PHYTON

ANNALES REI BOTANICAE

VOL. 47, FASC. 1–2 PAG. 1–337 19. 12. 2007

Phyton (Horn, Austria)	**Vol. 47**	Fasc. 1–2	1–46	19. 12. 2007

Dedicated to Univ.-Prof. Dr. Friedrich EHRENDORFER (Vienna)
on the Occasion of his 80th Birthday

Polyad Development and Karyology in *Inga* and *Calliandra (Mimosaceae-Ingeae)*: A Reply to a Recent Paper in Flora.

By

Herwig TEPPNER*)

With 102 Figures

Received August 28, 2007

Keywords: *Acacia, Acacieae, Calliandra angustifolia, Inga feuillei, Ingeae, Leguminosae, Mimosaceae, Mimosoideae.* – Embryology, karyology, chromosome numbers, meiosis, microsporogenesis, pollen adhesive, pollenkitt, polyads, prepolyad, tapetal membrane.

Summary

TEPPNER H. 2007. Polyad development and karyology in *Inga* and *Calliandra (Mimosaceae-Ingeae)*: A reply to a recent paper in Flora. – Phyton (Horn, Austria) 47 (1–2): 1–46, with 102 figures. – English with German summary.

Inga and *Calliandra* anthers have transverse septa of parenchymatous tissue; in each locule-half one archesporial cell is present at the beginning. In *Inga* mitotic di-

*) Pens. Univ.-Prof. Dr. Herwig TEPPNER, Institute of Plant Sciences, Division of Systematics and Geobotany, Karl-Franzens University Graz, Holteigasse 6, 8010 Graz, Austria, Europe; e-mail: herwig.teppner@uni-graz.at

visions and cytokinesis produce prepolyads of 8–12 pollen mother cells (PMCs), whereas in *Calliandra* one division takes place and only 2 PMCs are formed. In the PMCs meiosis occurs in the usual way with simultaneous cytokinesis and wall formation. In *Inga* the position of metaphase II-spindles and thus also the order of the pollen grains in the tetrads is variable (most frequent is the tetrahedral orientation with two grains peripheral in the plane of the polyad). In *Calliandra* all spindles lie parallel to the plane of the polyad and thus all grains lie in the same plane. The postmeiotic secreted tapetal membrane with sporopollenin persists till maturity and in the open theca it is strongly appressed to the valves in both taxa. In *Calliandra* the apical grain of the polyad finally bears a drop of pollen adhesive which originates by postmeiotic lysis of descendents of middle layer cells within the narrowed proximal end of the cavity of the locule (mucilage chamber).

Small amounts of pollenkitt can be verified by the contact zones of polyads in air hanging on the under side of a cover slip. The adherence for the polyad presentation is reached by pollenkitt in *Inga* and *Calliandra*. *Acacia* clearly possesses pollenkitt as well.

The chromosome number is 2n = 26 and n = 13 in *Inga feuillei* and 2n = 16 and n = 8 in *Calliandra angustifolia*. *C. houstoniana* var. *calothyrsus* shows 2n = 20 chromosomes.

It was stated in a recent paper, that in *Calliandra* the primary cell per locule-half is a PMC, that the first wall is oblique, the meiotic wall formation is successive and that the drop of pollen adhesive is solid at the beginning. These statements are incorrect and will be corrected in the present paper.

Zusammenfassung

Teppner H. 2007. Polyaden-Entwicklung und Karyologie bei *Inga* und *Calliandra* (*Mimosaceae-Ingeae*): Eine Entgegnung auf eine kürzlich in Flora erschienene Publikation. – Phyton (Horn, Austria) 47 (1–2): 1–46, mit 102 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Bei *Inga* und *Calliandra* sind die Antheren durch Parenchym quer septiert; in jeder Lokulament-Hälfte ist zunächst eine Archesporzelle vorhanden. Durch Mitosen und nachfolgende Zellteilungen enthalten die Präpolyaden bei *Inga* schließlich 8–12, bei *Calliandra* stets nur zwei Pollenmutterzellen (PMZ). In den PMZ läuft die Meiose in der üblichen Weise ab, gefolgt von simultaner Wandbildung. Bei *Inga* ist die Stellung der Metaphase II-Spindeln variabel, so daß verschiedene Anordnung der Pollenkörner (PK) in den Tetraden möglich ist (am häufigsten tetrahedral mit 2 PK peripher in der Ebene der Polyade). Bei *Calliandra* liegen alle Spindeln parallel zur Fläche der Polyade und daher auch alle 8 PK in einer Ebene. Die postmeiotisch abgeschiedene Tapetum-Membran aus Sporopollenin persistiert bei beiden Taxa bis zur Reife und ist in der geöffneten Theka völlig den Valven angepreßt. Bei *Calliandra* trägt das apikale Korn der Polyade schließlich einen Pollenklebstoff-Tropfen, der durch postmeiotische Lysis von Zellen, die von der Mittelschicht abstammen, im verschmälerten, proximalen Ende des Raumes im Lokulament (Schleimkammer) entsteht.

Geringe Mengen an Pollenkitt können durch die Kontakthöfe trockener Polyaden in Luft, die an der Unterseite eines Deckglases hängen, nachgewiesen werden.

Das Haften für die Präsentation der Polyaden wird sowohl bei *Inga* als auch bei *Calliandra* durch Pollenkitt erreicht. *Acacia* besitzt ebenfalls Pollenkitt.

Die Chromosomenzahlen sind 2n = 26 bzw. n = 13 bei *Inga feuillei* und 2n = 16 bzw. n = 8 bei *Calliandra angustifolia*. *C. houstoniana* var. *calothyrsus* weist 2n = 20 Chromosomen auf.

Die in einer kürzlich erschienen Publikation aufgestellten Behauptungen, daß die primäre Zelle in der Lokulament-Hälfte eine PMZ sei, daß die erste Querwand schief angelegt werde, die Meiose mit sukzedaner Wandbildung verbunden sei und daß der Pollenklebstoff-Tropfen am Anfang fest sei, sind unrichtig.

1. Introduction

In a recent issue of Flora (201: 570–587, 2006) appeared a paper on 'Ontogeny of the *Calliandra*-massulae (*Mimosaceae: Ingeae*), and the associated viscin body' by R. GREISSL. The main results of this paper are:

1) A single polyad should originate from one PMC (pollen mother-cell), the primary cell in each locule-half.

2) Wall formation in this assumed PMC is supposed to be successive, which is very unusual for dicotyledons (e. g., some Magnoliids). The first wall is supposed to be oblique. In the gones (= meiotic products) successive divisions should give the eight cells of each polyad.

3) The drop on the apical grain is – even if this is not explicitly said – supposed to be produced by this grain and this 'drop' is supposed to be solid when the anther opens.

These results are in contrast to what is known from other *Mimosaceae* since the 19th century and also to observations of other scientists in *Calliandra*. Therefore they seem remarkable and interesting. But are these results correct ? The aim of this paper is to answer this question. The existing literature would have been sufficient as basis for a critical statement to the above points. However, before a discussion it seems to be useful to demonstrate some developmental facts for two examples. The misuse of the terms massula and viscin body in the case of *Calliandra* was discussed in a previous paper (TEPPNER 2007), where the terminology used here is discussed.

2. Material and Methods

The plants were grown in the greenhouse of the Institute for Plant Sciences of the University of Graz, Austria, Europe. The origin is as follows:

Inga feuillei DC.: Seeds purchased in Lima, Peru, June 12, 1979, H. TEPPNER 79/428 & K. KEPLINGER (see TEPPNER 1998: 38–39 and TEPPNER & STABENTHEINER 2006: 142–143).

Calliandra angustifolia BENTHAM: Peru, Dpt. Pasco, Pozuzo, ca. 820 m, August 30, 1981, H. TEPPNER 81/518 & K. KEPLINGER, live plant.

Calliandra haematocephala HASSKARL var. *haematocephala*: Purchase from the Emil KUR collection, Czech Republic, received July 1998, as *C. emarginata* and *C. inaequilatera*, respectively, three live plants.

Calliandra houstoniana (MILLER) STANDLEY var. *calothyrsus* (MEISNER) BARNEBY: Dominican Republic. – Chiltern Seeds, Cumbria, England, 2006: 234T (as *C. calothyrsus*), sown March 22, 2006, start of germination April 1, 2006, c. 30–37 cm high seedlings only.

Acacia caven (MOLINA) MOLINA: Origin not known, old stock.

Acacia celastrifolia BENTH.: Western Australia, 'Collected in South-west Botanical Province'. – Kings Park & Bot. Garden, Perth, Western Australia 1995: 2358 (seeds received as *A. myrtifolia*; det. G. PRENNER), sown August 2, 1995.

Acacia longifolia (ANDREWS) WILLD.: Bot. Garden Berlin-Dahlem 1978: 2230 (seeds received as *A. latifolia* BENTH.; det. G. PRENNER), sown February 1979.

Since for defining and recognising PMCs meiosis is an important and essential fact, it was decided to use a fixation for the flower buds adequate for chromosome studies – even though it is not well suited to discern cell borders and thin primary cell walls. Inflorescence buds in different stages of development were fixed in ethanol : chloroform : acetic acid 5 : 3 : 1 and stained with aceto-carmine solution in the usual way (e. g., DARLINGTON & LA COUR 1963, SHARMA & SHARMA 1965); then the anthers were dissected on the slide in a drop of acetic acid; squash preparations were also made. Observations and photos were made with a 'Zeiss Photomikroskop III'; Agfapan APX 100 professional was used. The negatives were scanned with Epson Perfection 2400 Photo and the images were edited with Adobe Photoshop 7.0.

The diameter of small inflorescences was measured without the surpassing bracts; the length of flower buds was measured along a straight line, even when arched.

The detection of a thin film of pollenkitt surrounding the polyads (in other cases single pollen grains) can be difficult as in the case of small polyads as in *Acacia*. The fact, that the viscous liquid pollenkitt gets in contact with glass in a physically characteristic way, offers a method for proving its presence. When grains in air come close to a glass plate, the pollenkitt leaps to the glass forming a contact zone between the glass and the grain. In the LM this zone is brighter than the surroundings, appears homogenous (without any structure of the exine visible) and, furthermore, is bordered by a dark line with a thin, bright halo (Fig. 100–101). Thus pollenkitt behaves as an optical gel; the same effect is shown macroscopically by a drop of immersion oil between two slides. One possibility to proceed this attachment would be to shake the polyads from open anthers (by pushing flowers or filaments with a needle) onto a slide, together with structures a little larger than the polyads (filaments, part of anthers, hairs or other material). A cover slip is fixed by paraffin or wax, then the slide is inverted and knocked against a table edge. Now, at least if pollenkitt is present, a part of the polyads will adhere to the cover slip and show distinct contact zones at all places of contact. So direct and easy comparison of polyads with contact zones to the cover slip on the upper side of the polyad, with others without contact on the upper side, lying on the slide only, is possible (such a control may be helpful in critical cases with few pollenkitt).

A polyad is defined as an assembly of pollen grains; thus, for the developmental stages from the archesporium to the PMCs the term p r e p o l y a d is used here.

Abbreviations used in the figures:

c callose
co connective
en endothecium
ep epidermis
m middle layer (1–3 layered)
s septum
t tapetum or tapetum remains
tm tapetal membrane

3. *Inga feuillei* DC.

3.1. Archesporium to PMCs

The four locules of *Inga* anthers have transverse septa of parenchymatous tissue, thus each locule consists mostly of two locule-halves (8 locule-halves per anther). Because of the high number of stamens (75–120) in the complex androeceum, the anthers in a bud show different sizes and developmental stages. At a bud length (= calyx length) of c. 3.5 mm (corolla c. 1.8 mm), in the locule-halves the first sporogenous cell (primary archesporial cell) becomes visible (Fig. 1). At a bud length of c. 4.5 mm (corolla c. 2.4 mm) one to four cells form the archesporium, whereas at a length up to c. 6 mm (corolla c. 3–4 mm) the number of cells is near eight or the final number of mostly eight cells (the PMCs) is reached. The primary archesporial cell in the locule-half is roundish to ellipsoid (Fig. 2) and becomes flattened; the nucleus divides mitotically (Fig. 3), cytokinesis is transversal in respect of the cell or the cavity in the locule-half. Surprisingly, the next divisions in these prepolyads clearly do not occur synchronously; at a given time usually only one mitosis per prepolyad can be observed. Since the cell walls appear blurred due to the chosen method, time was not wasted to clarify the sequence of divisions. It seems that at first a quadrant of cells is formed (Fig. 4) ($\pm$ round as seen from the flat side) and then these cells divide for reaching the final number of cells (Fig. 5, 6, 9, 10), the PMCs (usually eight, sometimes nine or ten, rarely twelve). The divisions are clearly mitotic as can be seen from the chromosome structure (doubled chromosomes, partite at the centromere; Fig. 3) and number ($2n = 26$). For an isolated cell with the plate in side view see Fig. 3, for a polar view within the intact prepolyad Fig. 7, 8 and 20. The order of the PMCs within a prepolyad in the case of eight PMCs is in four pairs (Fig. 15, 37, 39) or one single cell is in a terminal position at one or both ends (Fig. 21, 28).

Surrounded by the tapetum-sac, each prepolyad (now group of PMCs), increases up to c. 150 µm in length in premeiotic stage, which is approximately the final size of the polyad. The premeiotic phase needs a lot of time. Finally callose deposition begins (Fig. 9–11).

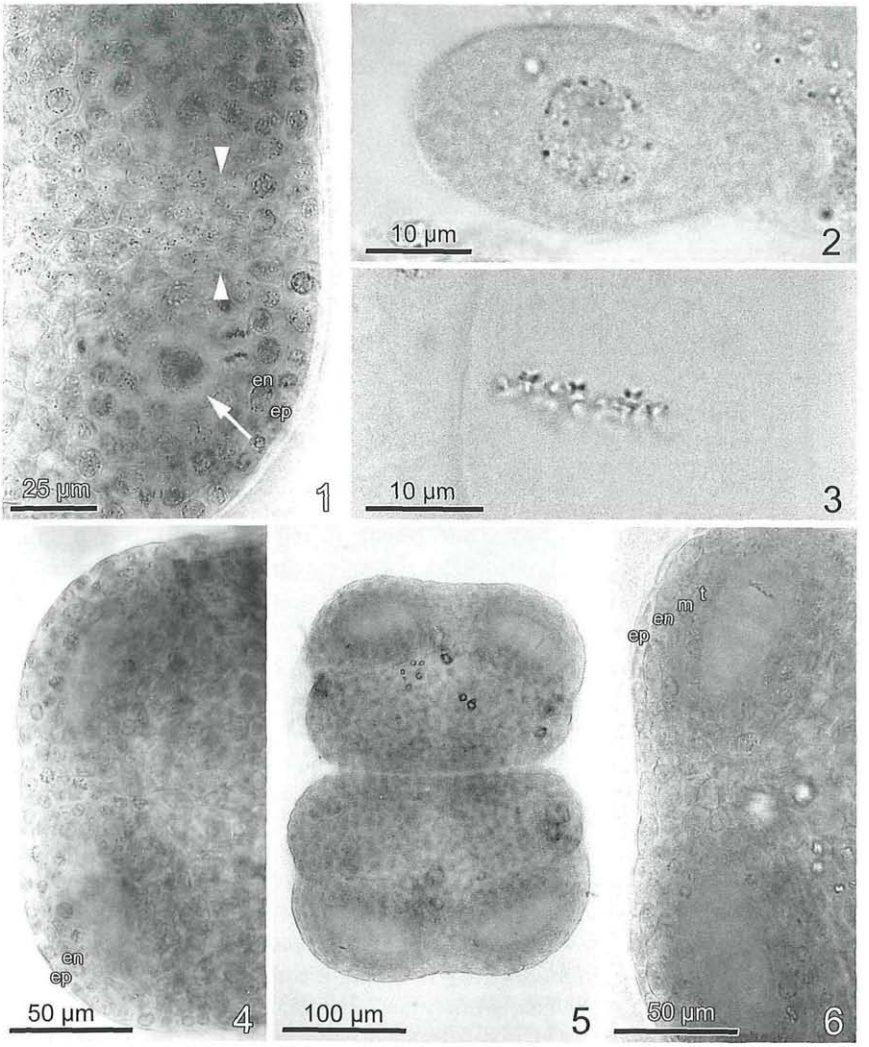


Fig. 1–6. *Inga feuillei*. Young anthers, stages from primary archesporial cell to prepolyads. – Fig. 1. Locule of a young anther, in the basal locule-half the primary archesporial cell. The arrow points to the large nucleus in mitotic prophase. Septum between the two locule-halves c. 4 cell-layers thick (between the arrowheads). Outer theca wall three layers thick. – Fig. 2. Primary archesporial cell squashed out from the locule-half. – Fig. 3. Mitotic metaphase plate in side view (the chromosomes consist of two chromatids) from an archesporial cell, most probably a primary one, squashed. – Fig. 4. Locule with c. 4-celled prepolyads in each locule-half. – Fig. 5. Young anther, upper end at the right side. In the outer locules c. 8- or nearly 8-celled prepolyads in each of the locule-halves discernible. Crystal-idioblasts in the connective. – Fig. 6. The uppermost locule from Fig. 5, the dark line near the upper end

