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Anther Development, Pollen Presentation and Pollen Adhesive of Parenchymatous Origin in *Calliandra angustifolia (Leguminosae-Mimosoideae-Ingeae)*

By

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With 7 Figures

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Summary

PRENNER G. & 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, with 7 figures. – English with German summary.

The genus *Calliandra* s. str. shows a unique mode of pollen presentation and production of pollen adhesive. Within a septate locule we find two drop-shaped polyads which are composed of eight pollen grains and which lie with their longitudinal axis parallel to the longitudinal axis of the dorsifixed anther. In the course of anther opening the polyads rise up in a right angle, facing with their apices outwards towards the floral visitor. Furthermore, on each apex a small amount of pollen adhesive is deposited.

To clarify the mechanism of pollen presentation, the mode of anther septation and the origin of the adhesive, anther development, morphology and anatomy of *Calliandra angustifolia* was studied using Scanning Electron Microscopy (SEM) and serial microtome sections. In these studies we show that the locules are septate with parenchymatous tissue, and that the pollen adhesive is a resolvent product of parts of this tissue. This is the first report of an extra-tapetal pollen sticker (namely pollen adhesive) in *Mimosoideae.* Furthermore, the mode of pollen presentation is a complex interaction of (1) lysigenous processes in and between the locules, (2) the appearance

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of interlocking hairs at the periphery of the stomium, (3) movements of the thecal valves, and (4) of the polyad's morphology. A correlation of locule septation and pollination ecology is demonstrated for the first time, and the isolated position of *Calliandra* s. str. within *Mimosoideae* is confirmed.

Zusammenfassung

PRENNER G. & TEPPNER H. 2005. Antherenentwicklung, Pollenpräsentation und Pollenklebstoff parenchymatischen Ursprungs bei *Calliandra angustifolia (Leguminosae-Mimosoideae-Ingeae)*. – Phyton (Horn, Austria) 45 (2): 267–286, mit 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Gattung *Calliandra* s. str. weist eine einzigartige Form der Pollenpräsentation, sowie die Produktion von Pollenklebstoff auf. In den septierten Lokulamenten finden sich je zwei achtzellige Polyaden von der Form eines abgeflachten Tropfens, die mit ihrer Längsachse parallel zur Längsachse der dorsifixen Anthere liegen. Im Zuge der Antherenöffnung richten sich die Polyaden um 90° auf, sodass sie mit der Spitze nach außen zum Blütenbesucher hin orientiert sind. Desweiteren findet sich an jeder Polyadenspitze eine kleine Menge von Klebstoff.

Um den Mechanismus der Pollenpräsentation, die Art der Antherenseptierung sowie die Herkunft des Klebstoffes zu klären wurden Entstehung, Morphologie und Anatomie der Antheren von *Calliandra angustifolia* mittels Rasterelektronenmikroskpie und Mikrotomschnitten untersucht. Diese Studie zeigt, dass die Lokulamente durch parenchymatisches Gewebe septiert sind und dass der Klebstoff ein Auflösungsprodukt eines Teiles dieses parenchymatischen Gewebes ist. Damit wird der Erstnachweis von nicht tapetogenem Pollen-Haftmittel (nämlich Pollenklebstoff) innerhalb der *Mimosoideae* erbracht. Schließlich können wir zeigen, dass die Pollenpräsentation ein komplexes Zusammenwirken ist (1) von lysigenen Prozessen innerhalb und zwischen den Lokulamenten, (2) von ineinandergreifenden Haaren am Rande des Stomiums, (3) von Bewegungen der Antherenwände und (4) von der Morphologie der Polyaden. Erstmals wird ein Zusammenhang von Lokulamentseptierung und Blütenökologie dargestellt. Desweiteren wird die isolierte Stellung der Gattung *Calliandra* s. str. innerhalb der *Mimosoideae* bestärkt.

1. Introduction

Calliandra s. str. is a neotropical genus of about 132 species (BARNEBY 1998). Some taxa are frequently cultivated as ornamental shrubs or trees, or used for fuel wood and as a forage crop (eg., NEVLING & ELIAS 1971, MACQUEEN & al. 2001). Nevertheless, details of the unique mode of pollen presentation, and some remarkable features in anther morphology are still unexplored.

