, 2003

This study investigated patterns of cytoskeletal organization during microsporogenesis in *Chondrilla juncea* L., an autonomous apomict with a triploid chromosome number (2n = 15). The distribution of microtubules and organelles is not typical. The microtubules do not form a normal phragmoplast and consequently the organelle equatorial plate observed in many taxons is not present. The organelles are dispersed randomly in both the central and peripheral parts of the cytoplasm.

Key words: *Chondrilla juncea* L., immunofluorescence, microsporogenesis, phragmoplast, microtubular cytoskeleton, organelle aggregation.

INTRODUCTION

Microtubules are highly dynamic structures that play a major role in cell motility, organelle movement, cell division and cell wall synthesis. The cytoskeleton plays a fundamental role throughout meiosis, forming spindles, the phragmoplast and microtubular network in the cortical cytoplasm as well as on the surface of the nuclear envelope (Hogan, 1987; Brown and Lemmon, 1988; 1996; Van Lammeren et al., 1985; 1989; Cai and Cresti, 1999).

Two basic types of meiotic cytokinesis are generally recognized (Periasamy and Swamy, 1959; Sampson, 1969). One is successive cytokinesis, with cell plates forming after each round of meiosis, which is typical for most monocotyledons, Bryophyta, Pteridophyta and Gymnospermae. This kind of cytokinesis resembles vegetative meristem cell division in that cell plates form in association with centrifugally expanding phragmoplasts after each round of nuclear division (Brown and Lemmon, 1996). The other is simultaneous cytokinesis, in which infurrowing of the cytoplasm in the division planes achieves quadripartitioning of the coenocyte after meiosis (Brown and Lemmon, 1991a). Usually an equatorial plate of organelles is formed during such cytokinesis (Rodkiewicz et al., 1989) between telophase I and II nuclei.

It is well known that the planes of cell division in sporogenesis and microsporogenesis depend on the deposition of nuclei, which define cytoplasmic domains via a radial system of microtubules (Brown and Lemmon, 1991a,b, 1992, 1996).

Chondrilla juncea belongs to a group of plants with simultaneous-type cytokinesis. As the activity of the cytoskeletal apparatus in apomictic plants is poorly understood, we decided to determine the activity of the cytoskeleton apparatus of *C. juncea*, an autonomous apomict with a triploid chromosome number (2n = 15).

Previous studies noted disturbances of meiosis in anthers. In some meiocytes in metaphase I the

* e-mail: m.pajak@iphils.uj.edu.pl

PL ISSN 0001-5296

chromosomes were scattered along the spindle and subsequently surrounded by the common nuclear membrane. The second division gave rise to dyads instead of tetrads. In the other meiocytes the first meiotic division continued to the end, but disturbances in anaphase I and the subsequent second division gave rise to tetrads with cells of unequal size. In both types of loculi, monads, triads, pentads and other configurations of microspores were present (Kościńska-Pająk, 1996, 2000).

In this paper we report on microtubular configurations in successive stages of meiosis and on the development of two forms of phragmoplasts during telophase in *C. juncea*.

MATERIALS AND METHODS

The study used plants of *C. juncea* growing on an experimental field in Modlnica. Anthers in different stages of development were fixed for 4 h in a freshly prepared solution of 4% paraformaldehyde and 0.25% glutaraldehyde in phosphate buffer saline (PBS). After fixation the anthers were rinsed three times with PBS and dehydrated in a graded ethanol series. Then the plant material was infiltrated with polyethylene glycol (PEG) according to van Lammeren et al. (1985). After polymerization of the PEG, 2 µm thick sections were prepared, rinsed (3 ×) for 5 min in PBS, treated for 15 min with 0.1 M NH_4Cl, and blocked with 0.1% bovine serum albumin (BSA) prior to the application of antisera. For immunocytochemistry the samples were first incubated in a humid chamber for 90 min at 37˚C with monoclonal antimouse-β-tubulin (Sigma-Aldrich Co.) diluted 300 times with 0.1% BSA in PBS. After 3 rinses with 0.1% BSA in PBS the sections were incubated overnight at 4˚C with secondary antibody conjugated with FITC (Sigma-Aldrich Co.) diluted 200 times with blocking buffer. The slides were rinsed in PBS, and the nuclei stained with 4'6'-diamino 2 phenylindole (DAPI). Callose and cellulose were identified with aniline blue and calcofluor white solution.