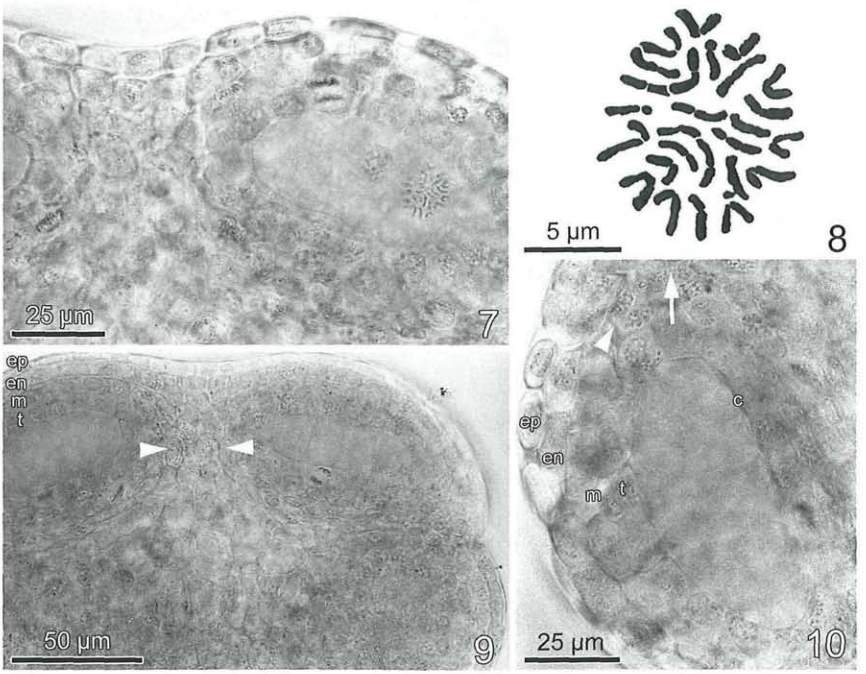
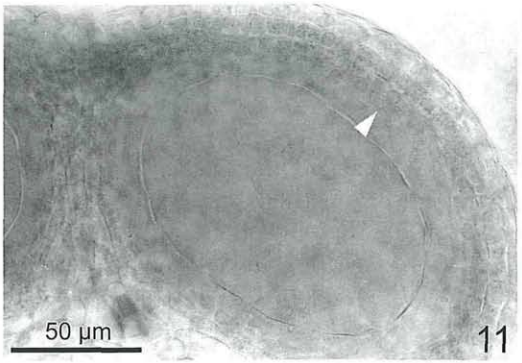
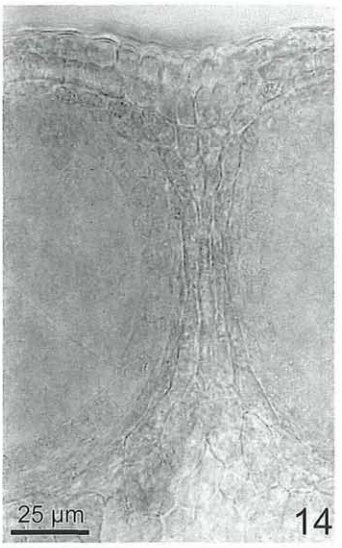


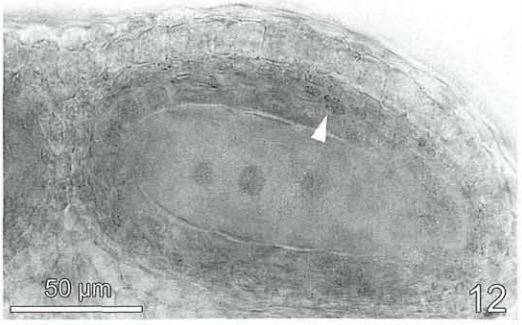
Fig. 7–10. *Inga feuillei.* Young anthers with prepolyads with the final number (or nearly so) of PMCs. – Fig. 7. Locule-half with the last mitotic divisions, a metaphase plate in face view (parallel to the longitudinal axis of the prepolyad) at the right, a prophase nucleus in the next of the four segments. – Fig. 8. Drawing of the mitotic metaphase plate with 2n = 26 chromosomes from Fig. 7. – Fig. 9. A locule with c. 8-celled prepolyads and the first signs of callose deposition. Septum c. 4-layered (between the arrowheads). – Fig. 10. Prepolyad with PMCs surrounded by callose. Middle layer already in part 2-layered (arrowhead). Slight distortion of epidermal cells due to pressure. Arrow: Limit between middle layer and septum.

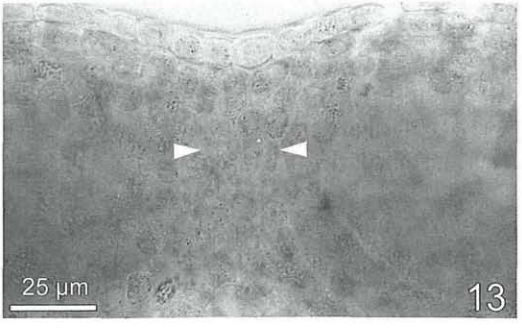
---

of the upper prepolyad is a mitotic metaphase plate in side view, perpendicular to the long axis of the prepolyad. The outer theca wall is 4-layered, i. e., the middle layer and the tapetum are already differentiated. The septum is c. 4-layered. – Abbreviations at the end of chapter 2.









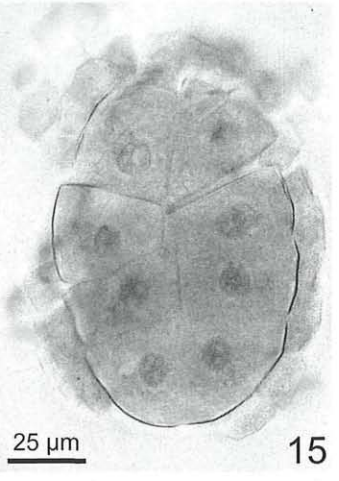


Fig. 11–15. *Inga feuillei*. Anthers with fully developed prepolyads with the final number of PMCs at premeiotic stage. – Fig. 11. Prepolyad in face view, in situ in the locule-half, surrounded with callose. Middle layer in part 2-layered (arrowhead). – Fig. 12. Prepolyad in side (lateral) view, in situ in the locule-half, surrounded with callose. The middle layer in part 3-layered (arrowhead). – Fig. 13. Centre of a locule with a 4-layered septum (between the arrowheads). Prepolyads distinct due to the callose layer, in face view. – Fig. 14. Centre of a locule with the whole septum and the adjacent connective. – Fig. 15. Prepolyad consisting of eight PMCs with callose (dark), surrounded by fragments of the tapetum-sac, squashed-out from the locule-half.

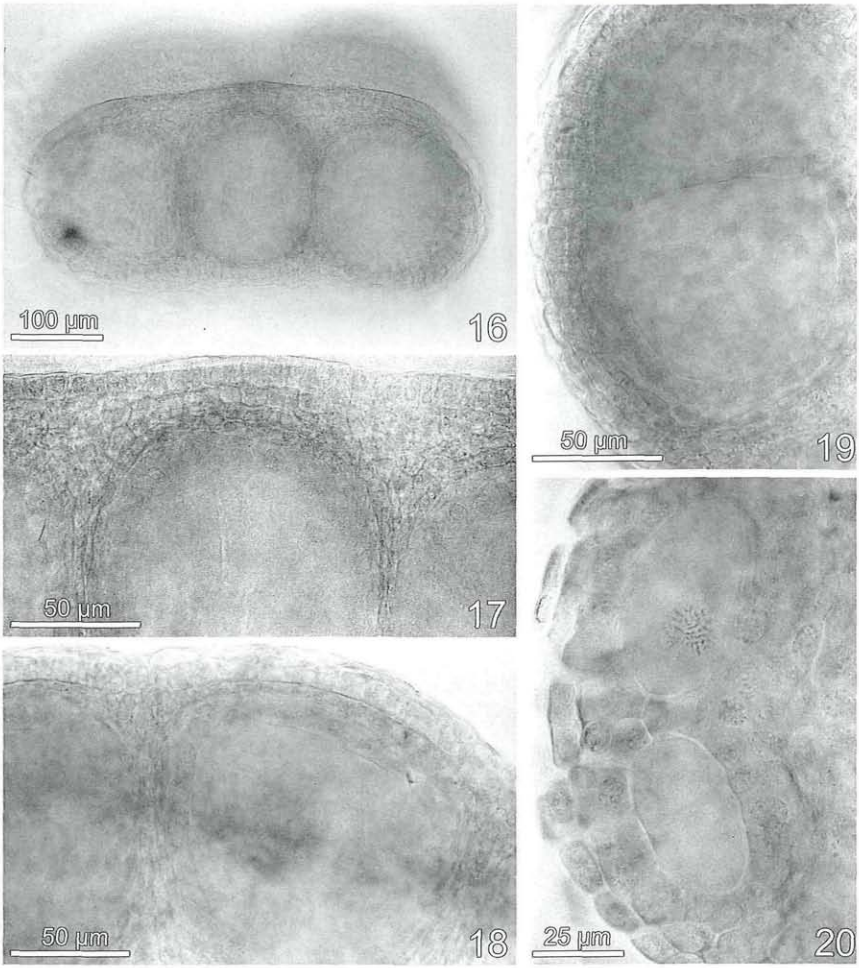


Fig. 16–20. *Inga feuillei*. Locules with three polyads. – Fig. 16. One of the four locules of an anther with exceptionally three similar cavities with premeiotic PMCs. – Fig. 17. Detail of Fig. 16, showing the central cavity (locule-third) and the adjacent parenchymatous septa. The left septum in the thinnest part 3-4-layered, the other (the additional one ?) 2-layered only. – Fig. 18. Central locule-third with premeiotic prepolyad of another anther, also with parenchymatous septa. – Fig. 19. A locule-half with two premeiotic prepolyads with callose, partite by a 1-layered tapetal bridge. – Fig. 20. A locule half with two prepolyads with callose, one with the last mitosis, separated by a 2-layered tapetal bridge.

Sometimes in the one or another locule-half two prepolyads are developed (Fig. 19–20). In such a case, a layer of tapetum lies between the two and divides the lumen of the locule-half, i.e., each prepolyad is completely surrounded by tapetum.

The parenchymatous transversal septum between the two locule-halves is c. four-layered (e. g., Fig. 1, 9, 13). Rarely two parenchymatous septa divide one locule, so that it contains three cavities each with a polyad (Fig. 16–18), evenly distributed along the locule; this was the case at times in only one locule of an anther. A case of four polyads per locule in a mature anther was seen only once, unfortunately the character of the septation was not discernible.

When development of the archesporium starts, the theca wall is three-layered (epidermis, endothecial layer, third layer; Fig. 1) but soon the tapetum is formed by divisions in the third layer and finally it is well developed as a fourth layer (Fig. 6). Now the third layer is the middle layer, which, itself, becomes two- to three-layered (Fig. 11, 12). The outer walls of the epidermis cells are flat or only slightly arched.

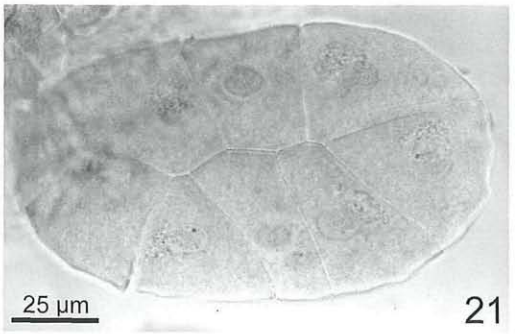
3.2. Meiosis

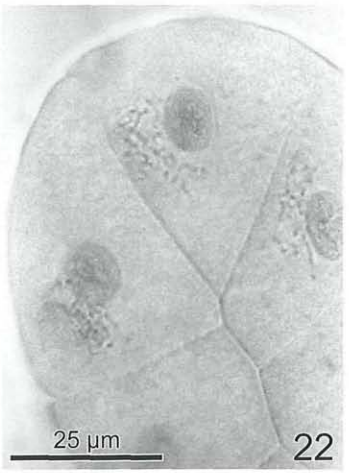
In flower buds of c. 6.0 – 7.0 mm in length (corolla c. 4.0 – 4.7 mm, length of anthers c. 0.3 mm) meiosis takes place.

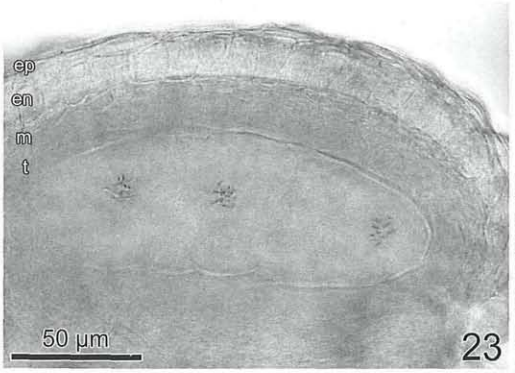
The prepolyads are enclosed in the tapetum-sac and at this stage it is relatively easy to separate the whole sac by dissection of the anther (e. g., Fig. 24, 28, 29). Each PMC is surrounded by a callose layer. Meiosis occurs synchronously within all eight (or up to twelve) cells of a prepolyad (Fig. 24, 28, 29, 34). At metaphase I all spindles lie approximately parallel

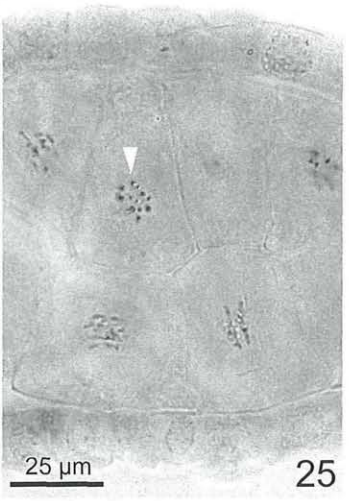
---

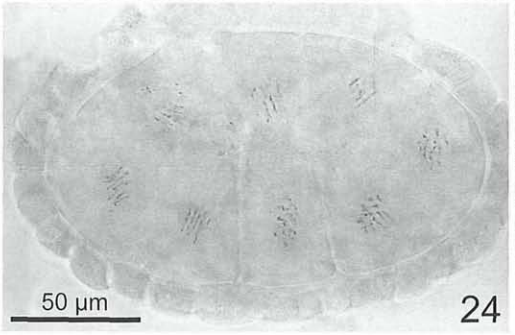
Fig. 21–27. *Inga feuillei*. Anthers at Meiosis I. – Fig. 21. Prepolyad consisting of eight PMCs at pachytene, squashed-out from the locule-half; with thin callose and few remains of the tapetum sac. – Fig. 22. Detail of Fig. 21, within the nuclei the threads of the pachytene bivalents and the nucleoli. – Fig. 23. Prepolyad in lateral view at metaphse I, with callose, in situ in the locule-half. – Fig. 24. Prepolyad with 9 PMCs at metaphase I, within the tapetum-sac, squashed-out from the locule-half. Variability in the orientation of the metaphase I-spindles. – Fig. 25. Detail of a prepolyad within the tapetum-sac, squashed out from the locule-half. Variation in the orientation of the spindles: nearly parallel to the plane of the polyad below and above left and perpendicular to the plane of the polyad (arrowhead). – Fig. 26. Details of two PMCs in late metaphase I, with spindles parallel to the plane of the polyad (thus bivalents in lateral view) and more (left) or less radially. Above left a tapetal cell and callose. – Fig. 27. The n = 13 bivalents of a late metaphase I plate in lateral view. Bivalents laying superimposed, drawn side by side.

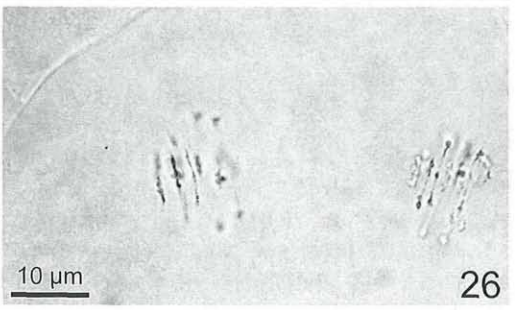


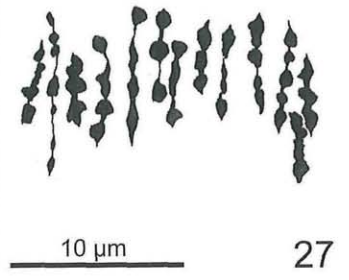


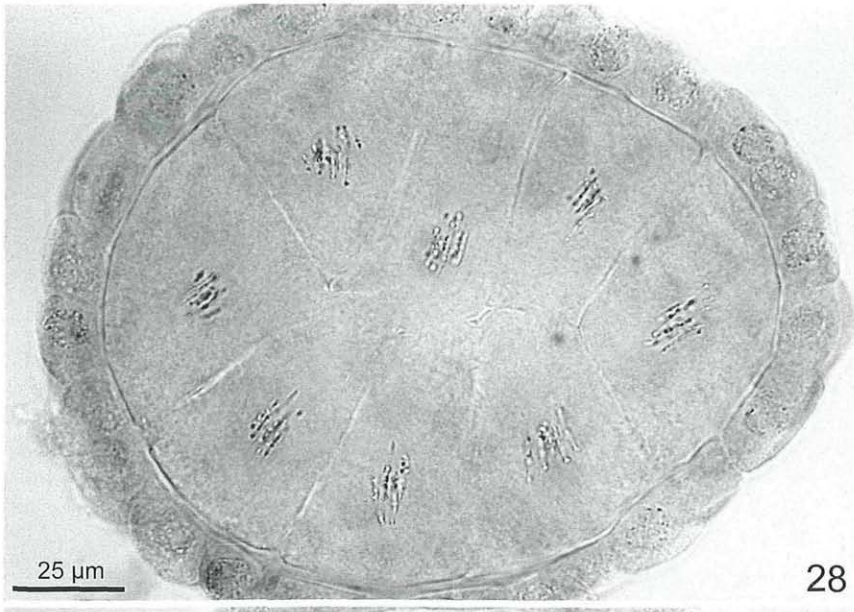












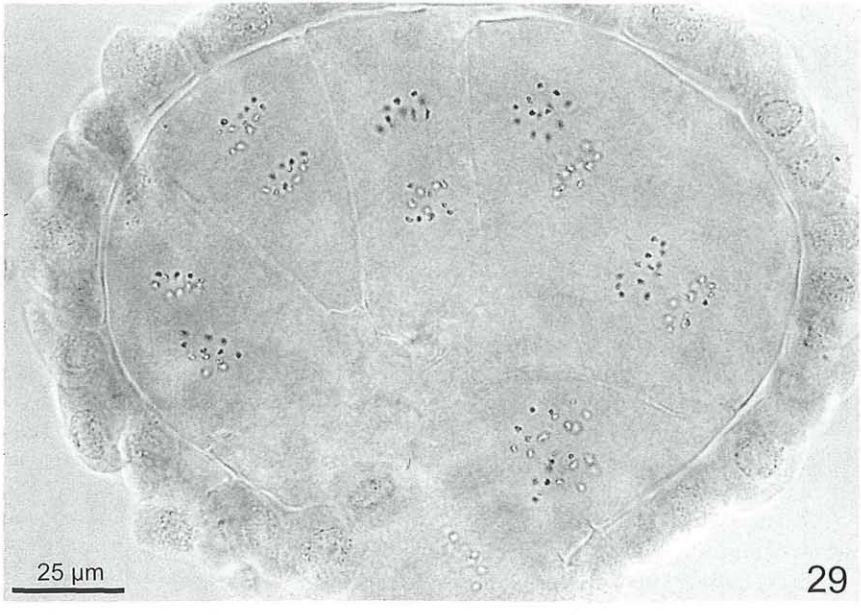


Fig. 28–29. *Inga feuillei*. Meiosis I. Prepolyads with 8 PMCs with callose in the tapetum-sac, squashed out from the locule-halves. – Fig. 28. PMCs at metaphase I, all spindles more or less parallel to the plane of the prepolyad and radially oriented. – Fig. 29. PMCs at anaphase I, prepolyad a little damaged at the bottom due to squashing. Orientation of the spindles similar to those in Fig. 28, a few a little more oblique (above right).