In the dorsifixed anthers each transversally septate locule bears two drop-shaped and eight-celled polyads. The polyads are calymmate (GUINET 1965) and lie with their longitudinal axis parallel to the longitudinal axis of the anther, showing with their tips against each other. In the course of anther dehiscence the polyads raise up in a right angle, and a small amount of adhesive can be observed on the tips of the polyads. The line drawings of MOHL 1834 are the first documents, showing the polyads with

a small amount of adhesive on their apical ends. In the following decades the anthers, polyads and their appendage where discussed by several authors (ENDLICHER & UNGER 1843, ROSANOFF 1865, ENGLER 1876, MÜLLER in LUDWIG 1897, GOEBEL 1923, RICHTER 1929, DNYANSAGAR 1958, NEVLING & ELIAS 1971, ENDRESS & STUMPF 1990). But the mode of anther septation (either parenchymatous or tapetal only), the exact mechanism of polyad erection, and the origin of the pollen adhesive remain unknown.

The aim of this paper is to study anther morphology and anatomy in detail, to clarify the origin of the adhesive, and to investigate the particular mode of pollen presentation in *Calliandra angustifolia*. The results will be discussed with respect to recent results of floral ontogeny (PRENNER 2004) and with respect to the systematic position of the genus *Calliandra* s. str.

2. Material

Calliandra angustifolia SPRUCE ex BENTH. was collected on August 30th 1981 by H. TEPPNER & K. KEPLINGER in Peru, Dep. Pasco, Prov. Oxapampa, Pozuzo. According to its natural habit on river banks (TEPPNER 1983: 324), the plant is cultivated in the Neotropics part of the new greenhouses (Botanical Garden, Institute of Plant Sciences, Karl-Franzens-University Graz, Austria, Europe).

3. Methods

3.1. Stereomicroscopic Observations

Anther dehiscence was observed under a Leica M3 stereomicroscope. Macro photographs were taken under a Leica Wild MZ3 stereomicroscope with phototubus and a Nikon F 70 camera.

3.2. Scanning Electron Microscopy (SEM)

For SEM inflorescences of different sizes were collected, immediately fixed in FAA (5 parts formalin: 5 parts acetic acid: 90 parts 70 % ethanol) for at least 24 hours, and stored in 70 % ethanol. Anthers were dissected in alcohol of the same concentration under a Leica MZ6 stereomicroscope. The specimens were dehydrated in formalindimethylacetal (FDA) for at least 24 hours and afterwards critical point dried with liquid CO_2 in a Polaron 7010 CPD. Dried specimens were mounted on aluminium stubs with nail polish and dissection was completed on the stubs. The specimens were coated with gold in a MED 010 sputter coater and studied and micrographs were taken in a Zeiss DSM 950 at 15 kV at the Institute for Cell Biology, Histology and Embryology (Medical University of Graz, Austria, Europe).

3.3. Serial Microtome Sections

For serial microtome sections buds of different sizes were collected and immediately fixed in FAA for at least 24 hours. The small anthers were not treated individually, but whole floral buds of different sizes were cut. In this way the anthers are stabilised within the bud, and several anthers can be cut in one run. Disadvantage of this method is that several buds have to be investigated for appropriate cuttings. The buds were dehydrated, cleared with tertiary butanol and embedded in

Histosec. Sections of 6–7 µm were stained either with safranin-fastgreen or auramine-astrablue. The sections were enclosed in Entellan and observed under a Zeiss Axioskop 20 microscope. Drawings were made with a compatible drawing unit and LM-micrographs were taken under a Zeiss Axiophot 2.

3.4. Dissolubility of the Pollen Adhesive

To test the dissolubility of the pollen adhesive, tweezers with adhering polyads were exposed for five minutes in the xylolsubstitute XEM 200 (a 100% lemon extract, used in the microtome process), in formalindimethylacetal (FDA, used in the course of the SEM studies), and in 96% ethanol. Afterwards the tweezers where scanned for adhering polyads using the stereomicroscope.

3.5. Terminology

Tobe & Raven 1986 as well as Endress & Stumpf 1990 termed septate anthers as polysporangiate and thus canceled the synonymy of locule and microsporangium (cf., Green 1980, Wagenitz 1996: 224 and 297, D'Arcy 1996: 5). Green 1980 suggests the term locellus for such cases of septate locules. In the present paper the term locule is used synonymous with pollen sac and microsporangium. Thus in the case of the septate locules in *Calliandra* each half is named locule-half instead of microsporangium.