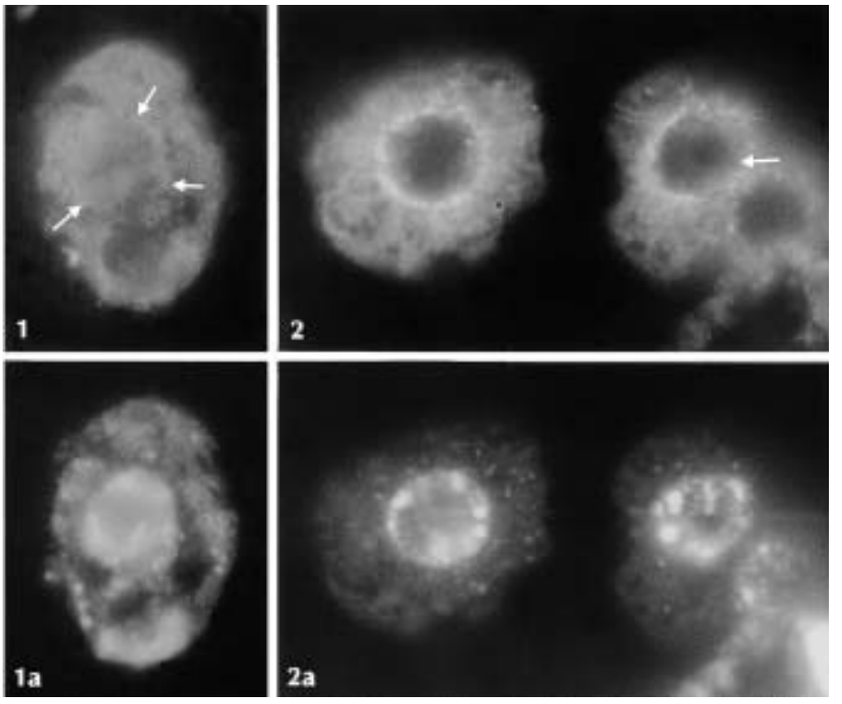
Fluorescence was observed with a Nikon Eclipse E 400 epifluorescence microscope. Photographs were taken on Kodak T-max film, ASA 400.

RESULTS

The anthers of *C. juncea* are tetrasporangiate with a secretory-type tapetum. In young archesporial cells the spherical nuclei are situated in dense cytoplasm. The microtubules cluster around the nucleus and close to the cell wall. DAPI reagent disclosed the DNA in the nucleus and nucleoids of cell organelles. The organelles were scattered randomly throughout both the central and peripheral parts of the cytoplasm (Figs. 1, 1a). A densely structured nucleus with nucleolus was visible in the central part of the cell.

During prophase I, short perinuclear microtubules became more prominent (Fig. 2). At that time the chromocentric parts of chromosomes appeared close to the nucleus envelope (Fig. 2a). In some pollen chambers, both mononuclear and binuclear microsporocytes were observed (Figs. 2, 2a). At zygotene the number of cytoplasmic microtubules increased. Significantly, they were arranged in a network stretching from the nucleus surface to the cell wall, but with a tendency to form a radial arrangement. At that stage the microtubular network was asymmetric, which might be connected with the transition of the nucleus from the central part of the cell towards the cell wall (Figs. 3, 3a). At a later stage of prophase I the nucleus moved from the cell periphery towards the meiocyte center. The microtubules of the peripheral parts of the cytoplasm depolymerized, and only short pieces were visible on the nuclei surface (Figs. 4, 5). After DAPI treatment, condensed chromosomes and loose aggregations of organelles were visible close to the nuclear envelope. The lateral part of the cell was almost without organelles (Figs. 4a, 5a). At the transition from diakinesis to prometaphase I the nuclear membrane disappeared, and microtubules radiating from the polar center to the chromosomes could be seen (Figs. 6,7). DAPI enabled us to confirm that organelles were not present in the vicinity of chromosomes (Figs. 6a, 7a). At an early stage of telophase I, the remnants of the spindle were visible both in the polar areas and between the formed nuclei (Fig. 8). In these cells the nucleoids of organelles were scattered around the chromosomes and along the polar microtubules of the spindle (Fig. 8a).

At telophase, two forms of phragmoplasts appear in *C. juncea* meiocytes. In one case a large phragmoplast with numerous microtubules was initiated in the perinuclear zone of a sister nucleus at a cell pole, and expanded towards the equatorial plane of the sporocyte. In this type of phragmoplast the microtubules occupied the entire cell width (Figs. 9, 10). The other type of phragmoplast was narrower, and was composed of a smaller number of microtubules. It seems that in this phragmoplast the bundles of microtubules originate from the karyokinetic spindle (Figs. 11, 12).