to the plane and are oriented more or less radially. The formation of bivalents prove these divisions as meiotic and thus the cells as PMCs. Usually rod bivalents dominate; n = 13 can be easily counted at metaphase I (Fig. 25–27) and in the later stages (Fig. 29, 32, 34–36). The outer walls of the PMCs are more distinct than the adjacent ones. Due to the orientation of the metaphase I and anaphase I spindles, one of the two interkinesis nuclei in each PMC lies near the outer side, the other near the inner side of the PMC (Fig. 33). In the metaphase II and anaphase II the two spindles within one PMC usually lie approximately at right angles: the peripheral one lies in the plane of the polyad whereas the spindle near the centre is perpendicular (Fig. 34 arrows, Fig. 35 left). There is some variability, e. g., in a smaller number of cells both plates can also lie parallel to the plane of the polyad (Fig. 34 arrowheads, Fig. 35 right) and intermediate positions also occur. Postmeiotic cytokinesis is simultaneous. The order of the pollen grains in the tetrad within the PMC, and thus in the polyad, depends on the spindle orientation and as a consequence of it, the position of the telophase II nuclei. Usually the two grains from the peripheral dyad of a PMC lie in the plane of the polyad, whereas the other two are oriented perpendicular in two planes (as in six PMCs or tetrads, respectively, in Fig. 37). The next frequent possibility is, that the grains from both dyads lie parallel and perpendicular to the plane of the polyad, so that in two planes two grains are visible (as in four cases in Fig. 38).

The outer walls of the epidermal cells are flat to arched, endothecial cells show the start of the thickening of the baseplate. The middle layer consists of three cell layers. The tapetum is well developed.

3.3. Postmeiotic Development

In buds of c. 8 mm length (corolla c. 5 mm) development has largely progressed. In the polyads pollen grain exine is well developed (Fig. 40, 42), as well as the pores [(4?-)6–8 per grain (Fig. 42)]. The callose layer is reabsorbed, the tapetum is still well developed and has secreted a thin, hyaline, distinctly granulated membrane (tapetal membrane with orbicules) on its inner side (Fig. 39–41); the orbicules sit on the inner (polyad) side of the membrane (Fig. 40, 41); secretion also takes place in the furrows between the tapetum cells; thus the outlines of the tapetum cells are clearly figured on the membrane (Fig. 41). In part it is already loosened from the tapetum (spontaneous or due to the preparation ?). Epidermal papillae start to develop, the formation of the endothecial thickenings progresses.

At 14 mm bud length (corolla nearly of the same length as the calyx) anthers are fully developed, the tapetum has disappeared, only the tapetal membrane is present and appressed to the middle layer or directly to the

endothecium or the transversal septum (Fig. 43–46). The middle layer shows 2–3 layers of cells with more or less degenerated content or has disappeared.

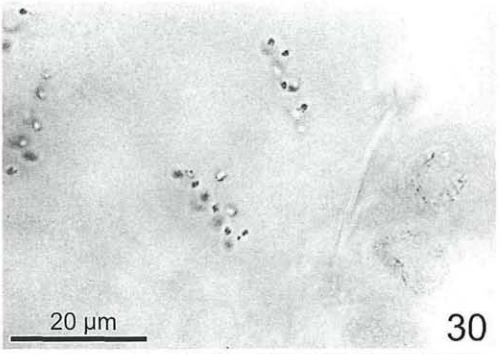
Before anther opening approximately the half of the longitudinal septum along the stomium and the central part of the transversal septum are dissolved. In the mature, opened anthers the tapetal membrane is so strongly and completely appressed to the inner side of the remaining theca wall, that it was not observed by TEPPNER & STABENTHEINER 2006: 146. Higher magnification show the fine granulation on the inner side of the valves, proving the persistence of the tapetal membrane (Fig. 47). Anther opening and polyad presentation is described in TEPPNER & STABENTHEINER 2006.

4. *Calliandra angustifolia* BENTH.

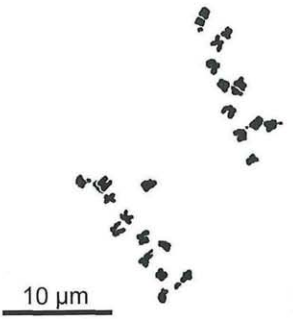
4.1. Archesporium to PMCs

Just like *Inga, Calliandra* also shows transverse septate locules and therefore also has two locules or four locule-halves per theca. At a bud length of c. 1.1–1.5 mm [inflorescence (head) diameter c. 2.5–3.5 mm], the anthers on very short filaments fill only the lower part within the corolla. In each locule-half the first cell, an archesporial cell, can be observed; it is roundish or rather elliptic in outline (Fig. 48–50). The first division of the nucleus is clearly mitotic (metaphase plate in side view: Fig. 51) and because of the mitotic spindle lying in the longitudinal axis of the archesporial cell, the wall separating the two daughter cells is perpendicular at right angle to the longitudinal axis of the primary cell (Fig. 49, 52) and to the theca wall. As shown later, the two resulting cells are PMCs already. In the next developmental phase the cells become flattened and the elliptic

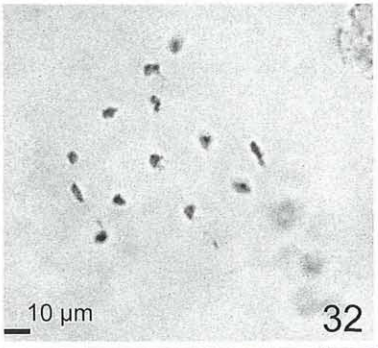
Fig. 30–36. *Inga feuillei.* Meiosis I and II in the anthers. – Fig. 30. PMC with an anaphase I nucleus in lateral view. On the right a fragment of callose layer and tapetal cell nuclei. – Fig. 31. Drawing of Fig. 30, the two plates drawn approached and all chromosomes (consisting of two chromatids each) drawn in one plane. – Fig. 32. A single anaphase I plate with n = 13 chromosomes in face (polar) view. – Fig. 33. Prepolyad with 9 PMCs within the tapetum-sac, squashed out from the locule-half, at interkinesis (two nuclei in each PMC). – Fig. 34. Prepolyad with 8 PMCs at metaphase II, within the tapetum-sac, at the left end a little damaged by the preparation. The two spindles perpendicular to each other [the outer one parallel, the central one perpendicular to the plane of the polyad (arrows)] or both spindles more or less perpendicular to the plane of the polyad (arrowheads). – Fig. 35. Detail of two PMCs at metaphase II, the two extreme orientations of the spindles described for Fig. 34 side by side (the outer spindle parallel to the plane of the polyad: left). – Fig. 36. Drawing of a PMC at metaphase II with the spindles of the two nuclei perpendicular to each other.



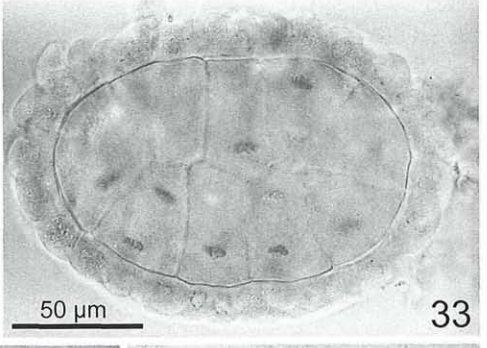
30



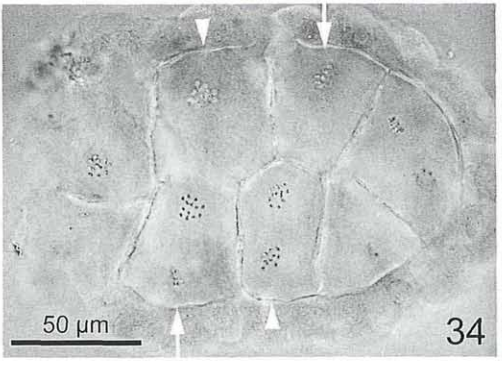
31



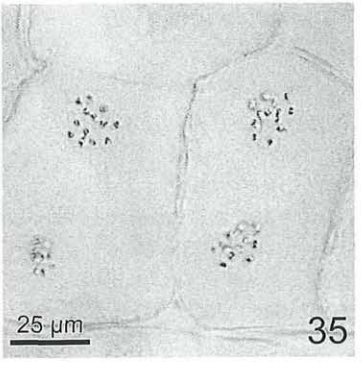
32



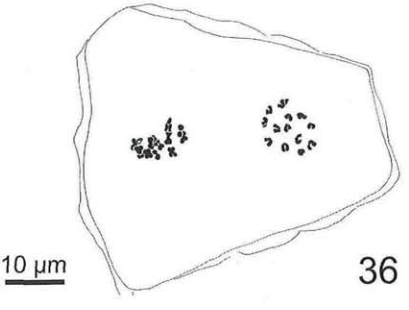
33



34



35



36

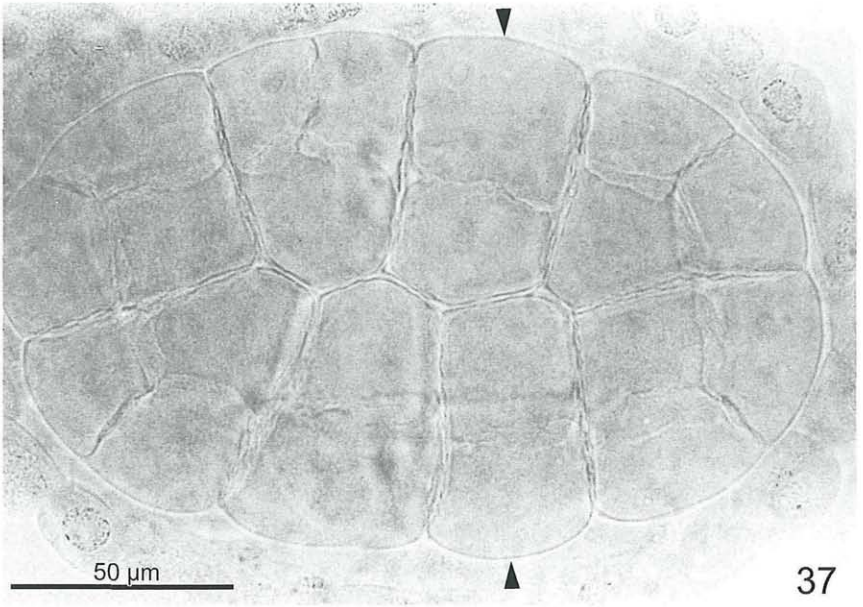
shape of the two PMCs changes to heteropolar and asymmetric in face view: one cell (at the distal end of the locule-half) remains rounded (later the basal end of the polyad) whereas the other at the proximal end of the locule-half, becomes more or less acute (later the apical end of the polyad). The wall between the two cells of the prepolyad becomes oblique: it inserts at the outer margin (stomium side) nearer the base and passes to the inner side (connective-side) nearer to the apex. The final form and size is reached at premeiotic stage (Fig. 53–56) and thus, the final shape of the polyad already is discernible at this early stage. Since the young PMCs fill the lumen of the locule-half without any interstice, the change of the shape of the PMCs is probably affected by changes of the shape of the cavity by growing of the locule walls (accompanied by shifts by the growth of the PMCs itself ?).

The theca wall during archespore and young PMC stage is three-layered (Fig. 49), during the early growth of the PMCs the fourth layer, the tapetum originates (Fig. 54). The growth of the anther causes many divisions in the theca walls, thus often the somatic chromosome number of 2n = 16 can be counted (Fig. 48, insert).

4.2. Meiosis

In buds of c. 1.0–1.6 mm in length (inflorescence diameter c. 3.5–3.8 mm), in which the anthers fill the space within the corolla, premeiotic PMCs, meiosis and postmeiotic polyads can be observed.

Meiosis begins synchronously in the two PMCs of one locule-half (Fig. 57–59), but may not be exactly synchronous in the whole locule, theca or anther. Metaphase I spindles lie parallel to the plane of the PMCs and approximately parallel to each other. Because of the oblique position of the separating wall, one spindle pole is nearer to this wall on the outer side in the apical PMC and on the inner side in the basal PMC (Fig. 57, 59, 64–67). This causes also the position of the daughter-nuclei in the following stages, e. g., Fig. 64-66. The result of the following anaphase I is interkinesis with two nuclei in each PMC (Fig. 63). The spindles of metaphase II and consequently also anaphase II lie parallel to the face of the cells as well and more or less oblique to the metaphase I spindles (Fig. 64–67). Thus all spindles lie in the same plane. At telophase II four nuclei are formed in each of the PMCs (postmeiotic: Fig. 70), and as a consequence, all 8 nuclei lie also in the same plane. After meiosis furrowing and formation of the walls between the four gones (later pollen grains) are clearly simultaneous in both PMCs (Fig. 71–74). [For a review of the cytokinesis types in PMCs see, for e.g., BHANDARI 1984: 106–107]. Thus the final form and position of the pollen grains is formed. During meiosis (from premeiotic to postmeiotic stage), relatively thin but distinct amounts of callose usually surround the



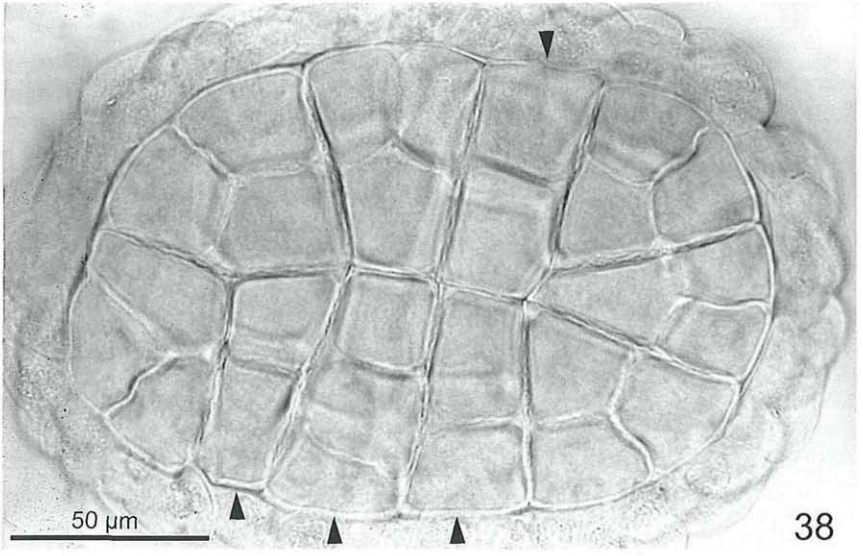


Fig. 37–38. *Inga feuillei.* Postmeiotic polyads, still with callose around the pollen grains, within the tapetum-sac. The majority of the PMCs show the normal order of grains with the two peripheral grains side by side in the plane of the polyad whereas the two grains on the centre side are superimposed. In the remaining PMCs (arrowheads) the peripheral and the central grains are superimposed. – Fig. 37. A polyad with 8 PMCs. – Fig. 38. A polyad with 10 PMCs.

PMCs (e. g., Fig. 55, 71), to a smaller extent callose can be found also in the cleft between the two neighbouring PMCs (e. g., Fig. 60, 63, 65, 73). Rarely thick layers of callose can be observed (Fig. 75). These break down postmeiotically. In all suited stages the meiotic chromosome number of n = 8 is easy to count (e. g., Fig. 58–62, 64–69).

The epidermal cells are still smooth, the endothecium has no thickenings. The tapetum is well developed since premeiotic stages. Prepolyads and polyads liberated during preparation frequently remain included in the tapetum-sac (e. g, Fig. 55, 71, 74).

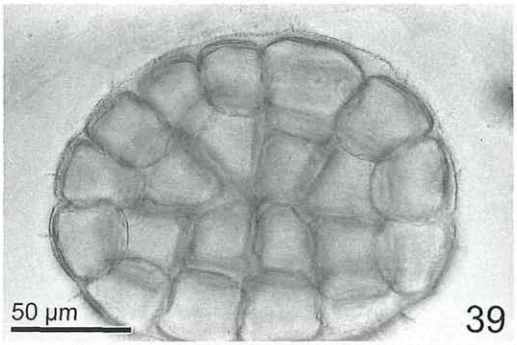
4.3. Postmeiotic Development

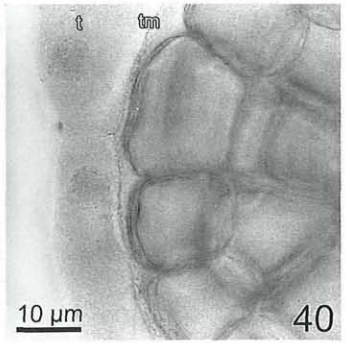
In the postmeiotic phase, in flower buds from c. 1.5 mm onwards (inflorescence diameter c. 3.5–5.0 mm) the rapid formation of the exine is very prominent (Fig. 75–85). In the relevant epidermal cells the growth of the papillae begins.