To emphasize the difference to pollenkitt, which is defined as a complex mixture of lipid viscous substances, produced by the plastids of the anther tapetum (Hesse 1981, Pacini & Hesse 2005), we use the term pollen adhesive (Pollenklebstoff) sensu Vogel 2002 (original term: extra-tapetal pollen adhesive) for sticky substances of other origin than the tapetum. The sole function of pollen adhesives is sticking pollen to a vector, whereas pollenkitt is also responsible for adhering pollen in the open anther or on another place of pollen presentation (see also Pacini & Hesse 2005). If necessary, as an umbrella term for both, the term pollen sticker (Pollen-Haftmittel) would be applicable.

4. Results

4.1. Anther Development and Microsporogenesis

Anther development sets in at a stamen length of c. 50 µm. First the apical part of the stamen broadens tangentially, which strengthens its bifaciality (Fig. 1A). Soon afterwards the two thecae are formed and separated by a deep constriction (Figs. 1B-D). By this time the dorsifixed character of the anther (dorsal insertion of the filament) becomes discernible.

Next the two pollen sacs are differentiated and transversely parted with a parenchymatous tissue (Figs. 2A-C). In each locule-half one pair of pollen mother cells is formed, and enclosed by a one-layered tapetum (Fig. 2B). The pollen mother cell pair of one locule-half is flattened drop-shaped, with one cell in form of a flattened hemisphere and the other of a flattened cone (Fig. 2B). Within each locule, the pollen mother cell pair is oriented with its tip looking against each other. Hence, the two pollen mother cell pairs per locule lie against one another and the two pollen mother cell pairs of neighbouring locules lie parallel to each other (Fig. 2C).

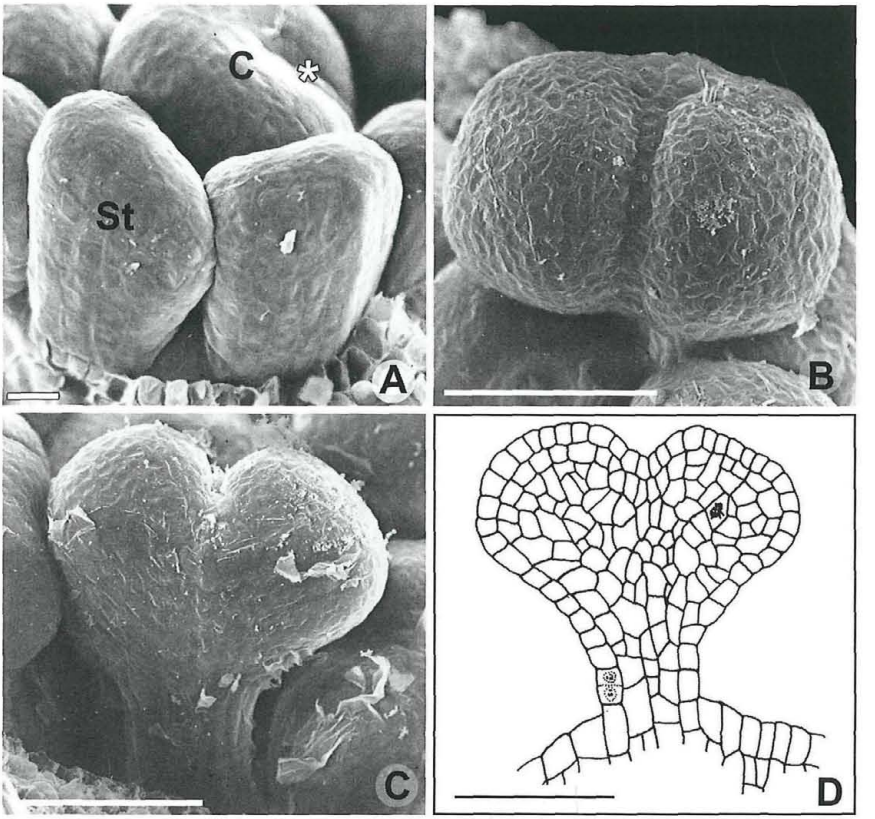

Fig. 1. *Calliandra angustifolia*, early anther development. – A. The young stamen primordia (St) broaden tangentially. In parallel, the cleft of the carpel (C) is formed (asterisk). – B. Young anther from above, with the two thecae separated by a deep constriction. – C. Side view on a young anther of the same developmental stage as B. – D. Longitudinal section of a young anther before the formation of pollen mother cells sets in. – Scale bar = 10 μm in A, 50 μm in B-D.