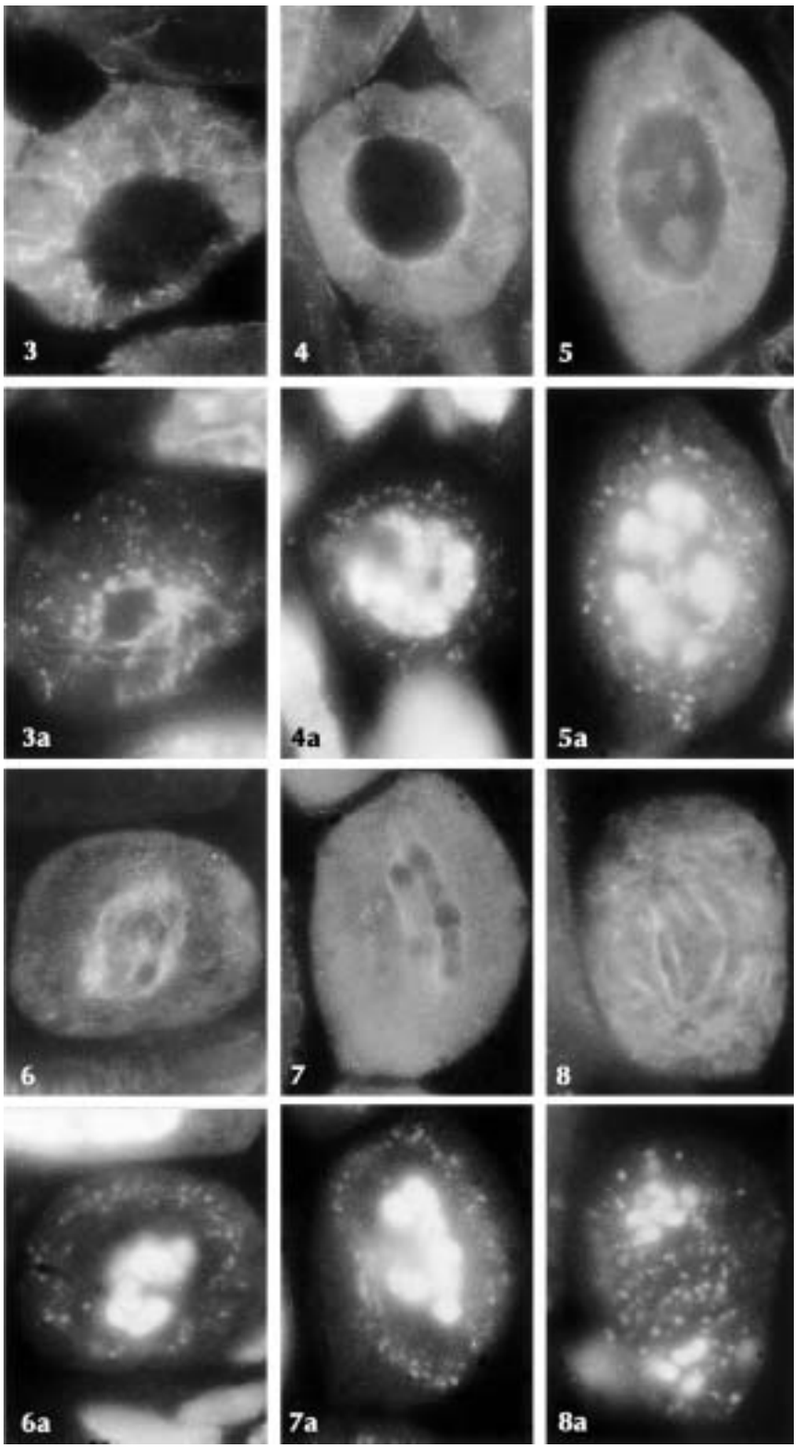
Figs. 1–2. *Chondrilla juncea* L. Early prophase I. **Fig. 1.** Short cytoplasmic microtubules visible near the spherical nucleus (arrows). **Fig. 1a.** Nucleoids of cell organelles scattered in the cytoplasm, visualized by DAPI staining. **Fig. 2.** Radiating arrangement of microtubules in mononucleate and binucleate meiocytes (arrow); denser concentration of microtubules around the nuclei, less dense in cortical cytoplasm. **Fig. 2a.** Nuclei after DAPI staining. All figures × 1500.