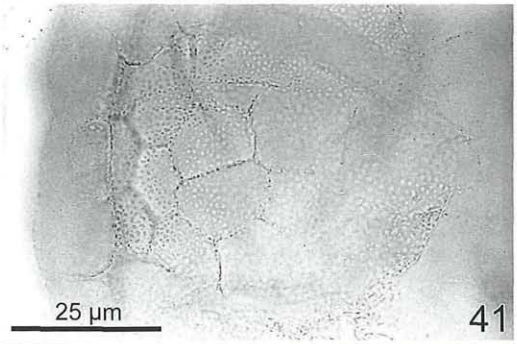
In the second (endothecial) layer in the arched parts of the theca walls and in a bridge between them [narrower (5–6 cells high) on the outer side of the theca (Fig. 86), wider (9–10 cells high) on the inner side (Fig. 87)] the thickenings of the endothecium are formed (with the baseplate characteristic for *Mimosaceae*) (Fig. 97). The middle layers, at least the outer one, remain intact. The originally intact tapetum secretes a thin, hyaline tapetal membrane with orbicules (Fig. 82–91), which surrounds the polyads like a close-fitting sac, which is attached to the polyad at the tip of the apical

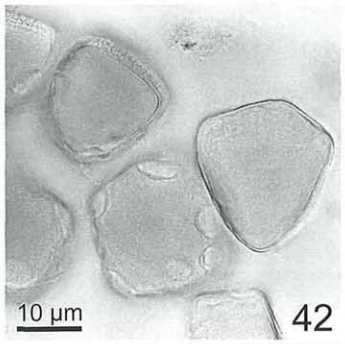
---

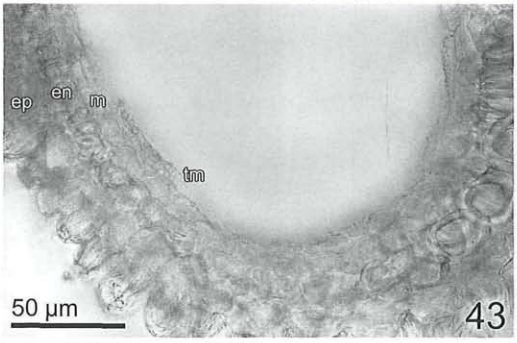
Fig. 39–47. *Inga feuillei*. Postmeiotic polyad and anther development. – Fig. 39. A polyad with 32 grains (from 8 PMCs) within tapetum-sac and tapetal membrane. Exine well developed, callose disintegrated. – Fig. 40. Detail of Fig. 39, tapetum cells still intact, tapetal membrane largely adhering to the inner side of the tapetum-sac, thick exine in the outer walls of the pollen grains only. – Fig. 41. Tapetal membrane, left optical section through tapetum cells and adhering tapetal membrane, otherwise surface view of the membrane at different levels in relation to the optical plane. – Fig. 42. Pollen grains, loosened from the polyad, in surface view and optical section. In one grain at least 6 pores discernible. Others show the different thickness of the exine around the grain. – Fig. 43. Fully developed anther, shortly before anthesis. Optical section through a locule-half, polyad removed. Tapetum completely disappeared, tapetal membrane, spreading the cavity, appressed to the inner middle layer. – Fig. 44 and 45. Tapetal membrane at the inner middle layer. – Fig. 46. Middle layer reduced, tapetal membrane nearly at the endothecium. – Fig. 47. ESEM image of the inner side of the valve of an open anther after the night of anthesis. Tapetal membrane with orbicules strongly appressed to the valve. Above part of a polyad, below the margin of the valve with shrivelled epidermal papillae. Similar view as in Fig. 43 in TEPPNER & STABENTHEINER 2006: 155 (phot. STABENTHEINER).

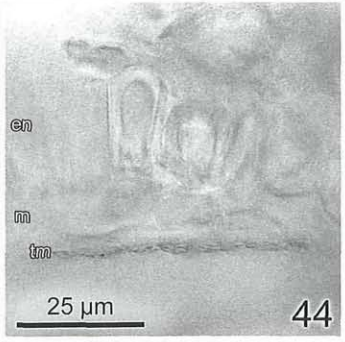


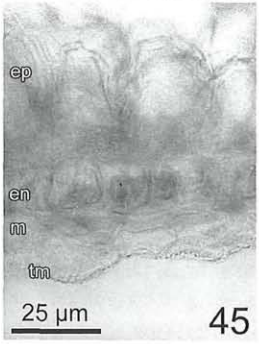


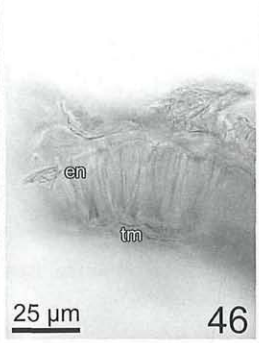


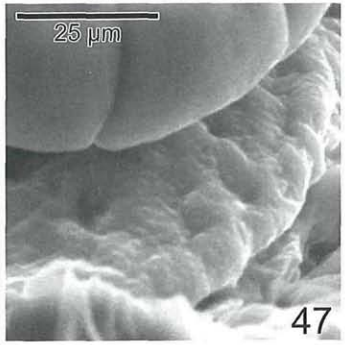


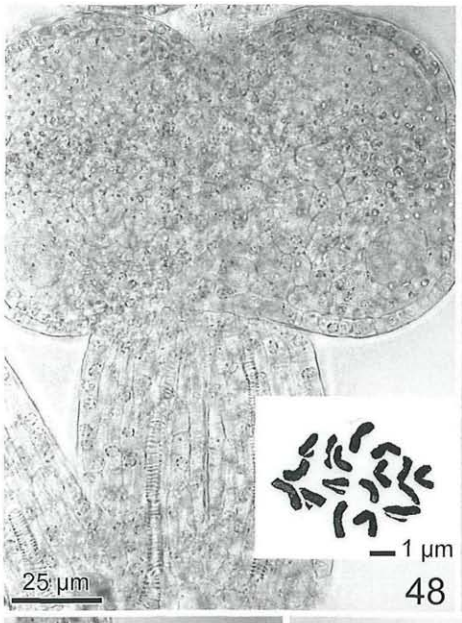




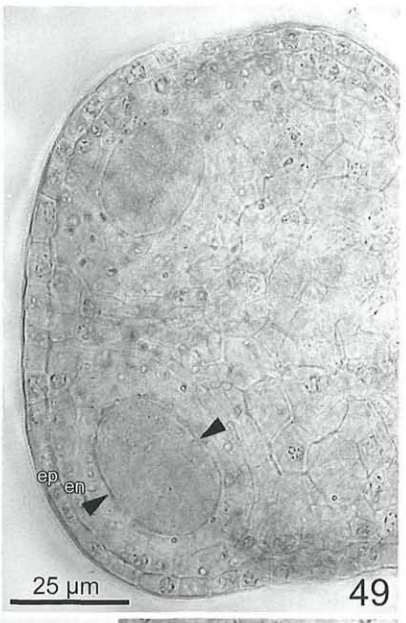




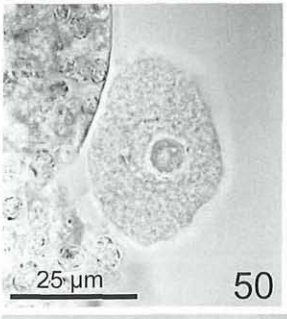




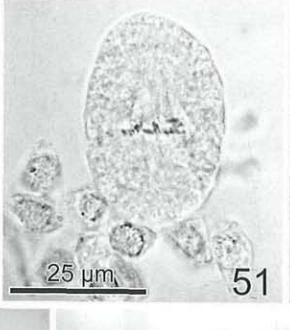
48



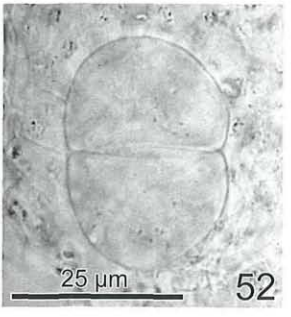
49



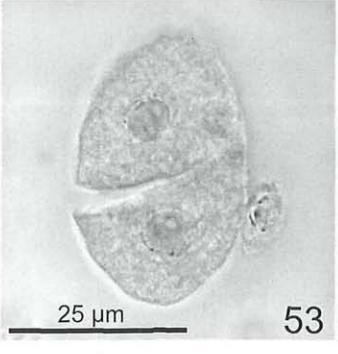
50



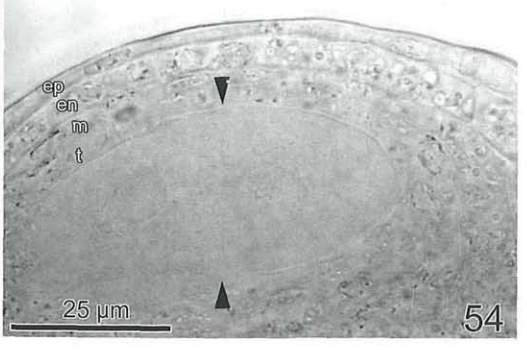
51



52



53



54

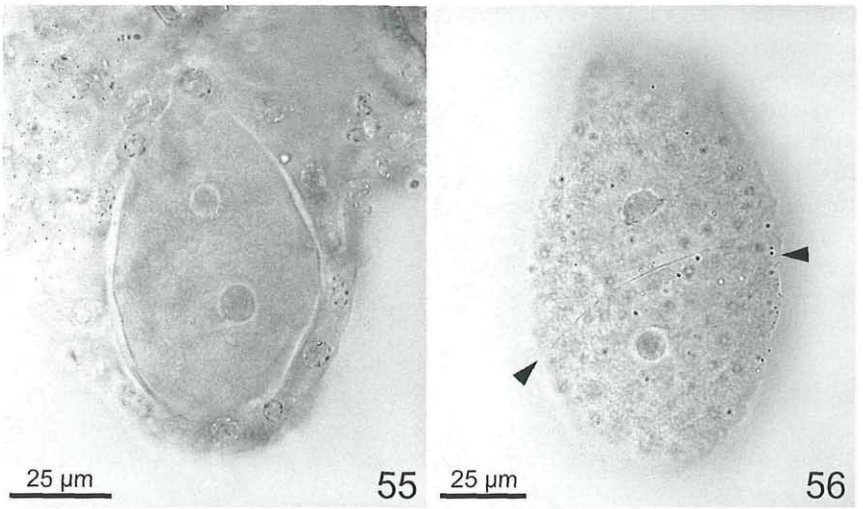


Fig. 55–56. *Calliandra angustifolia*, premeiotic prepolyads. – Fig. 55. Not fully grown prepolyad (two PMCs, separating wall here indistinct) within the tapetum-sac and a thin callose layer. – Fig. 56. Fully grown prepolyad, squashed out from tapetum and callose. Surface covered with strongly light refracting particles. The dark line between the two nucleoli is part of the oblique, separating wall (arrowheads). – Stomium side of the prepolyads left.

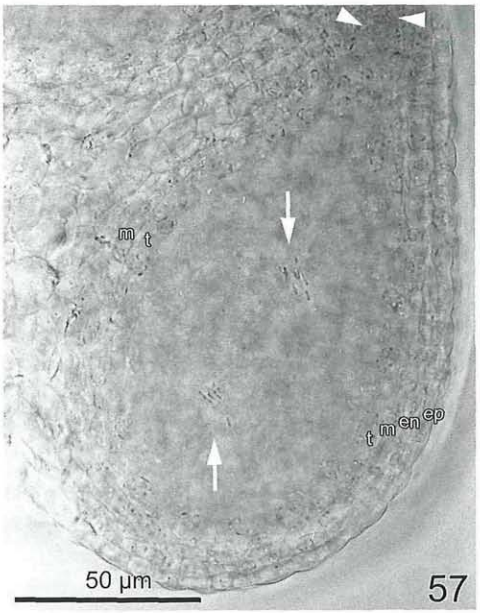
Fig. 48–54. *Calliandra angustifolia* anthers, premeiotic development. – Fig. 48. Young anther with very short filament, in the two lateral locules the primary archesporial cells visible in the basal locule-halves. Insert: a mitotic metaphase plate from the anther wall, with 2n = 16 chromosomes. – Fig. 49. Locule a little larger than in Fig. 48, in the apical locule-half an archesporial cell, in the basal one two young PMCs already; a weak transversal wall separating the PMCs between the arrowheads. Theca wall three-layered, septum two- to three-layered, epidermis and endothecial layer already developed. – Fig. 50. An archesporial cell squashed out from the anther. – Fig. 51. Archesporial cell with the mitotic metaphase plate in side view. – Fig. 52. Mitosis and cytokinesis completed: two young PMCs, largely liberated from the locule-half tissue during the preparation. – Fig. 53. Young prepolyad consisting of two PMCs after some further development, when the separating wall between the two PMCs become oblique. The basal cell at the bottom, the apical one above, both a little separated by the mechanics of squashing. – Fig. 54. Further developed prepolyad with two PMCs, in the intact locule-half. Apical end left. Between the arrowheads the wall separating the PMCs. Theca wall four-layered, middle layer (two-layered itself) and tapetum well developed already.

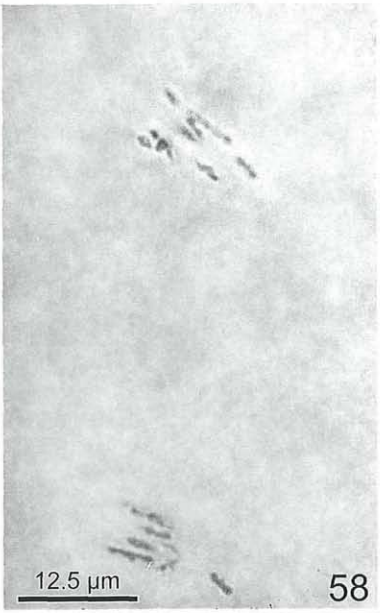
grain (Fig. 89, 90, 98). Then the tapetum progressively reduces to a degenerated thin layer with flattened nuclei (Fig. 81–85). Soon after meiosis the lysis of the potential mucilage cells begins and thus the border between these cells and the transversal septum becomes clearer (Fig. 75). It becomes apparent that the small septum between two opposite mucilage chambers has 2–4 layers of small cells (Fig. 75–85) and only this part is homologous to the septum as in *Inga*. Inwards, the septum dilates abruptly to a wide structure of 7–8 layers of much larger cells (Fig. 75–85); this is an extension (protrusion) of the connective. If this interpretation is correct, than the mucilage chamber is the narrowed, proximal end of the cavity of the locule-half.

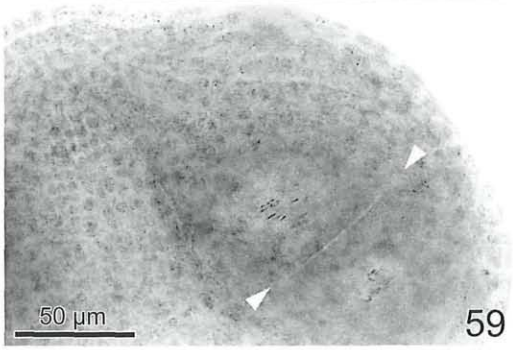
According to the investigations the mucilage cells are descendents of cells of the middle layer. C. 10–20 small cells are involved. The lysis begins always soon after meiosis at the periphery, with the cells adjacent to the septum (Fig. 76) and progresses towards the polyad tip (Fig. 77). Lysis occurs whereas the tapetum is still intact (Fig. 77, 78, 80). The tapetum disintegrates considerably later and apparently in another way.

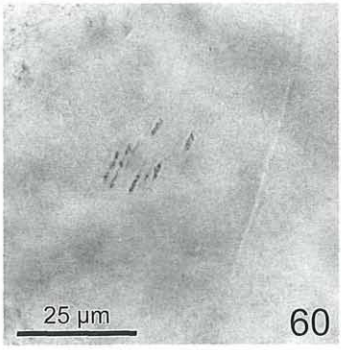
At a flower bud total length of c. 2.8–3.3 mm (inflorescence diameter c. 6 mm) the buds are arched distinctly claw-like. The corolla surpass the calyx tips for c. 0.5–1 mm, the filaments are long and coiled and the style shows 1 1/2 – 2 1/2 screw-turns. In the anthers the epidermal papillae are already well developed. The endothecial thickenings are fully developed, traversing the transversal septum as a smaller zone of cells, especially on the outer side (Fig. 86), where the zone of cells with thickenings is c. half as wide only, compared to the inner side (Fig. 87). The middle layer is fully intact. The tapetum has disappeared (Fig. 88), only the granulated tapetal membrane is still present. The mucilage drops are fully developed, some subdivisions as relics of the previous cellular structure may be still discernible (Fig. 98). With the applied methods it is difficult to discern, if the mucilage drop is attached to the polyad tip at this stage or later on. It is not impossible, that this takes place during the early stages of anther opening.