After meiosis the tetrads stay fused and eight-celled polyads emerge, the cells laying in one plane (Figs. 3B-D). Due to formation of a uniform tectum, the mature polyads are finally calymmate. The pollen grains of each polyad have a characteristic heteromorphic habit, with two central cells and six distinct peripheral cells (Figs. 3C-D). The drop-shaped basic form of the mature polyads resembles that of the pollen mother cell pairs. Finally, four flattened polyads can be found in the two locules of each theca, oriented in the same manner as are the pollen mother cells, with the apices lying against each other (Fig. 2C).

In this context the high economy in respect of pollen production should be highlighted: Only 64 pollen grains per anther are formed. Con-

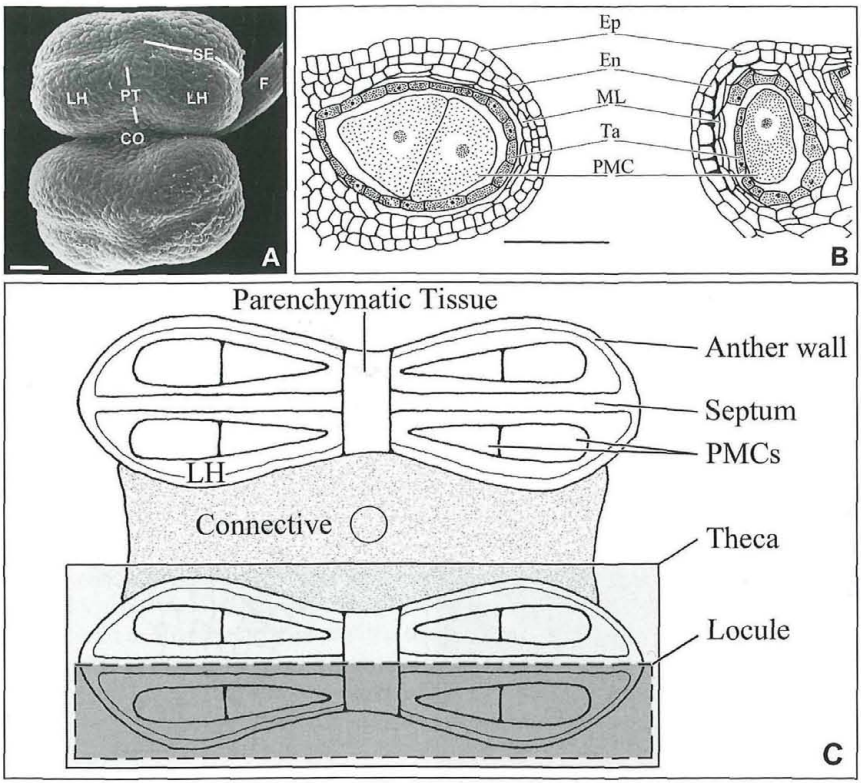

Fig. 2. *Calliandra angustifolia*, pollen mother cells. – A. Young anther out of a c. 5 mm long bud. The two thecae are already differentiated and the locules contain pollen mother cells. Each theca shows a longitudinal constriction (SE), which parts the theca into two locules, and a radial constriction (PT), which parts the locules into two locule-halves (LH). – B. Longitudinal section (left) and cross section (right) of a young anther, which is of the same developmental stage as the anther in Fig. A. In each locule-half two pollen mother cells are formed (PMC), which are surrounded by a one-layered tapetum (Ta). Furthermore, the middle layer (ML) is visible as well as the young endothecium (En) and the epidermis (Ep). – C. Schematic longitudinal anther section (details of the anther wall and parenchymatous tissue omitted). Partitioning of the anther by the connective into two thecae, partitioning of the thecae by the septum into two locules, and transversal partitioning of the locules by the parenchymatous tissue into two locule-halves (PMCs = two pollen mother cells in one locule-half). – Scale bar = 50 μm in A-B.