In numerous PMCs, the congression of chromosomes in the metaphase plate was irregular, and some chromosomes were situated outside the plate or even outside the spindle. Single lagging chromosomes or small groups of chromosomes (Fig. 9a) were sometimes surrounded by the nuclear membrane, giving rise to microcytes. DAPI showed that nucleoids of organelles were scattered throughout the cytoplasm in the middle plane of the cell as well as around the sister nuclei at telophase I (Figs. 10a, 11a, 12a). In cells in which a conspicuous large microtubular cytoskeleton was formed, narrowing at the equatorial plane was observed, suggesting cell wall formation as in successive cytokinesis. The disappearance of the peripheral microtubules caused the previously wide phragmoplast to narrow. Simultaneously, the depression of cytoplasm increased (Figs. 13, 13a, 14). In such cells the second meiotic division was omitted and dyads were formed (Fig. 15).

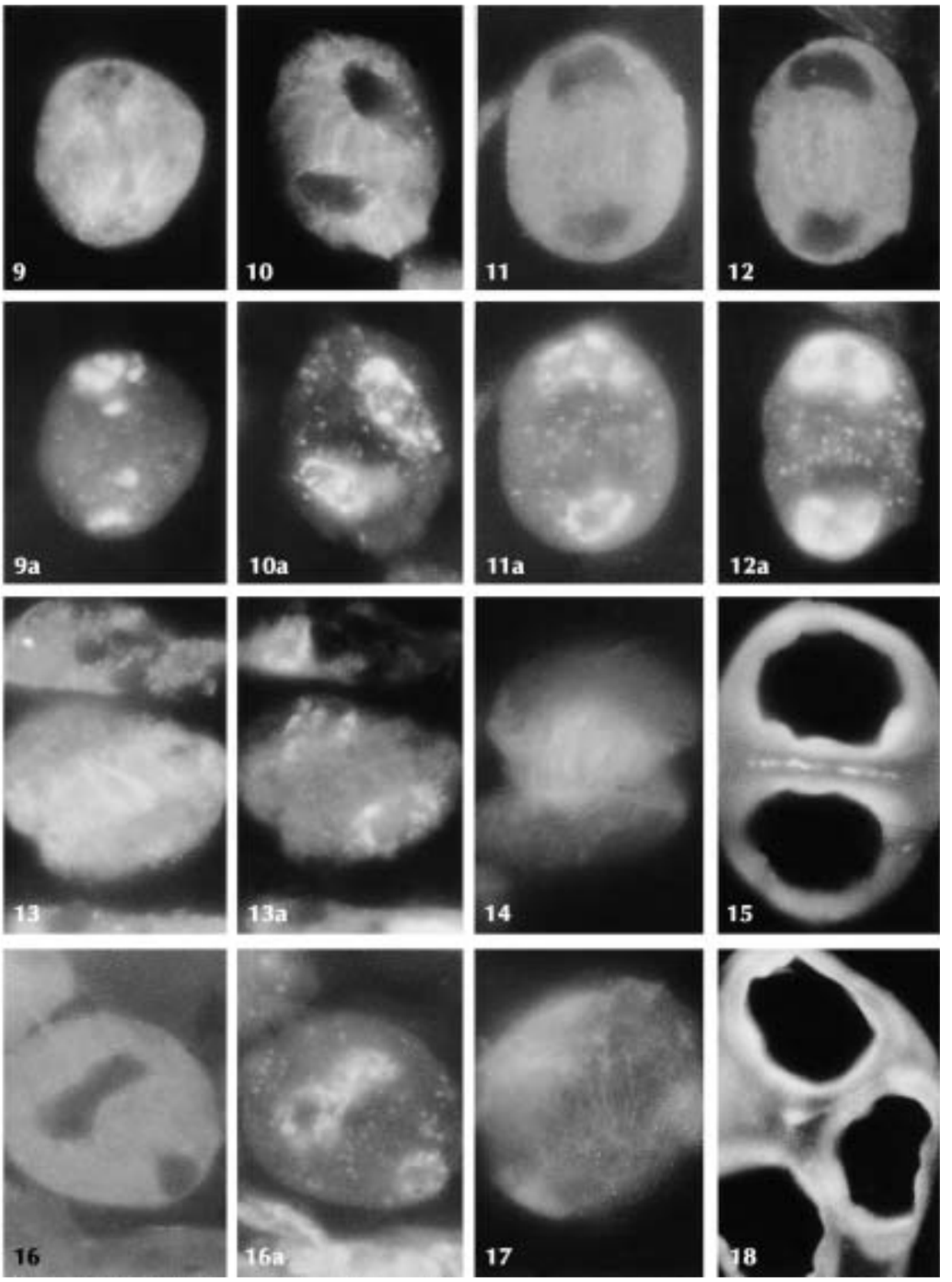
In some pollen chambers a small number of cells had cytoskeletons without phragmoplasts (Figs. 16, 17) and organelles scattered throughout the cytoplasm (Fig. 16a). In such cells no telophase organelle plate or cell wall formed after the first meiotic division. The chromosomes were divided into four groups, and coenocytic cells with four nuclei were formed. The newly established phragmoplast was situated between the nuclei, and cell walls were formed between the four microspores (Fig. 18).