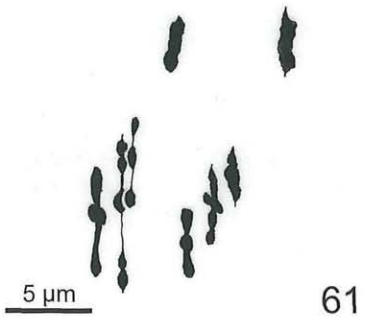
Fig. 57–62. *Calliandra angustifolia*. PMCs at meiosis I: metaphase I. – Fig. 57. Survey of a locule-half with the two PMCs, synchronously at metaphase I (arrows); separating wall indistinct here. The two arrowheads point to the limit between septum and the later mucilage cells. – Fig. 58. Detail of Fig. 57 with the two nuclei at metaphase I (8 bivalents each). – Fig. 59. Another locule-half with the two PMCs at late metaphase I, separating wall distinct here (between the arrowheads). – Fig. 60. Detail of Fig. 59 with the upper left metaphase I plate. – Fig. 61. Drawing of the n = 8 bivalents in the late metaphase I-nucleus of Fig. 60. – Fig. 62. Prepolyad with two PMCs at metaphase I squashed-out from the anther. The arrowhead points to the position of the separating wall.

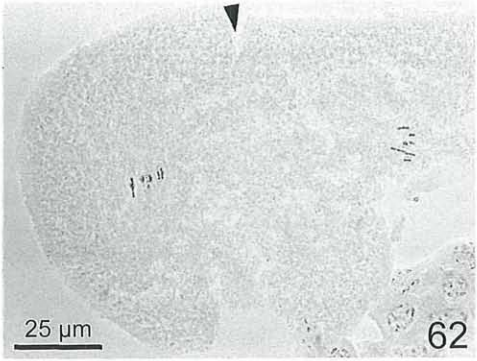












At a bud length of c. 3.8–4 mm (corolla surpassing the calyx-tips for c. 1.4–1.8 mm, inflorescence diameter c. 7 mm) no dramatical changes were observed except that from the polyads removed from the locule-halves the percentage with mucilage drops is higher.

In buds of c. 4.5 – 5 mm in length (corolla surpassing the calyx-tips for c. 2 – 2.5 mm, inflorescence diameter c. 8 mm) the middle layers are in the process of degeneration; often the cells show hyaline, homogenous, strongly refracting drops as content (Fig. 95). They remain intact for longer period at the outer side near the polyad apex (Fig. 92). The tapetal membrane is appressed to the remains of the middle layer (Fig. 88, 92–95).

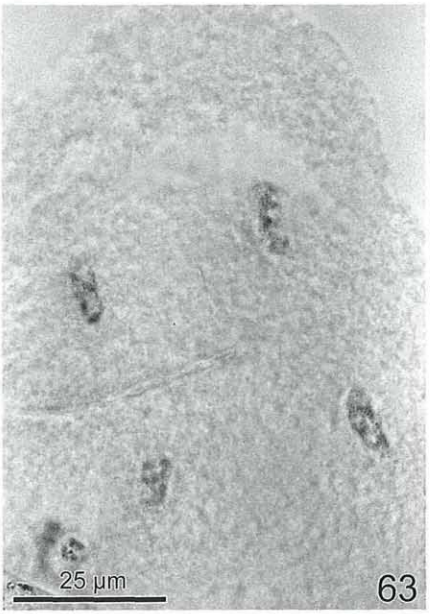
Fully developed buds of *C. angustifolia* were no longer available when finishing this paper. Thus buds of *C. haematocephala* at the day before anthesis were used. The cells of the middle layer are largely degenerated (Fig. 96–99), cells are usually discernible, sometimes the remains are only a thin layer. The content of the cells mostly forms one to many drops which are strongly light refracting. The tapetal membrane is still appressed to the remains of the middle layer and only by the mechanics of preparation sometimes loosened (Fig. 96, 97, 99). Epidermis and endothecial layer show seemingly normal nuclei, usually, especially in the region of the eye in the centre of the stomium, otherwise the cell content is degenerated in the same manner as described for the middle layer. The transversal septum appears ruptured (mechanically by the preparation or spontaneously by lysis ?).

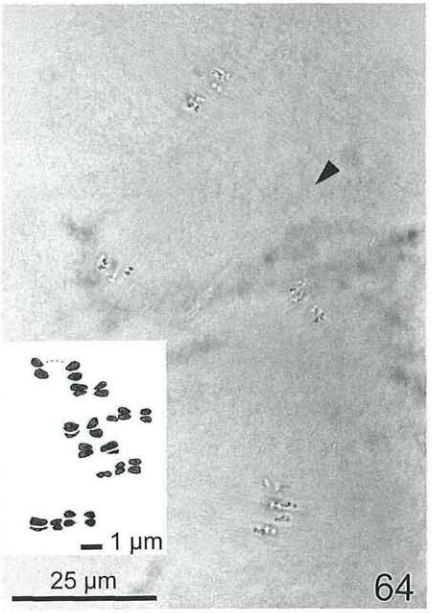
5. Discussion

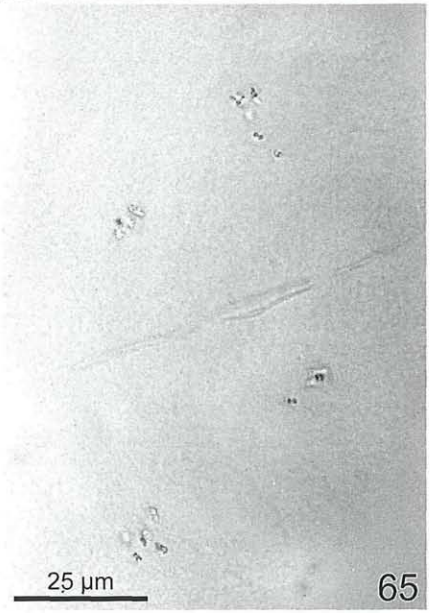
5.1.

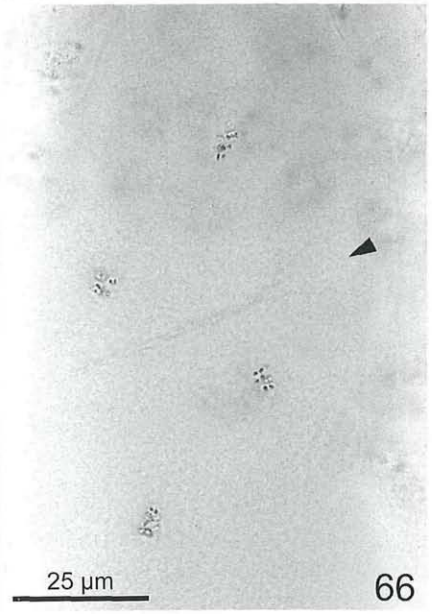
The ontogeny of the *Calliandra angustifolia* flowers is presented in PRENNER 2004. For Inga no such studies seem to exist but the paper of VAN HEEL 1993 on the related genus *Archidendron* gives an impression of the possible flower development.

Fig. 63–66. *Callianadra angustifolia*, PMCs at meiosis: interkinesis and metaphase II. – Fig. 63. The two PMCs of one locule-half at interkinesis, squashed out, apical PMC above. Nuclei a little distorted during preparation. – Fig. 64. The two PMCs (prepolyad) at metaphase II, squashed out from the locule-half. All four plates in side-view (spindles parallel to the plane of the prepolyad), apical PMC above, separating wall (with callose) distinct (arrowhead). Insert: Drawing of one metaphase II-plate (the one below right), the chromosomes consisting of two chromatids partite by the centromere. – Fig. 65. Another prepolyad at metaphase II, squashed plates in side view, apical PMC above, separating wall distinct. – Fig. 66. Ditto, photographed in two optical planes, the arrowhead points to the position of the separating wall. – In all four figures the stomiumside of the prepolyads left. Position of the nuclei and orientation of the spindles always basically the same.









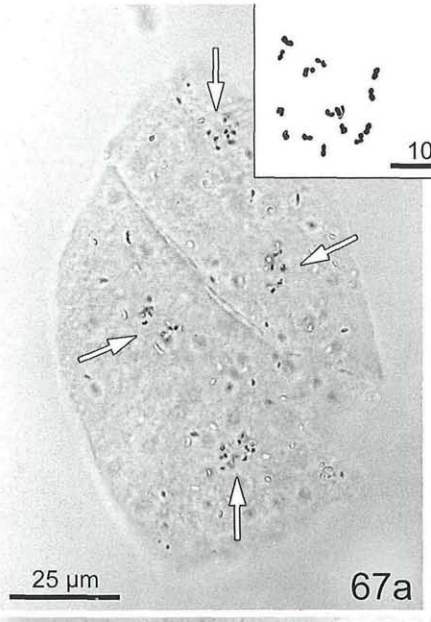
Some fundamentals of the polyad development in *Mimosaceae* are already known, at least since ROSANOFF 1866 who studied six species (*Inga* and *Calliandra* included), and observed the development in *Acacia*. He described one 'Mutterzelle' per locule-half and a granulated membrane, which clearly can be assigned as the tapetal membrane with orbicules. More comprehensive and precise is the treatment of ENGLER 1876 (*Inga* and *Calliandra* mainly p. 282–284): In the cases concerning us here, the development within one locule-half starts with one primary archesporial cell each, the 'Urmutterzelle' of the old German literature. By one to some divisions groups of two to more PMCs ('Pollenmutterzellen') are formed. ENGLER clearly states, that a 'Pollengruppe' (now polyad) is composed of two to some pollen tetrads and postulates that the inclusion of the archesporial cells in its own tapetum is the prerequisite for the formation of polyads; a good summary is included in ENGLER 1887: 152–153. An exact and clear presentation of the first developmental stages from the primary archesporial cell per locule-half to the four PMCs in *Albizia* is included in YAMASAKI 1956. Obviously *Acacia* (*Acacieae*) has found greater interest (for e. g., NEWMAN 1933, FITZGERALD & al. 1993). A short review about microsporogenesis in the family is included in PRAKASH 1987: 242, 252–253 (YAMASAKI 1956 not considered).

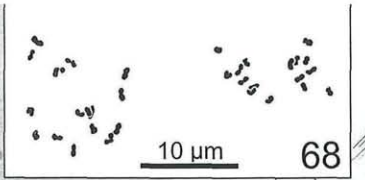
For the tapetum cells which surround the prepolyads, especially the meiotic ones, excised from the locule-halves, the term tapetum-sac is very obvious and therefore this self-explanatory term is used here. However it is necessary to avoid confusion with the 'culture sac' (= a peritapetal membrane in the sense of BHANDARI 1984: 85) of HESLOP-HARRISON 1969: 542 or the tapetal membrane; the latter is often named spore sac by *Acacia* researchers (e. g., KENRICK 2003: 121). The baseplate in the endothecial cells is a characteristic of *Mimosaceae* according to MANNING & STIRTON 1994: 145.

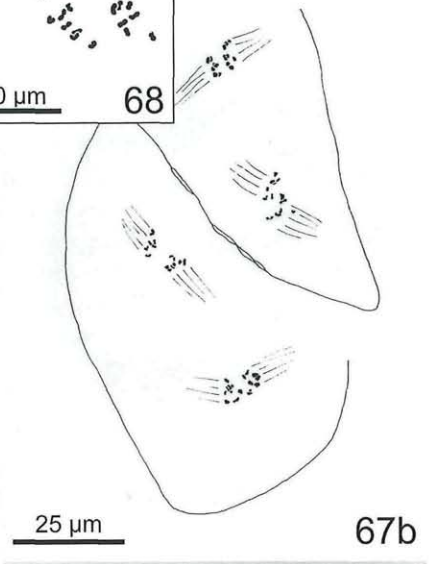
Sometimes three (rarely four) polyads per locule are formed in *Inga feuillei*. This is possible in two manners. One of the locule-halves can

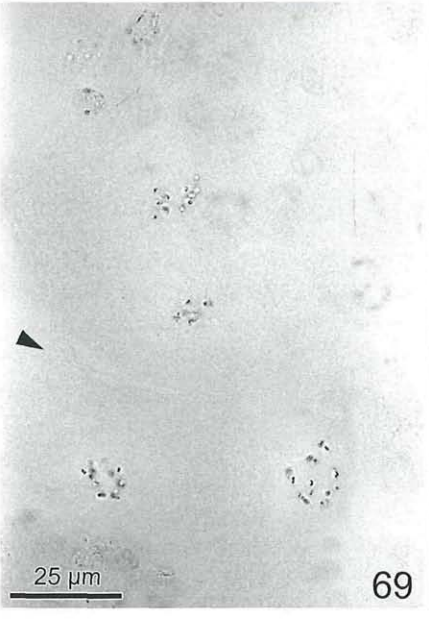
---

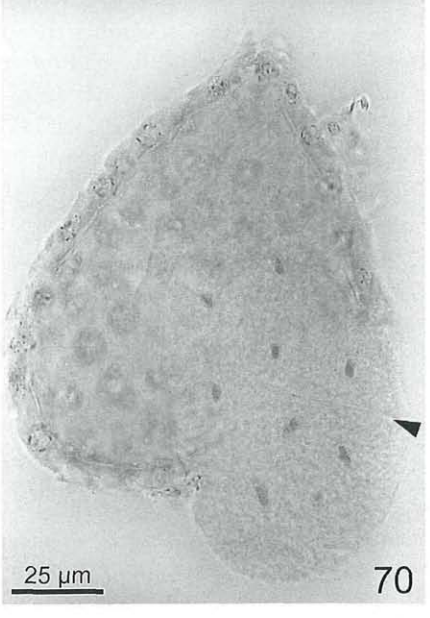
Fig. 67–70. *Calliandra angustifolia*, PMCs at meiosis II: anaphase II and telophase II. – Fig. 67 a. Prepolyad at anaphase II squashed out from the locule-half, apical PMC above. Surface of the PMCs convered with light refracting particles, arrows point to the four anaphase II – nuclei. Fig. 67 b. Drawing of Fig. 67 a. – Fig. 68. Two anaphase II nuclei with n = 8 undoubled chromosomes in each of the four plates. – Fig. 69. Prepolyad at anaphase II squashed out from the locule-half and photographed in two optical planes. The arrowhead points to the position of the separating wall between the two PMCs. – Fig. 70. The prepolyad at late telophase II, apical cell above, the nucleoli (four per PMC) reappearing, largely squashed out from the tapetum-sac. The arrowhead show the position of the separating wall between the two PMCs, no walls within the PMCs. – In all four figures the stomium-side of the prepolyads right.











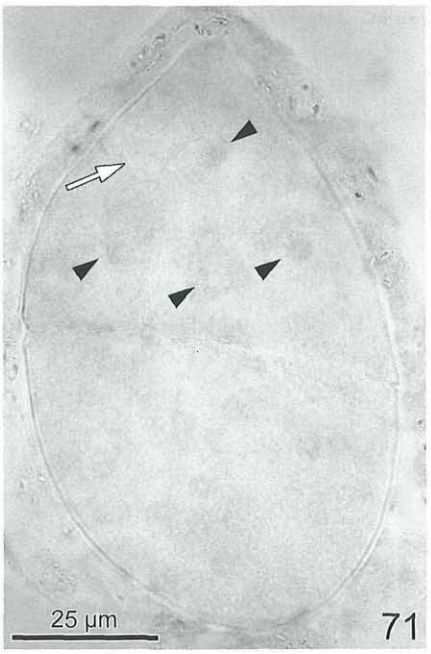
be parted into two compartments separated by a layer of tapetum (Fig. 19, 20). Otherwise, a second parenchymatous septum can be developed, so that three similar compartments (locule-thirds) arise within a locule (Fig. 16-18). Two conclusions can be drawn. 1) According to ENGLER 1876: 282 *I. affinis* and *I. edulis* possess 4–5 polyads per locule (and some other *Mimosaceae* much more; compare ENGLER 1876, DNYANSAGAR 1954, 1970: 93–95, SEIJO & SOLÍS NEFFA 2004); thus the condition of two locule-halves per locule and of one archesporial cell and polyad, respectively, per locule-half are clearly derived by reduction. 2) In studies of *Mimosaceae*, when the locules are organized in compartments, it is very important to distinguish between septation of locules by parenchymatous tissue or by tapetal layers (tapetal bridges). In the latter case it can be discussed, if the term septum is applicable for these ephemeral cells. At least an adjective would be obligatory for distinction such as parenchymatous septum and tapetal septum, respectively.

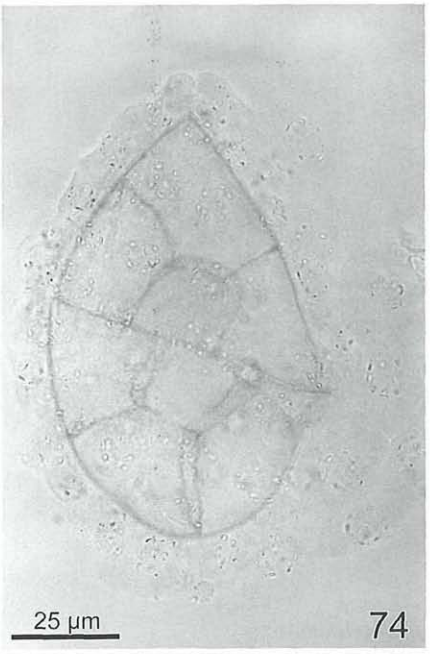
Whereas anther development in *Inga* seems not to be treated since ENGLER 1876, *Calliandra* has found more interest. Especially in DNYANSAGAR 1958: 4–6, 11–12 one primary sporogenous cell and two PMCs per locule-half forming a polyad are clearly and correctly described and figured.