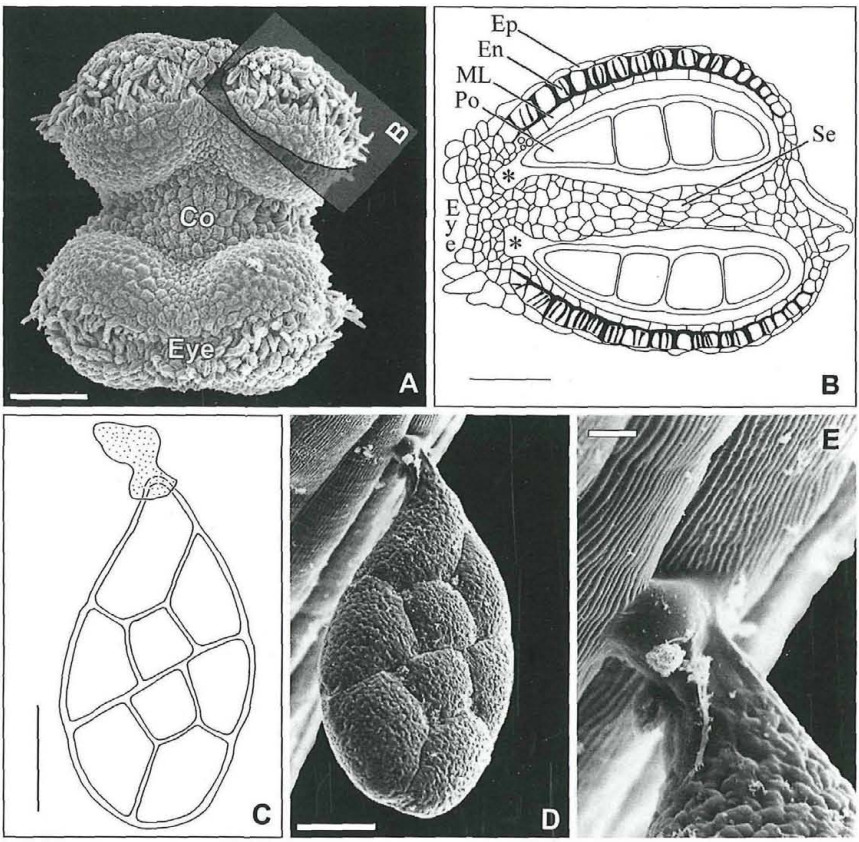

Fig. 3. *Calliandra angustifolia*, anther maturation. – A. Anther out of an about 6 mm long floral bud showing the distinct connective (Co) and rows of short hairs, accompanying the stomium. The hairs are absent around the stomium's centre (eye). The rectangle marks the section level of the section in Fig. B. – B. Section through one locule-half as marked in Fig. A, showing two neighbouring polyads (one marked with Po). At the tips of the polyads two cavities have been formed (asterisks) due to partial lysis of the parenchymatous transversal tissue. The product of the lysis is washed out in the course of the microtome preparation processes. The tapetum is already used up, and the middle layer (ML) is reduced. Endothecium (En) with partially thickened cell walls and reduced epidermis (Ep). – C. Line drawing of a mature polyad, showing characteristic heteromorphic pollen cells, with two distinct central cells, which are surrounded by six different peripheral cells. Pollen adhesive is found on the polyad's tip. – D. SEM micrograph of a mature polyad sticking on the surface of a filament. – E. Detail of D, showing the pollen adhesive on the apical cell of the polyad. – Scale bar = 100 μm in A, 50 μm in B-D, 5 μm in E.