Microtubules were also observed in the tapetal cells from the beginning of meiosis. They were similar to the cortical network and occurred more frequently near the cell wall adjoining the middle layer of the anther. Microtubules entered the tapetal cell cytoplasm and radiated to the cell nuclei and towards the part of the cell adjacent to the pollen chamber. The microtubules radiated as single units, and continued to be arranged in a crisscross pattern or were organized into short bundles (Fig. 19). Nuclei at interphase stage were visible after DAPI treatment. They did not separate at a great distance and formed pairs (Fig. 19a). In some tapetal cells, instead of a microtubular cytoskeleton only small aggregations of microtubules between pairs of nuclei were observed (Fig. 20). The structure and volume of these nuclei suggested the endomitotic or post-telophase stage (Fig. 20a).

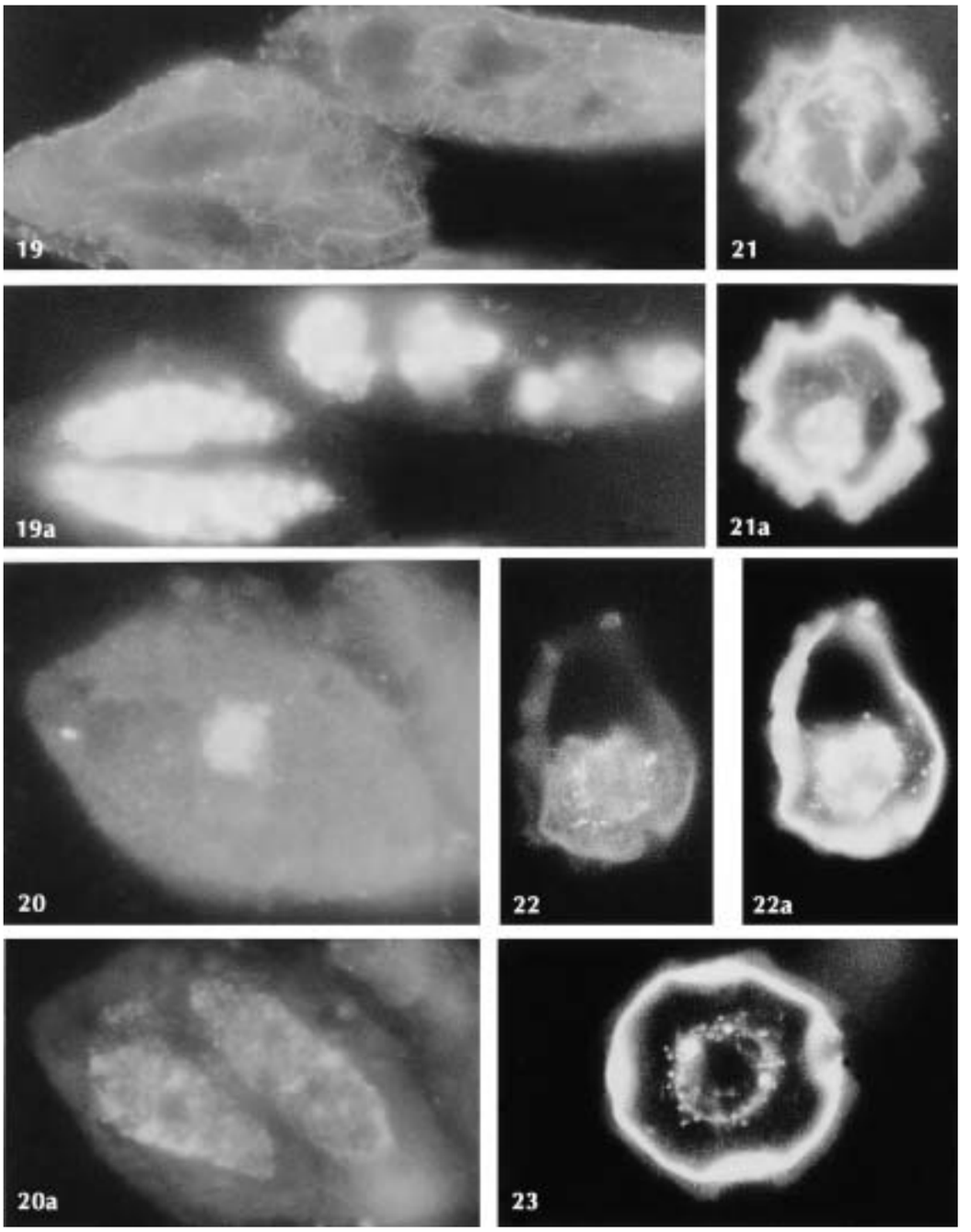
Microspores formed after meiosis were unequal in size, and although at the same developmental stage they differed in the thickness of the sporoder-