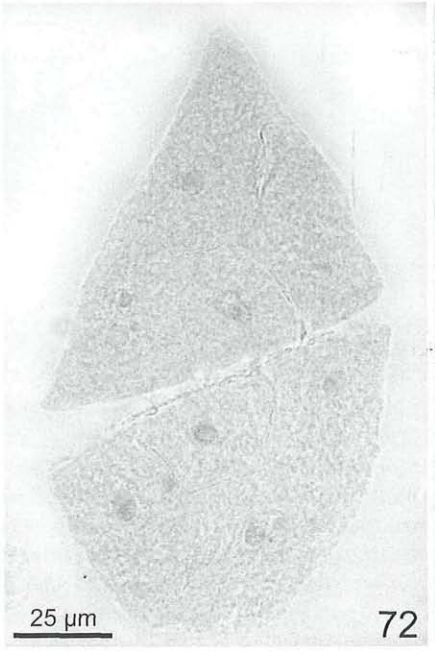
For summaries about different types of pollen adhesive see VOGEL 1984 and 2002. In *Calliandra* the pollen adhesive is formed by lysis of descendents of the middle layer (chapter 4.3.), neither from the tapetum, as supposed by RICHTER 1929, nor from the septum as believed by PRENNER & TEPPNER 2005; NEVLING & ELIAS 1971: 79 noted only, that the mucilage is formed between the apices of the two opposed polyads in a locule. Thus, the pollen adhesive of *Calliandra* can be added as a forth type to the three enumerated by VOGEL 2002.

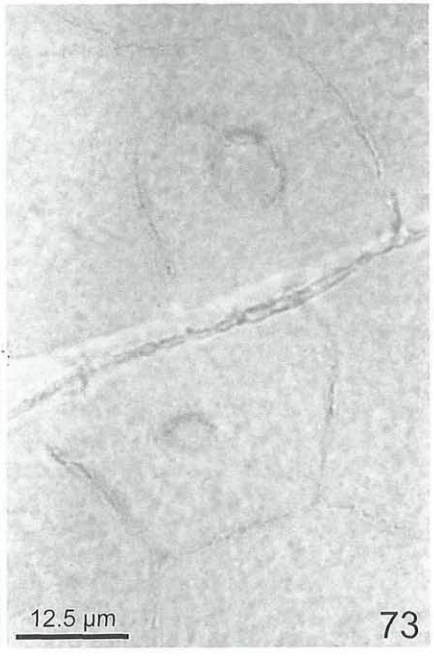
As I am aware, many details of the polyad development in *Inga* and *Calliandra* are not described till now, this is especially true for meiosis and the existence of a tapetal membrane with sporopollenin [cutinisation in the older literature (e.g., MAHESHWARI 1950: 36, 37, KOSMAT 1927); general: BHANDARI 1984: 83–85, PACINI 1997: 1453, HUYSMANS & al. 1998, 2000].

Fig. 71–74 (numbered in the ontogenetic order). *Calliandra angustifolia*, meiosis: simultaneous cytokinesis. – Fig. 71. Prepolyad, covered with a thin callose layer, within the tapetum sac. In the apical PMC arrowheads are marking the four nucleoli. The arrow points to the first sign of furrowing. – Fig. 72. The two PMCs with callose between them and with furrowing and begin of wall formation within the PMCs. – Fig. 73. Detail of Fig. 72 with the two central cells (later pollen grains). – Fig. 74. Polyad within the tapetum sac with the primary walls of the four cells (the pollen grains) within each PMC. – Outer margin (stomium-side) of the polyad at the right side in Fig. 71 and 74, left in Fig. 72 and 73.









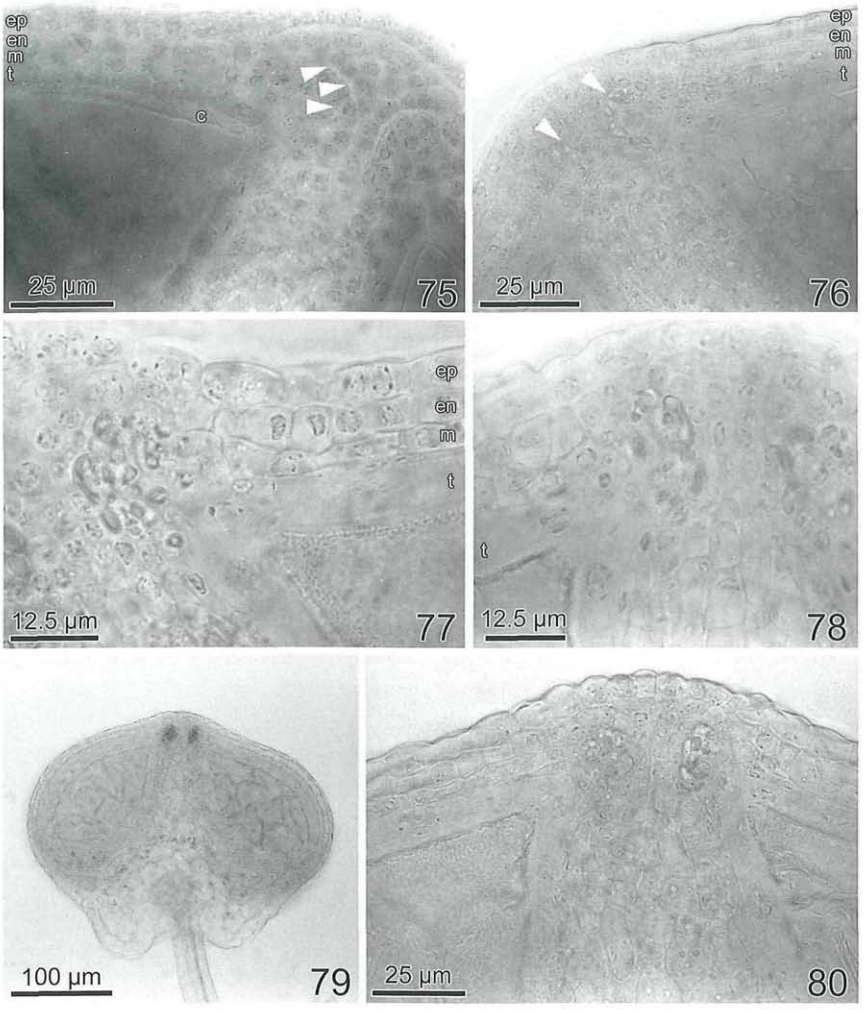


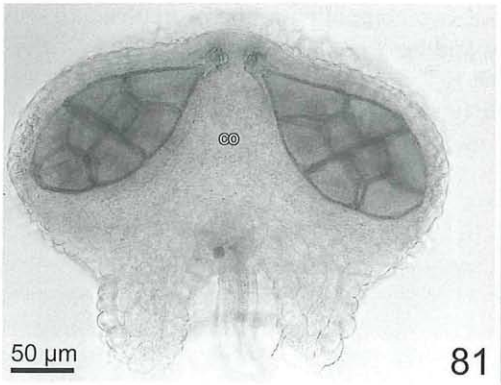
Fig. 75–80. *Calliandra angustifolia*, forming of the mucilage cells originating from the middle layer, immediately after meiosis. – Fig. 75. The difference between the cells of the septum (in the centre two layers) and the potential mucilage cells becomes distinct (marked with arrowheads at the left side). – Fig. 76. Degeneration of cells and mucilage formation begins in the cells adjacent to the septum (arrowheads). – Fig. 77. Mucilage production progressed in the direction to the polyad, tapetum still intact, also over the tip of the polyad. Septum in the centre three-layered. – Fig. 78. Ditto, especially the intact tapetum distinct. – Fig. 79. A postmeiotic anther somewhat after the start of mucilage production (dark dots). – Fig. 80. Detail of Fig. 79 showing the intact tapetum and the septum which is in its centre, between the mucilage cell groups, three-layered.

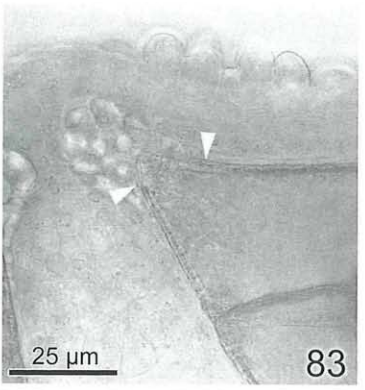
The tapetal membrane consists of two components, the orbicules and the orbicular membrane, connecting and bearing the orbicules (e. g., EL-GHAZALY & NILSSON 1991). In contrast to *Acacia* (e. g., KENRICK & KNOX 1979), in *Inga* and *Calliandra* the thin tapetal membrane is so strongly united with the theca-valve, that it is easy to overlook in the open anther. The tapetal membrane which covers the degenerated remains of the middle layer, is possibly important for the surface properties of the inner side of the open valves in connection with the adherence of the presented polyads (for *Inga* see TEPPNER & STABENTHEINER 2006, for *Calliandra* TEPPNER & STABENTHEINER 2007). Functions of the orbicules associated with the dispersal of pollen grains [in cases with single grains (monads)], are discussed sometimes earlier, e. g., ECHLIN 1971: 53, KEIJZER 1987: 502, 503, PACINI & FRANCHI 1993: 5, PACINI 2000: 33 and HUYSMANS & al. 1998: 248–249. Especially non-wettability is discussed for angiosperms (e. g. KEIJZER 1987: 502, 503).

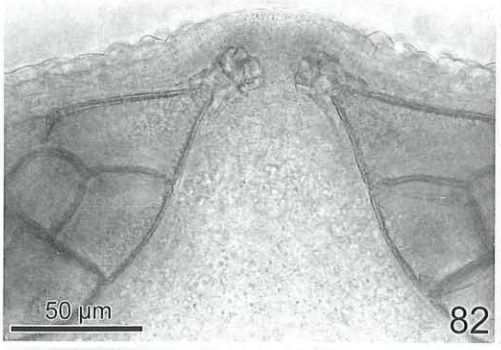
In *Inga feuillei* there is some variability in the number of PMCs per prepolyad (8–12) and consequently also in the number of pollen grains (32–48), but 32 is undoubtedly the most frequent one. Usually the order of the pollen grains in the polyad reflects the former PMCs clearly in both genera and shows also high variability in *Inga*; here, within the PMC, the most abundant bauplan has its origin in a tetrahedral order of the telophase II nuclei in the PMCs and corresponds to the model of grain orientation drawn by WODEHOUSE 1935: 433 for 16-grained polyads in *Acacia* (two grains from each PMC peripheral in the plane of the polyad, the other two, the central ones, perpendicular in two planes). Because of the many deviations which are possible, counting of the grains on both sides of the polyad (in young or cleared polyads) is necessary for a correct number of pollen grains. In the scheme Fig. 20 h-l in YAMASAKI 1956: 439 the natural three-dimensional order of the grains in the 16-grained polyads of *Albizia glabrior* is masked, because all spindles and grains are drawn in one and the same plane.

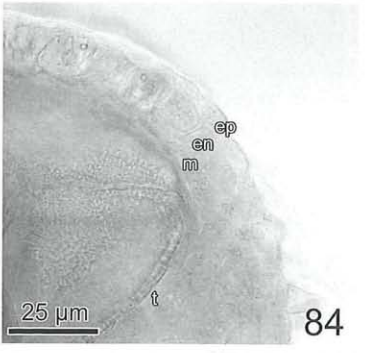
It is supposed that the positions of the meiotic spindles in the PMCs (in all its variability) and the resulting positions of the telophase II nuclei are sufficient conditions for the configuration of the grains within the tetrad. It does not seem to be necessary to assume postmeiotic movements of the cells under the effects of surface tension (e. g., MELVILLE 1981). Furthermore, in *Inga* and *Calliandra* such movements would be hindered by the lack of space.

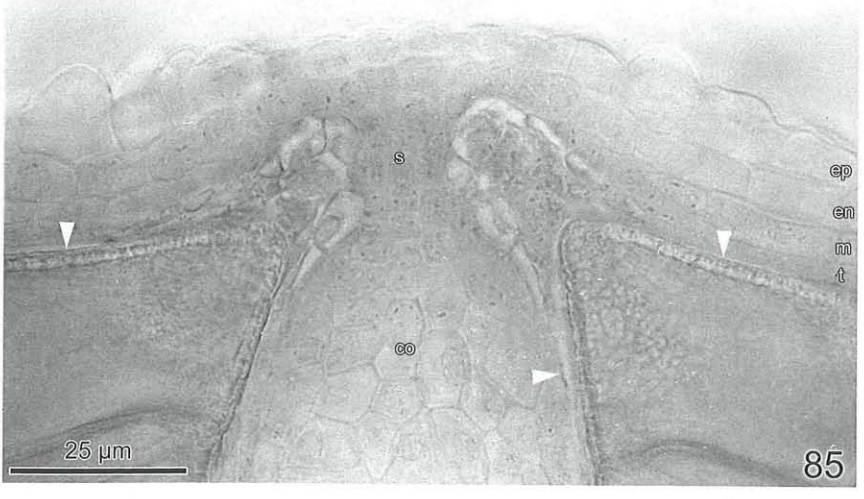
The TEM study of *Acacia paradoxa* by FITZGERALD & al. 1993 shows the details of polyad development very well. Surprising are the premeiotic degeneration of tapetum and middle layer (!), but compare the doubts on such an early degeneration of the tapetum in ROWLEY 1993: 36 as well as SANTOS & al. 2003: 196. Comparable TEM studies do not exist for *Inga* and *Calliandra*.

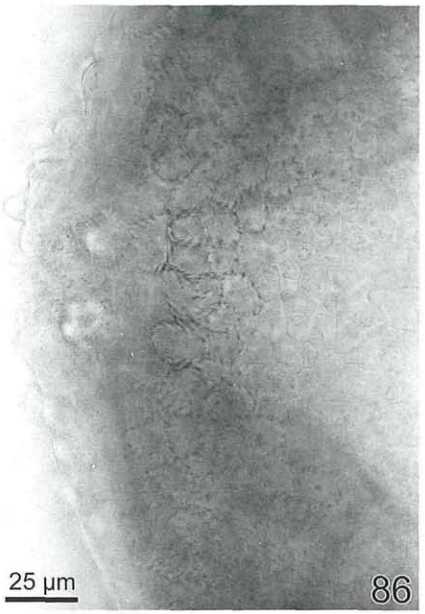












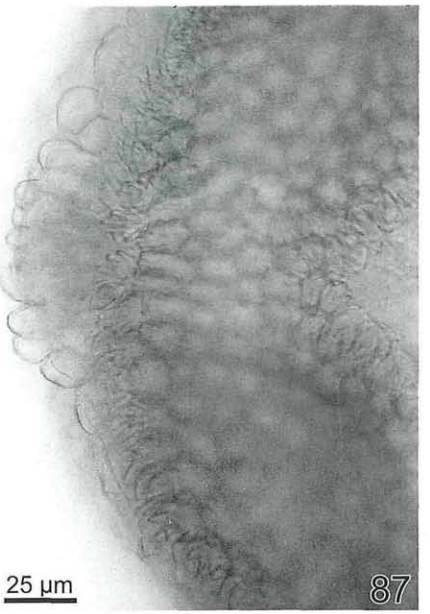
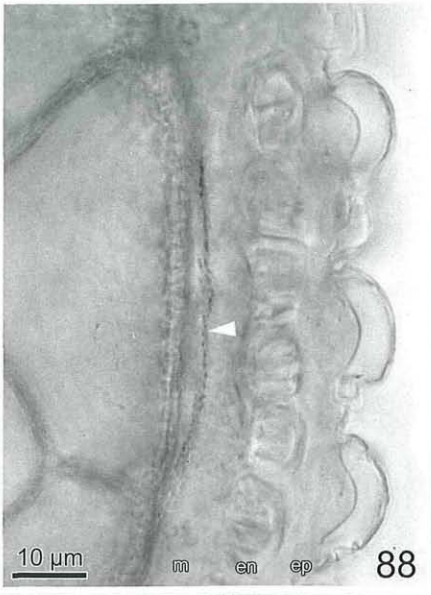
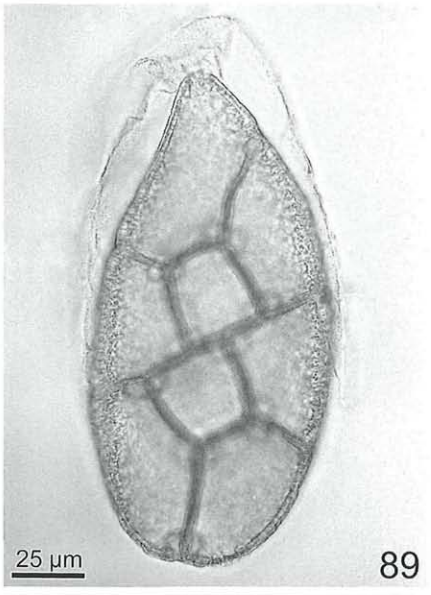
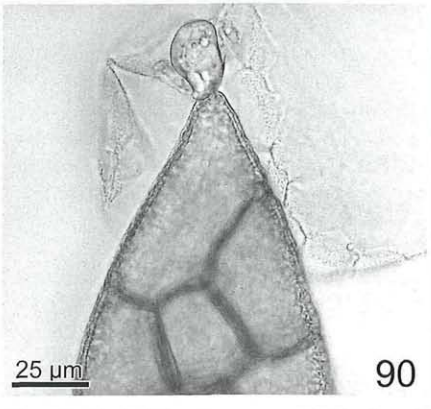


Fig. 86–87. *Calliandra angustifolia*. Distribution of endothecial thickenings. – Fig. 86. Between the locule-halves, endothecial cells with thickenings (dark dots in optical section) form a narrow band only on the outer side of the theca. – Fig. 87. A wide zone of endothecial cells with thickenings is developed on the inner valve of the theca. In the figure, the marginal ones only lie in the optical plane (as a consequence of the arching of the valve).

Fig. 81–85. *Callianadra angustifolia*. Anthers with tapetum reduced to a thin layer, with tapetal membrane, with fully developed mucilage and the growing of the epidermal papillae. – Fig. 81. Longitudinal optical section through the whole anther showing the two polyads of one locule, the massive connective and the filament isthmus. – Fig. 82. Detail of Fig. 81 with the mucilage drops in the chambers below the 'eye', the septum between the drops and the connective protrusion below. – Fig. 83. Details of 'eye', mucilage drop, polyad tip, septum and connective protrusion. In situ the tapetal membrane is often difficult to discern (arrowheads). – Fig. 84. Basal end of a polyad with tapetal membrane distinct. Endothecial thickenings can be seen in this optical section. – Fig. 85. As Fig. 83. Septum between the mucilage drops three-layered, arrowheads point to the tapetal membrane.