Figs. 3–8. *Chondrilla juncea* L. First meiotic division of microsporogenesis. **Fig. 3.** Asymmetric network of microtubules. **Figs. 4–5.** Short microtubules closely associated with nucleus surface. **Figs. 3a–5a.** Diplotene chromosomes after DAPI staining. **Figs. 6–7.** Prometaphase I; microtubules in meiotic spindle. **Figs. 6a–7a.** DAPI-stained nuclei of prometaphase I cells disclosed organelles at some distance from chromosomes. **Fig. 8.** Early telophase I with remnants of the spindle. **Fig. 8a.** Dispersed proplastid nucleoids after DAPI treatment. All figures × 1500.



Figs. 9–18. *Chondrilla juncea* L. Latter stages of microsporogenesis. **Figs. 9–10.** Large phragmoplast with numerous microtubules occupied the whole cell width. **Figs. 11–12.** Narrow phragmoplast with remnants of the karyokinetic spindle. **Fig. 9a.** Lagging chromosomes between two telophase groups. **Figs. 10a–12a.** Nucleoids of organelles scattered throughout the cytoplasm. **Fig. 13.** Wide phragmoplast with numerous microtubules. **Fig. 13a.** Matching stage of nuclei after DAPI staining. **Fig. 14.** Phragmoplast after the disappearance of peripheral microtubules in central part of cell. **Fig. 15.** Callosic cell wall in dyad of microspores detected after aniline blue treatment. **Fig. 16.** Meiocyte without phragmoplast. **Fig. 16a.** Nucleoids of organelles after DAPI staining. **Fig. 17.** Young coenocytic tetrad. **Fig. 18.** Fragment of tetrad cell walls after aniline blue treatment. All figures × 1500.



Figs. 19–23. Fluorescence of tapetal cells and microspores of *Chondrilla juncea* L. **Fig. 19.** Arrangement of microtubules entering tapetal cells. **Fig. 19a.** Matching stage of nuclei after DAPI treatment. **Fig. 20.** Small aggregation of microtubules in tapetal cell. **Fig. 20a.** Pair of nuclei visible after DAPI staining. **Fig. 21.** Radial arrangement of microtubules surrounding the nucleus. **Fig. 21a.** Plastids scattered in the cytoplasm. **Fig. 22.** Microspore with asymmetric arrangement of microtubules and exocentric position of nucleus (Fig. 22a). **Fig. 23.** Atypical microspore with centrally placed nucleus and organelles gathered around the nucleus. All figures × 1500.

mis. The nuclei were surrounded by a radial arrangement of microtubules (Fig. 21). Plastids (after DAPI staining) were scattered all over the cytoplasm (Fig. 21a). It is clear that the asymmetric arrangement of microtubules was responsible for the exocentric position of the nucleus. The microtubules were longer at one pole of the cell than at the other (Fig. 22). A vacuole was formed at the same time (Fig. 22a). In some cases an unusual symmetrical arrangement of the cell nucleus was observed (Fig. 23).

DISCUSSION

During sporogenesis and microsporogenesis of plants, meiosis is completed by simultaneous or successive cytokinesis. Such processes are connected with chromosome division, major transformations of the cytoskeleton, regular movements of cytoplasmic organelles, and the formation of tetrad cell walls.

The cytoskeleton configurations and the pattern of cytoplasmic organelle movements are specific to the types of cytokinesis. For simultaneous cytokinesis, the pattern of plastid and mitochondria aggregations and disaggregations is quite similar in the meiosis of many plants belonging to various taxonomical groups: the mosses *Rhynchostegium* (Brown and Lemmon, 1982a), *Amblystegium* (Brown and Lemmon, 1982b), *Atrichium* and *Entodon* (Brown and Lemmon, 1987a,b); the ferns *Onoclea* (Marengo, 1977) and *Pteridium* (Sheffield and Bell, 1979); other pteridophytes *Equisetum* (Bednara et al., 1986; Hiraoka, 1986) and *Selaginella* (Brown and Lemmon, 1985); the gymnosperms *Podocarpus* (Vasil and Aldrich, 1970), *Ginkgo* (Wolniak, 1976) and *Stangeria* (Rodkiewicz at al., 1988); the angiosperms *Ribes* (Geneves, 1976), *Impatiens* (Dupuis, 1978), *Lycopersicum peruvianum* (Pacini and Juniper, 1984), *Lycopersicon esculentum* (Hogan, 1987), *Nymphaea* (Rodkiewicz at al., 1989) and *Doritis* (Brown and Lemmon, 1989), and others.

Generally, organelles are scattered in the cytoplasm at the beginning of meiosis, subsequently aggregate at the nucleus, and later disaggregate until early telophase I. During telophase they form a conspicuous organelle band positioned in the equatorial space between the nuclei. Finally, in telophase II the aggregation changes shape and forms four postmeiotic nuclei.

The prophase aggregation forming a cap on the nucleus is connected with plastid divisions (Birky, 1983) but the equatorial band made up of a variety of small components in various taxa may play a role in organelle migration, isolation of nuclear domains, and elaboration of cell walls in cytokinetic processes.

The organelle layer develops along the line of the future cell wall between telophase I and II nuclei, probably on the edges of nuclear domain boundaries. The phragmoplasts form at the same time, starting as a nuclear-based radial system emanating from sister nuclei (following telophase I) and from both sister and non-sister nuclei (after telophase II).

The organelle band has been observed in meiosis of several species with simultaneous cytokinesis: bryophytes, pteridophytes, gymnosperms and angiosperms. In *Ginkgo biloba*, during band formation an increasing number of phragmoplast microtubules was reported, as was cross-linking between microtubules and organelles; it was interpreted as a sign of band stability and as a modality of organelle migration (Wolniak, 1976).

The studied *C. juncea* has meiosis with simultaneous cytokinesis. Unexpectedly, the plant does not form an organelle band in telophase I and II. Organelles such as plastids and mitochondria are dispersed randomly throughout the microsporocyte cytoplasm.

This situation is quite similar to the arrangement of organelles in meiocytes with successive cytokinesis.

The reasons for this phenomenon are not clear, but we may assume that the dispersed arrangement of organelles is due to the slightly abnormal phragmoplast, which looks more like a loose remnant of the meiotic spindle rather than the normal dense brush-shaped phragmoplast with newly polymerized microtubules. A mature and properly functioning phragmoplast can be covered with cyclin CycA I (John et al., 2001). It would be desirable to check its content in the *C. juncea* phragmoplast.

Chondrilla juncea is a triploid plant in which meiotic division is partly defective. The plant does not form normal bivalents, so some of the microsporocytes do not undergo complete meiosis resulting in the formation of four postmeiotic cells. *C. juncea* is an apomictic plant, and postmeiotic cells do not play any vital role in fertilization.

REFERENCES

BEDNARA J, GIEŁWANOWSKA I, and RODKIEWICZ B. 1986. Regular arrangements of mitochondria and plastids during sporogenesis in *Equisetum*. *Protoplasma* 130: 145–152.

BIRKY WC. 1983. *The partitioning of cytoplasmic organelles at cell division*. International Review of Cytology. Suppl. 15. Academic Press. Inc.

BROWN RC, and LEMMON BE. 1982a. Ultrastructural aspects of moss meiosis: cytokinesis and organelle apportionment in *Rhynchostegium serrulatum*. *Journal of the Hattori Botanical Laboratory* 53: 41–50.

BROWN RC, and LEMMON BE. 1982b. Ultrastructure of sporogenesis in the moss *Amblystegium riparium*. I meiosis and cytokinesis. *American Journal of Botany* 69: 1096–1107.

BROWN RC, and LEMMON BE. 1985. A cytoskeletal system predicts division plane in meiosis of *Selaginella*. *Protoplasma* 127: 101–109.

BROWN RC, and LEMMON BE. 1987a. Division polarity, development and configuration of microtubule arrays in bryophyte meiosis I. Meiotic prophase to metaphase I. *Protoplasma* 137: 84–99.

BROWN RC, and LEMMON BE. 1987b. Division polarity, development and configuration of microtubule arrays in bryophyte meiosis II. Anaphase I to the tetrad. *Protoplasma* 138: 1–10.

BROWN RC, and LEMMON BE. 1988. Microtubules associated with simultaneous cytokinesis of coenocytic microsporocytes. *American Journal of Botany* 75: 1848–1856.

BROWN RC, and LEMMON BE. 1989. Minispindles and cytoplasmic domains in microsporogenesis in orchids. *Protoplasma* 148: 26–32.

BROWN RC, and LEMMON BE. 1991a. Pollen development in orchids 1. Cytoskeleton at the control of division plane in irregular patterns of cytokinesis. *Protoplasma* 163: 8–18.