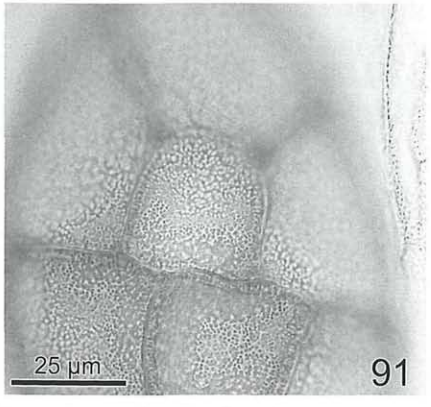


Fig. 88–91. *Calliandra angustifolia*, tapetal membrane shortly after degeneration of the tapetum. – Fig. 88. Polyad tip in situ, the tapetal membrane between exine and middle layer or adjoining to the middle layer (arrowhead) where it is a little separated during preparation. – Fig. 89. Tapetal membrane enveloping the polyad, liberated from the locule-half. The tapetal membrane is attached to the tip of the polyad and thus a little invaginated like a glove-finger by the preparation. – Fig. 90. The mucilage drop adheres to the tapetal membrane fixed at the polyad's tip. – Fig. 91. Detail of a polyad with the ornament of the columellae in the exine and a fragment of the adhering tapetal membrane.

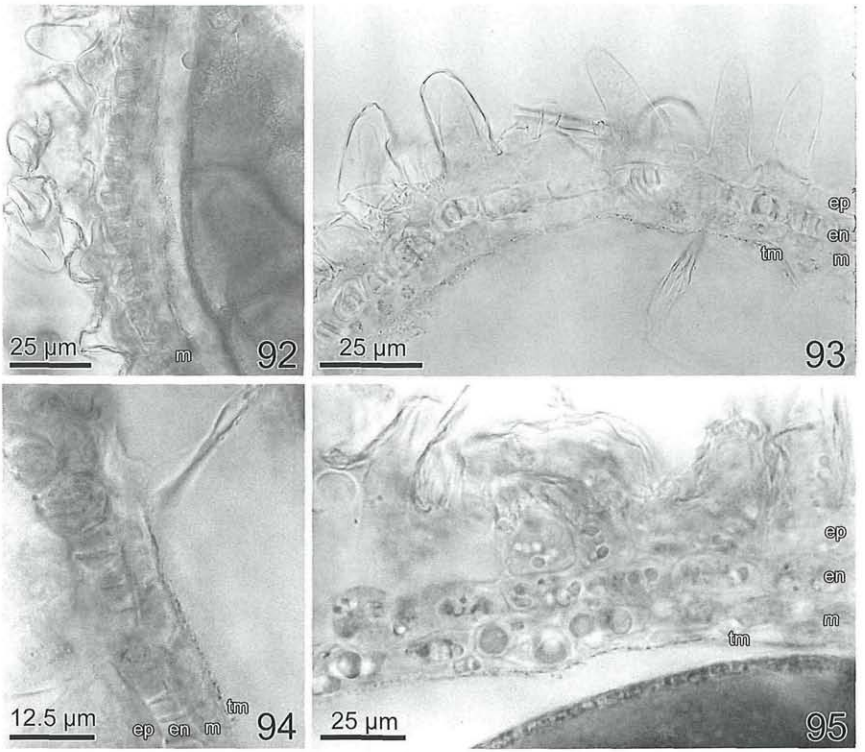


Fig. 92–95. *Calliandra angustifolia*. Anthers with completely disappeared tapetum and content of the middle layer cells more or less in degeneration. Tapetal membrane adjoining or appressed to the middle layer. – Fig. 92–94. Middle layer cells with distinct nuclei. – Fig. 95. Content of middle layer, endothecial and epidermis cells completely degenerated to strongly light refracting drops.

In respect of the few investigated examples, the conclusion of PACINI 1997: 1453 that orbicules 'are absent in species with strictly entomophilous [should probably be replaced by zoophilous in contrast to anemophily ?] pollination like *Cucurbita pepo* in which pollenkitt is present' seems to be overhasty. *Inga* as well as *Calliandra* possess orbicules and pollenkitt. Thus the older formulation in PACINI & FRANCHI 1993: 5 seems to be more appropriate (' ... in most entomophilous taxa they coexist with pollenkitt. ...'). In *Acacia* the situation concerning pollenkitt seems to be not so clear. PACINI 1997: 1455 cites KENRICK & KNOX 1989 for the completely lack of pollen coating in polyads, but no relevant phrase was found in this paper. It is BERNHARDT 1989: 266, 268 who writes that polyads appear to lack copious deposits of pollenkitt and about absence of pollenkitt and yet figures small droplets of pollenkitt in *Acacia pycnantha* (in or on the

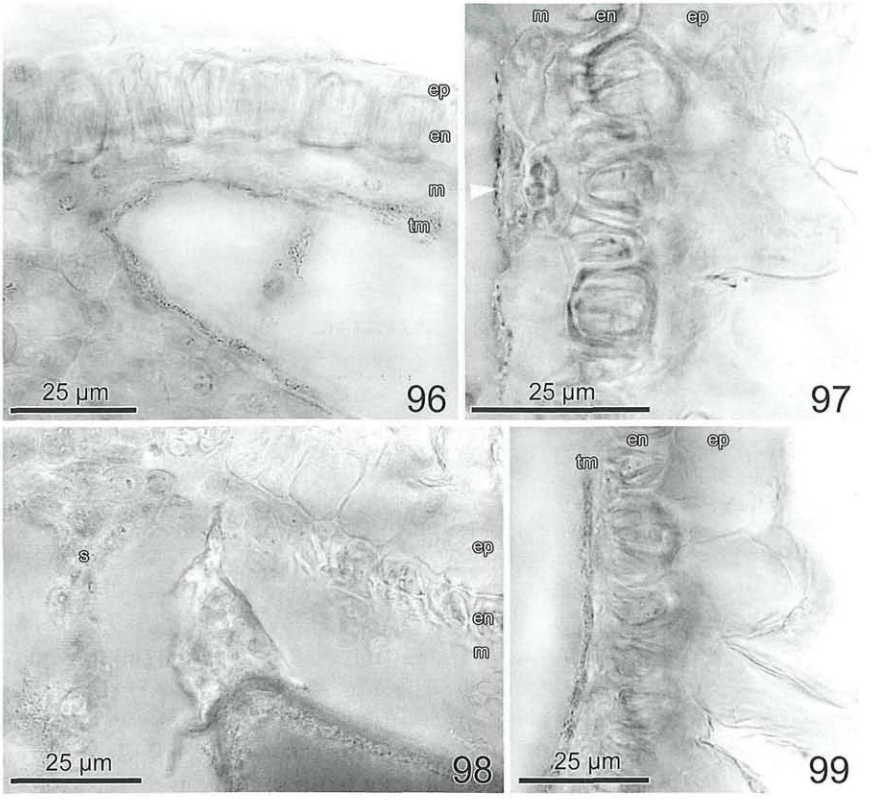


Fig. 96–99. *Calliandra haematocephala*. Ripe anthers at the day before anthesis. Cells of middle layer with degenerated content or nearly completely reduced. – Fig. 96. Polyad removed from the locule-halve, cavity coated with the tapetal membrane, one fragment of it broken away by the preparation. – Fig. 97. Middle layer distinctly two layered (arrowhead) with degenerated cell content. In some endothecial cells the baseplate discernible. – Fig. 98. The left mucilage chamber empty by the preparation, in the right one, the mucilage drop a little attached to the wall has stringed a thread (thus it is liquid). Tapetal membrane between mucilage drop and tip of the apical cell of the polyad. – Fig. 99. Middle layer largely lacking, tapetal membrane nearly at the endothecium.

pores ?). KNOX 1984: 218 himself writes, that the polyads of *Acacia* have sticky pollen-coat materials. According to the literature (e. g. KENRICK & KNOX 1979: 415) and own observations (ESEM, with E. STABENTHEINER, mainly on *Acacia celastrifolia, A. longifolia* and *A. caven*), the tapetal membrane is more robust and less strongly connected with the valve in melittophilous *Acacia*. The tapetal membrane-envelope of each polyad opens separately with or shortly after the start of anther-valves opening; the membrane may be more or less connected with the valve (and is then bent outwards) or stands free between valve and polyad. The polyads, with faces parallel to each other, loosely adhere with the margins, usually, in the depth between the inward bulges of the two valves of a theca. Because of the smallness of anthers and polyads I was not able to visualize pollenkitt directly. Excellent TEM images, e. g. FITZGERALD & al. 1993: 57, e. g. Fig. 17A, show no structure between tapetal membrane and pollen grain, but because of the dehydration with acetone this has no validity. From the observation of the bright contact zones of dry polyads in air on a cover slip (see methods; Fig. 100–101), it can be seen, that the *Acacia* polyads are covered throughout with a thin film of pollenkitt. Thus, also *Acacia* possesses orbicules and pollenkitt as well and the latter is responsible for the adherence of the polyads with their margins between the inward folded longitudinal bulges of the valves (or, after disturbance, with a face on a bulge) in the open anthers.

Because of the persistence of the tapetal membrane the pollenkitt must be secreted through the tapetal membrane (e. g., KEIJZER 1987: 501; compare also Fig. 15 and 16 in SANTOS & al. 2003). Beyond that, nothing can be said about the fate of the pollenkitt in our material because of ethanol and chloroform in the fixative.

The chromosome number in *Inga feuillei* with 2n = 26 and n = 13 is the same as in earlier reports for other species (HANSON 1997). Chromosome morphology in *I. feuillei* is treated by TEPPNER 1998: 43 from material of the same population. The number for *Calliandra angustifolia* (2n = 16, n = 8) is reported for the first time and is the same as indicated for the majority (6) of the counted species (9); one (*C. pittieri*) is tetraploid on this base: see the Index of Plant Chromosome Numbers at the Missouri Bot. Garden's web site www.mobot.org . The reports for sect. *Calliandra* ser. *Calliandra* only, seem to be problematic and need revision because n = 8 is reported for *C. houstoniana* and 2n = 22 for *C. houstoniana* var. *calothyrsus* (as *'C. confusa'*) and *C. physocalyx*. The result of the own counts on a number of good plates from root tip cells from one individual of *C. houstoniana* var. *calothyrsus* is 2n = 20 (Fig. 102)! Thus further investigations to clarify the appropriate chromosome number(s) in this group are needed urgently.

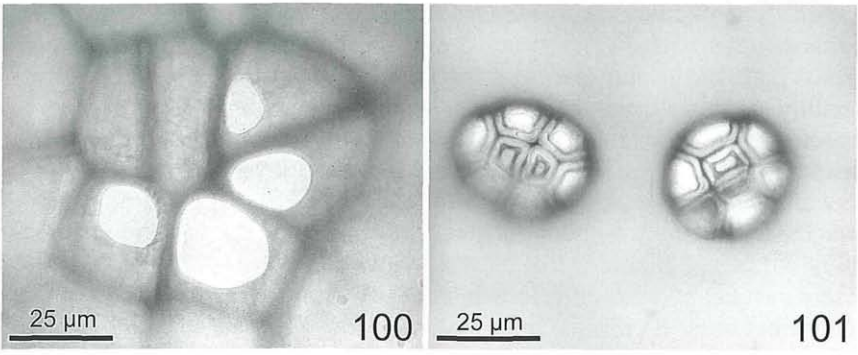


Fig. 100–101. Polyads in air, hanging on the under side of a cover slip, with plaques of pollenkitt at the contact zones. – Fig. 100. *Inga feuillei*. – Fig. 101. *Acacia celastrifolia*, the left polyad with 5, the right one with 6 contact zones.

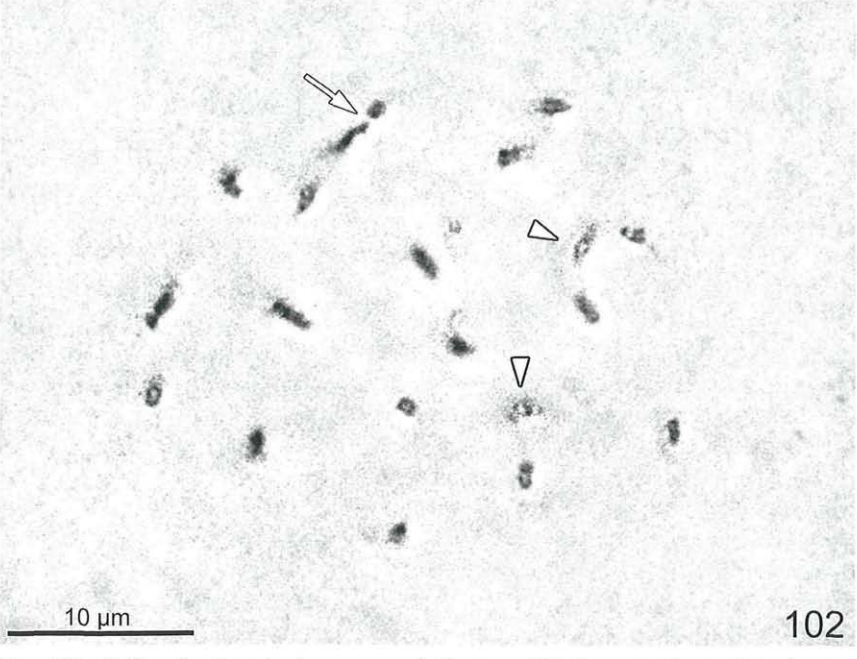


Fig. 102. *Calliandra houstoniana* var. *calothyrsus*. Mitotic metaphase plate from a root tip with 2n = 20 chromosomes. Arrowheads: chromosomes a little out of the optical plane. Arrow: space between two different chromosomes.

5.2. Comparision of *Inga* and *Calliandra*

The anther development and microsporogenesis in *Inga* and *Calliandra* are principally the same, but Calliandra shows remarkable specializations.

It is evident that the c. eight PMCs in each locule-half in *Inga* is a more primitive condition and the two PMCs in *Calliandra* are derived. In both genera metaphase I spindles lie in one plane. Metaphase II spindles are oriented perpendicular (decussate) within one PMC (as usual in many other dicots) in *Inga*, whereas all spindles are in the same plane in *Calliandra*, with the corresponding consequences for the order of the pollen grains. In *Inga*, because of the high number of grains, the polyad is complex with c. the half of the grains in two planes (e.g., the figures in TEPPNER & STABENTHEINER 2006: 153–156). In *Calliandra* the order is stronger with all grains in one plane, two central ones and one grain forming the apex of the polyad; deviations are relatively rare. Whereas the *Inga* polyads are roundish to elliptic and distinctly asymmetric in side view only, the polyads of *Calliandra* are asymmetric in all directions. The apparent heteropolar condition of the polyad in *Calliandra* is clearly a derived character also. The size of the 32-grained polyads in *Inga feuillei* and the 8-grained ones in *Calliandra angustifolia* is approximately the same (both c. 150 µm long). The association of the grains is much stronger in *Calliandra* than in *Inga*. The tapetal membrane with the sporopollenin orbicules has the same appearance and fate in both genera, but in *Calliandra* it must break around the base of the drop before or during anther opening. The drop of pollen adhesive in *Calliandra* is unique within the family. A possible presence in the related *Guinetia* can not be seen clearly from RICO ARCE & al. 2000. It may be permitted to speculate, that the mucilage drop could be a result of specialization during evolution. If so, a primitive, unspecialized condition could have been a middle layer (or more probably the inner layer of a two or three-layered middle layer) becoming mucilaginous at a large extent which covers the polyad largely with a continuous film of slime. Over restriction of the mucilage to the corner of the cavity near the septum the specialized condition of *Calliandra* could have been arisen. Thus, in further research in *Mimosaceae* it should be paid attention to the possibility of extratapetal mucilage occurrence. A thin layer of mucilage could be difficult to be distinguished from pollenkitt.

The parenchymatous, transversal septum in *Calliandra* remains very small in comparison to *Inga*, thus the lumen of the locule-half appears narrowed at the proximal end and forms the mucilage chamber. Whereas the transversal septum in *Inga* is dissolved as usual for the longitudinal septum (see figures in TEPPNER & STABENTHEINER 2006), this septum is degenerated in *Calliandra* in part, so that cell structure is no longer discernible and separates the two mucilage chambers of one valve. It could

not be decided if it separates from the connective protrusion before or during anther opening. From the connective protrusion the valve breaks most probably during anther opening.

An interesting candidate for a detailed comparison with *Calliandra* would be *Guinetia* with similar polyads, which '... shows a poorly developed basal cell and a very reduced sticky appendage, ...' (RICO ARCE & al. 2000: 977) (= a lesser differentiated apical cell and very few pollen adhesive in our terminology).

5.3. Comments to the *Calliandra* Paper in Flora

This paper contains a lot of incorrect statements and wrong conclusions. The corrections and the reasons for it are presented in this chapter.