BROWN RC, and LEMMON BE. 1991b. Pollen development in orchids 2. The cytokinetic apparatus in simultaneous cytokinesis. *Protoplasma* 165: 155–166.

BROWN RC, and LEMMON BE. 1992. Cytoplasmic domain: A model for spatial control of cytokinesis in reproductive cells of plants. *Electron Microscopy Society of America Bulletin* 22: 48–53.

BROWN RC, and LEMMON BE. 1996. Nuclear cytoplasmic domains, microtubules and organelles in microsporocytes of the slipper orchid *Cypripedium californicum* A. Gray dividing by simultaneous cytokinesis. *Sexual Plant Reproduction* 9: 145–152.

CAI G, and CRESTI M. 1999. Rethinking cytoskeleton in plant reproduction: toward a biotechnological future? *Sexual Plant Reproduction* 12: 67–70.

DUPUIS F. 1978. Etude ultrastructurale de la microgamétogenèse chez *l'Impatiens balsamina* L.: de la diade á la tetrade. *Bulletin de la Societe Botanique de France* 125: 19–25.

GENEVÉS L. 1976. Sur la répartition et lers mouvements des organites cytoplasmiques au cours de la méiose staminale, et principalement pendant les télophase héterotypique et homéotypique; dans le *Ribes rubrum*. *Comptes Rendus de l'Academie des Sciences Série* D 265: 1913–1916.

HIRAOKA T. 1986. Two types of cell organelles as polarity indicators in the bouquet stage; their ultracytochemical characterization. *Proceedings of the Eleventh Congress of Electron Microscope,* 1986, 3293–3294. Kyoto.

HOGAN CJ. 1987. Microtubule patterns during meiosis in two higher plant species. *Protoplasma* 138: 86–90.

JOHN PCL, MEWS M, and MOORE R. 2001. Cyclin/Cdk complexes: their involvement in cell cycle progression and mitotic division. *Protoplasma* 216: 119–142.

KOŚCIŃSKA-PAJĄK M. 1996. Embryological problems in the apomictic species *Chondrilla juncea* L. (Compositae). *Folia Geobotanica et Phytotaxonomica* 31: 397–403.

KOŚCIŃSKA-PAJĄK M. 2000. Microspores and pollen grain in triploid *Chondrilla juncea* L. from unpolluted and polluted areas. *Acta Biologica Cracoviensia Series Botanica* 42/2: 135–140.

MARENGO NP. 1977. Ultrastructural features of the dividing meiocyte of *Onoclea sensibilis*. *American Journal of Botany* 64: 600–601.

PACINI E, and JUNIPER B. 1984. The ultrastructure of pollen grain development in *Lycopersicum peruvianum*. *Caryologia* 37: 21–50.

PERIASAMY K, and SWAMY BGL. 1959. Studies in the Annonaceae – I. Microsporogenesis in *Cananga odorata* and *Miliusa wightiana*. *Phytomorphology* 9: 251–263.

RODKIEWICZ B, BEDNARA J, KURAŚ M, and MOSTOWSKA A.1988. Organelles and cell walls of microsporocytes in a cycad *Stangeria*. *Phytomorphology* 38: 99–110.

RODKIEWICZ B, DUDA E, and BEDNARA J. 1989. Organelle aggregations during microsporogenesis in *Nymphaea*. *Flora* 183: 397–404.

SAMPSON FB 1969. Cytokinesis in pollen mother cells of angiosperms, with emphasis on *Laurelia novae-zelandie* (Monimiaceae). *Cytologia* 34: 627–633.

SHEFFIELD E, and BELL PR. 1979. Ultrastructural aspect of sporogenesis in a fern *Pteridium aquilinum* (L.) Kuhn. *Annals of Botany* 44: 393–405.

VAN LAMMEREN AAM, KEIJZER CJ, WILLEMSE MTM, and KIEFT H. 1985. Structure and function of the microtubular cytoskeleton during pollen development in *Gasteria verrucosa* (Mill.) M. Duval. *Planta* 165: 1–11.

VAN LAMMEREN AAM, BEDNARA J, and WILLEMSE MTM 1989. Organization of the actin cytoskeleton during pollen development in *Gasteria verrucosa* (Mill.) M.Duval. *Planta* 178: 531–539.

VASIL IK, and ALDRICH HC. 1970. A histochemical and ultrastructural study of the ontogeny and differentiation of pollen in *Podocarpus macrophyllus* D. Don. *Protoplasma* 71: 1–37.

WOLNIAK SM. 1976.Organelle distribution and apportionment during meiosis in the microsporocyte of *Ginkgo biloba*. *American Journal of Botany* 63: 251–258.