Back to introduction, point 1): The primary cell in the locule half should be a PMC. It has been described and documented with photos in the previous pages, that the primary cell is always an archesporial cell which shows seven mitotic divisions (followed by cytokinesis) or few more in *Inga* and only one in *Calliandra*, thus, in the latter, two PMCs are formed in which then meiosis occurs. This is basically known since ENGLER 1876 and 1887 and well described in YAMASAKI 1956 for *Albizia* and in DNYANSAGAR 1958 for *Calliandra*. It is paradox, that the correct statement of DNYANSAGAR is explicitly contradicted by GREISSL 2006: 580 [citing the wrong page (p. 12), correct would be p. 6, among others with the phrase '... in *Calliandra* species, the number of microspore mother-cells formed per microsporangium is limited to 2' and the relevant figures.] Such apparent mistakes are rare, I know only two further ones: KAPP 1969: 245 in a wrong definition of polyad ('a symmetrical group of pollen grains which develop as a single unit, apparently from a single microspore mother cell') and CHEN 1973. The latter shows the basis of his conclusions by a series of photos of his cuts through anthers of *Calliandra haematocephala*, thus the paper is helpful for an understanding of the origin of such mistakes. CHEN describes primary archesporial cells (his Fig. 3–5) and the two young PMCs after the mitotic division of the archesporial cell (Fig. 6–12). [Fig. 5 is an excellent figure for a three-layered, Fig. 7 and 8 for a fully developed theca wall]. The archesporial cell is mistaken as PMC and consequently the two-celled stage is erroneously seen as meiotic product. Then, meiosis is overlooked. The following sequences (Fig. 13 onwards) are cuts through more or less fully developed polyads. From cuts in which not all grains of the polyads or wedges or degenerated grains are affected, he constructed a curious, non existing sequence of divisions. GREISSL has not demonstrated any basis for his conclusions, but the evidence suggest similarities, even when GREISSL 2006: 579 writes '... the details given by CHEN (1973) could not be confirmed'. The postulated sequences of divisions (p. 572, 574–575, 578–580) are unreal and not based on observations.

This first misinterpretation leads to a lot of further consequences. Among others, the first division and cytokinesis in the locule has nothing to do with meiosis. The furrowing or wall formation, respectively, in the two PMCs, which lead to the 2 × 4 pollen grains in the polyad is doubtless simultaneous, as nearly obvious in dicotyledons.

If, in *Mimosaceae*, in the primary cell in the locule-half meiosis should occur and the four (as consequence of meiosis) daughter cells should divide mitotically (e. g., p. 580: 'the cells of the tetrad secondarily increase in number') and result in other grains which produce the male gametophytes (other words for the theory in GREISSL 2006), then we would have a third generation between sporophyte and gamentophyte (a minute, haploid, second sporophyte): This would be very curious and interesting, though highly improbable for an angiosperm!!

Point 2) of the introduction: In the alleged PMC (in reality an archesporial cell) the first wall should be oblique (p. 572, 577–578) with an angle of 120° of the wall between the two new cells originating from the first division. What is the reference point for the angle ? Some papers, for example, SINNOT 1960 and WODEHOUSE 1935, are used for strengthening the reasons for an angle of 120°. At first the wall in the archesporial cell (in respect to the cell itself) is originally transversal. Secondly in SINNOT 1960: 46 the ominous 120° are mentioned for a completely other angle: in the example, a round cell is divided transversally as the archesporial cells (for e. g., our Fig. 49 and 52); in the case of free cells (not true in *Inga* and *Calliandra*) and theoretically with a liquid film surface, an intention is formed where the new wall meets the mother cell surface; thus at the meeting point an angle of 120° is formed between the transversal new wall and the tangent to the circumference circles of each daughter cell. Nothing is said about an oblique position of the first wall. Thirdly, to WODEHOUSE 1935. The reference pages are also not cited, but I think that p. 177–200 are meant, where he discusses the angle of 120° in describing the triradiate symmetry, for e. g., the arrangement of colpi on the surface of a pollen grain and the mode of contact of three cells such as pollen grains in a tetrad. To my opinion there is no connection of the mentioned results with the one wall separating the two PMCs after division of the archesporial cell.

Furthermore, on p. 572, 574 and 578 a 'tetrad stage' is stressed. If – as here – the first cell is counted as PMC, a postulated tetrad stage can not exist. Or the two PMCs at interkinesis together should be regarded as tetrad (four nuclei in the prepolyad) ? Also improbable, because there are no walls between the two nuclei in each PMC and the size of the tetrad is indicated as more than 50 % of the polyad, whereas it is 100 % at the interkinesis stage. So from the text it is not to be concluded what is meant by the 'tetrad stage'. 'The final, successive divisions [after tetrad stage] show a clear evidence of bipolarity'. The strong heteropolarity of the PMCs is fully

expressed at premeiotic stage. 'In *Calliandra*, polarity is induced by the theca. (DAWE & FREELING 1992)'. The cited paper concerns not *Calliandra* but exclusively *Zea mays* anthers; chimeric anthers were used to estimate the number of initial cells, the origin of the symmetry of the mature anther etc. Every anther or theca shows polarity, which is nearly isopolar in *Calliandra*; crucial is the heteropolarity of the locule-halves and in relation with the polyad in *Calliandra*, only these can be important for the induction of heteropolarity.

The reproduction of the figures of the polyad of *Calliandra houstoniana* var. *anomala* from MOHL 1834: Tab. V Fig. 11 (as *Inga anomala*) is inverted without notice. The two figures are praised as 'a beautiful drawing' (p. 574), but they are not fully correct because the side view is shown as exactly symmetrical. Matter of fact is that the *Calliandra* polyads are asymmetrical in side view, even when this can be lesser expressed in some species (for e. g., *C. haematocephala*).

On p. 575, 576, 580 stainability and small quantity of pollenkitt are discussed but there are no detailed indications of localization, amount and appearance of it.

To point 3): The origin of the drop of pollen adhesive is not mentioned explicitly. But phrases on p. 572 and p. 580 (for e. g., 'The viscin body is formed on the modified octad tip cell as a final step.') suggest, that a secretion from the apical grain is supposed. According to our investigations, the origin of the pollen adhesive has nothing to do with the polyad, its origin is clearly extratapetal (PRENNER & TEPPNER 2005, TEPPNER 2007: 234 and this paper chapter 4.3.). More than once it is stressed, that the pollen adhesive should be a solid body (for e. g., p. 572: 'When the theca opens, this drop-like body ... seems to be solid, ...'), but even with a good stereomicroscope, during anther opening, the character of a viscous fluid can be seen. The scheme of the behaviour of the drop of pollen adhesive during and after adherence on p. 582 is nice; what other a drop of similar viscosity should do according the physical laws ? Same is true for the Fig. on p. 580; additionally, comments on the potential function of pollenkitt are lacking here. Surprising is the viscosity diagram on p. 582 with the degree of viscosity and the time-scale as vectors. No methods for measurements of viscosity are indicated, although by the graph such measurements are suggested. The determination of melting points can not be a scale for viscosity. And the diagram with a solid initial phase is wrong in any case. Our impression (from the observation of the drops, without measurements) is, that the viscosity is lowest in the moment of anther opening and increases with desiccation. The diagram is imaginary according to my knowledge. A pity, that the author didn't have proper guidance.

6. Acknowledgements

The figures are an essential part of this paper; therefore specific, sincere thanks to Mr. P. KOSNIK for the developing of the negatives and the scans of the images and especially also to Ass.-Prof. Dr. W. OBERMAYER for the troublesome and time consuming editing of the figures. Without this help the original intention for this paper, to avoid further misunderstandings by the presentation of a lot of figures, would not have been possible to realize. My heartfelt thanks to Mag. Dr. U. BROSCH for procuring literature which was not available in our institute. Many thanks to Ass.-Prof. Dr. E. STABENTHEINER for an ESEM photo (Fig. 47) as well as for the possibility to observe *Acacia* anthers in the ESEM. I am grateful to Prof. Dr. V. RIBITSCH, Dr. A. BIZZARI and Mag. Dr. R. TEPPNER for helpful discussions concerning the interpretation of pollenkitt as an optical gel. Sincere thanks to Ass.-Prof. Dr. E. STABENTHEINER and Mag. P. HARVEY for the careful check of the language.

7. References

BERNHARDT P. 1989. The floral ecology of Australian *Acacia*. – In: STIRTON C. H. & ZARUCCHI J. L. (eds.), Advances in Legume Biology. – Monogr. syst. Bot. Missouri bot. Garden 29: 263–281.

BHANDARI N. N. 1984. 2. The Microsporangium. – In: JOHRI B. M. (ed.), Embryology of Angiosperms, p. 53–121. – Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.

CHEN Y.-Y. 1973. Studies on the development of polyad grains of *Calliandra haematocephala* HASSK. with electron microscopic technique. – Taiwania 17(1): 18–28.

DARLINGTON C. D. & LA COUR L. F. 1963. Methoden der Chromosomenuntersuchung. – Kosmos, Frankh'sche Verlagshandlung, Stuttgart.

DAWE R. K. & FREELING M. 1992. The role of initial cells in maize anther morphogenesis. – Development 116: 1077–1085.

DNYANSAGAR V. R. 1954. Embryological studies in the *Leguminosae*. VI. Inflorescence, sporogenesis, and gametophytes of *Dichrostachys cinerea* W. & A. and *Parkia biglandulosa* W. & A. – Lloydia 17(4): 263–274.

— 1958. Embryological studies in the *Leguminosae*. VIII. *Acacia auriculaeformis* A. CUNN., *Adenanthera pavonina* LINN., *Calliandra hematocephala* HASSK., and *Calliandra grandiflora* BENTH. - Lloydia 21(1): 1–25.

— 1970. *Leguminosae*. – Proceedings of the symposium of comparative embryology of Angiosperms. – Bull. indian nation. Sci. Acad. 41: 93–103.

ECHLIN P. 1971. The role of the tapetum during microsporogenesis of Angiosperms. – In: HESLOP-HARRISON J. (ed.), Pollen: Development and physiology, p. 41–61. – Butterworths, London.

EL-GHAZALY G. A. & NILSSON S. 1991. Development of tapetum and orbicules of *Catharanthus roseus* (*Apocynaceae*). – In: BLACKMORE S. & BARNES S. H. (eds.)., Pollen and Spores. – Systematics Association special Volume 44: 317–329. – Clarendon Press, Oxford.

ENGLER A. 1876. Beiträge zur Kenntniss der Antherenbildung der Metaspermen. – Jahrb. wiss. Bot. 10: 275–316, 5 plates.

— 1887. Angiospermae. – In: ENGLER A. & PRANTL K. (eds.), Die natürlichen Pflanzenfamilien, 2(1/Lief. 13): 145–176. – Engelmann, Leipzig.

FITZGERALD M. A., CALDER D. M. & KNOX R. B. 1993. Character states of development and initiation of cohesion between compound pollen grains of *Acacia paradoxa*. – Annals Bot. 71: 51–59.

HANSON L. 1997. Chapter 3. Cytology. – In: PENNINGTON T. D., The genus *Inga*. Botany, p. 31–33. – The Royal Botanic Gardens, Kew.

HEEL W. A. VAN 1993. Floral ontogeny of *Archidendron lucyi* (*Mimosaceae*), with remarks on *Amherstia nobilis* (*Caesalpiniaceae*). – Bot. Jahrb. Syst. 114(4): 551–560.

HESLOP-HARRISON J. 1969. An acetolysis-resistant membrane investing tapetum and sporogenous tissue in the anthers of certain *Compositae*. – Canad. J. Bot. 47: 541–542, 2 plates.

HUYSMANS S., EL-GHAZALY G. & SMETS E. 1998. Orbicules in Angiosperms: morphology, function, distribution, and relation with tapetum types. – Bot. Review 64(3): 240–272.

— , — & — 2000. Orbicules: still a well-hidden secret of the anther. – In: NORDENSTAM B., EL-GHAZALY G. & KASSAS M. (eds.), Plant systematics for the 21st century. – Wenner-Gren international Series 77: 201–212. – Portland Press, London.

KAPP R. O. 1969. Pollen and spores. – The picutred-key nature series. – Wm. C. Brown Comp. Publ., Dubuque, Iowa.

KEIJZER C. J. 1987. The processes of anther dehiscence and pollen dispersal. II. The formation and the transfer mechanism of pollenkitt, cell-wall development of the loculus tissues and a function of orbicules in pollen dispersal. – New Phytologist 105(3): 499–507, 4 plates.

KENRICK J. 2003. Review of pollen-pistil interactions and their relevance to the reproductive biology of *Acacia*. – Austral. syst. Bot. 16: 119–130.

— & KNOX R. B. 1979. Pollen development and cytochemistry in some Australian species of *Acacia*. – Austral. J. Bot. 27: 413–427.

— & — 1989. Pollen-pistil interactions in *Leguminosae* (*Mimosoideae*). – In: STIRTON C. H. & ZARUCCHI J. L. (eds.), Advances in Legume Biology. – Monogr. syst. Bot. Missouri bot. Garden 29: 127–156.

KNOX R. B. 1984. The pollen grain. – In: JOHRI B. M. (ed.), Embryology of Angiosperms, p. 197–271. – Springer-Verlag, Berlin, Heidelberg, New York.

KOSMAT L. 1927. Studien über das Antherentapetum. – Österr. bot. Z. 76(3): 235–241.

MAHESHWARI P. 1950. An introduction to the embryology of Angiosperms. – McGraw-Hill Book Company, New York, Toronto, London.

MANNING J. C. & STIRTON C. H. 1994. Endothecial thickenings and phylogeny of the *Leguminosae*. – In: FERGUSON I. K. & TUCKER S. (eds.), Adv. Leg. Syst. 6: 141–163.

MELVILLE R. 1981. Surface tension, diffusion and the evolution and morphogenesis of pollen aperture patterns. – Pollen et Spores 23(2): 179–203.

MOHL H. 1834. Ueber den Bau und die Formen der Pollenkörner. Mit VI lithographirten Tafeln. – Beiträge zur Anatomie und Physiologie der Gewächse, 1, IV + 130 Seiten. – Fischer, Bern.

NEVLING L. I. & ELIAS T. S. 1971. *Calliandra haematocephala*: History, morphology, and taxonomy. – J. Arnold Arboretum 52: 69–85.

NEWMAN I. V. 1933. Studies in the Australian Acacias. – II. The life-history of *Acacia Baileyana* (F. v. M.). Part I. Some ecological and vegetative features, spore production, and chromosome number. – J. linn. Soc. Bot. 49: 145–171, 4 plates.

PACINI E. 1997. Tapetum character states: analytical keys for tapetum types and activities. – Canad. J. Bot. 75: 1448–1459.

— 2000. From anther and pollen ripening to pollen presentation. – Plant Syst. Evol. 222: 19–43.

— & FRANCHI G. G. 1993. Role of the tapetum in pollen and spore dispersal. – Plant Syst. Evol., Suppl. 7: 1–11.

— , — & HESSE M. 1985. The tapetum: Its form, function, and possible phylogeny in *Embryophyta*. – Plant Syst. Evol. 149(3–4): 155–185.

PRAKASH N. 1987. Embryology of the *Leguminosae*. – In: STIRTON C. H. (ed.), Adv. Leg. Syst. 3: 241–278.

PRENNER G. 2004. Floral ontogeny in *Calliandra angustifolia* (*Leguminosae: Mimosoideae: Ingeae*) and its systematic implications. – Int. J. Plant Sci. 165(3): 417–426.

— & TEPPNER H. 2005. Anther development, pollen presentation and pollen adhesive of parenchymatous origin in *Calliandra angustifolia* (*Leguminosae-Mimosoideae-Ingeae*). – Phyton (Horn, Austria) 45(2): 267–286.

RICHTER S. 1929. Über den Öffnungsmechanismus der Antheren bei einigen Vertretern der Angiospermen. – Planta (Berlin) 8(1–2): 154–184.

RICO ARCE M. DE L., SOUSA S. M. & FUENTES S. S. 2000 ('1999'). *Guinetia*: a new genus in the tribe *Ingeae* (*Leguminosae: Mimosoideae*) from Mexico. – Kew Bull. 54(4): 975–981.

ROSANOFF S. 1866. Zur Kenntniss des Baues und der Entwickelungsgeschichte des Pollens der *Mimoseae*. – Jahrb. wiss. Bot. 4: 441–450, 2 plates.

ROWLEY J. R. 1993. Cycles of hyperactivity in tapetal cells. – Plant Syst. Evol., Suppl. 7: 23–37.

SANTOS R. P., MARIATH J. E. A. & HESSE M. 2003. Pollenkitt formation in *Ilex paraguariensis* A. ST.-HIL. (*Aquifoliaceae*). – Plant Syst. Evol. 237: 185–198.

SEIJO J. G. & SOLÍS NEFFA V. G. 2004. The cytological origin of the polyads and their significance in the reproductive biology of *Mimosa bimucronata*. – Bot. J. linn. Soc. 144: 343–349.

SHARMA A. K. & SHARMA A. S. 1965. Chromosome techniques. Theory and practice. – Butterworths, London.

SINNOT E. W. 1960. Plant morphogenesis. – McGraw-Hill Book Comp., New York, Toronto, London.

TEPPNER H. 1998. *Inga feuillei* DC. (*Mimosaceae-Ingeae*). – Samentauschverzeichnis 1998 (Bot. Garten Inst. Bot. Univ. Graz), p. 37–45.

— 2007. Notes on terminology for *Mimosaceae* polyads, especially in *Calliandra*. – Phyton (Horn, Austria) 46(2): 231–236.

— & STABENTHEINER E. 2006. *Inga feuillei* (*Mimosaceae-Ingeae*): Anther opening and polyad presentation. – Phyton (Horn, Austria) 46(1): 141–158.

— & — 2007. Anther opening, polyad presentation, pollenkitt and pollen adhesive in four *Calliandra* species (*Mimosaceae-Ingeae*). – Phyton (Horn, Austria) 47(1): 291–320.

VOGEL S. 1984. Blütensekrete als akzessorischer Pollenkitt. – In: EHRENDORFER F. & al. (eds.), Mitteilungsband. Kurzfassungen der Beiträge. Botaniker-Tagung in Wien. 9.–14. September 1984, p. 123.

— 2002. Extra-tapetal pollen adhesives: Where they occur and how they function. – In: SCHÖNENBERGER J., BALTHAZAR M. V. & MATTHEWS M. (eds.), Flowers, diversity, development & evolution. – Zürich Meeting 5 – 7 July, p. 48.

WODEHOUSE R. P. 1935. Pollen grains. Their structure, identification and significance in science and medicine. – McGraw-Hill Book Company, New York and London.

YAMASAKI N. 1956. Reifeteilung, Pollenkornteilung und Polliniumbildung an *Albizzia glabrior* OHWI var. *speciosa* KOIDZUMI. – Cytologia (Tokyo) 21(4): 433–441.