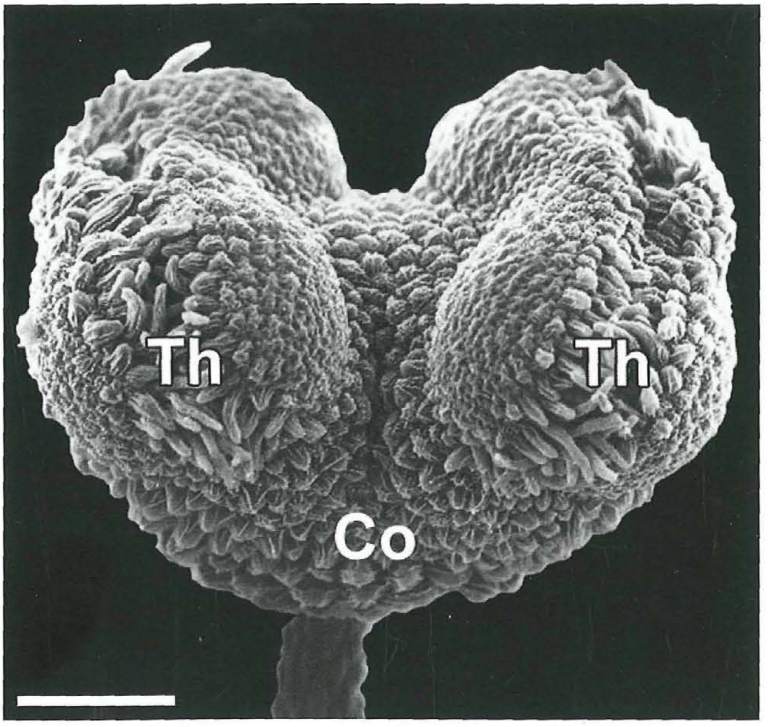

Fig. 4. *Calliandra angustifolia*, mature anther. – Mature anther with a distinct connective (Co) on which the two thecae sit in an angle of about 65°. – Scale bar = 100 µm

sidering an average of 9.5 stamens per flower (n = 711) and about 33 flowers per inflorescence (n = 28), only 608 pollen grains per flower and 20,064 pollen grains per inflorescence are produced.

From sporogenesis until anther dehiscence the following important processes within the anther go ahead:

1. Formation of an endothecium with partially thickened cell walls, running out in radial skinnier ridges (Figs. 2B, 3B).

2. Inside the locules: Subepidermally lysis of the parenchymatous transverse wall right under the eyelike broadening of the stomium. This process sets in at a bud length of c. 6 mm. The product of this lysis becomes the pollen adhesive, which is finally found at the tips of the polyads (Figs. 3B-E).

3. Reduction of the two to three cell-layers of the middle layer, and lysis of the one-layered tapetum during the formation of the polyad's tectum. In this process the tapetum is completely used up, and at a bud length of c. 6 mm it is no longer discernible (Figs. 3B, 6A-C).

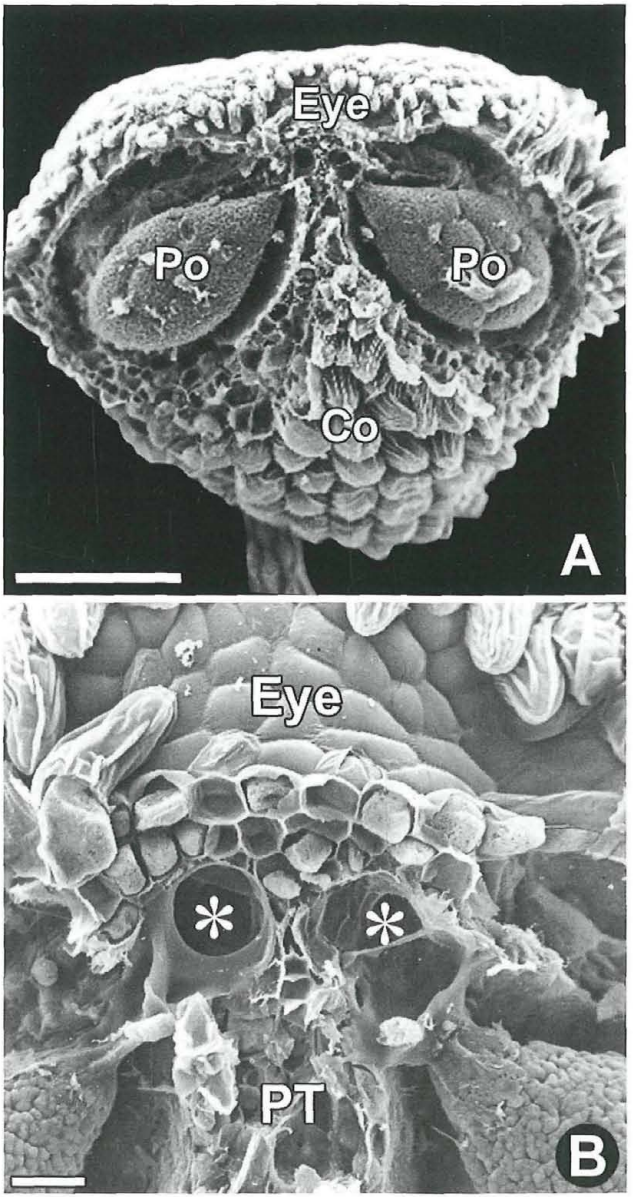

Fig. 5. *Calliandra angustifolia*, mature anther. – A. Longitudinally opened locule with massive connective (Co) and two polyads (Po), lying parallel to the anther's longitudinal axis. – B. Detail of A. At the central part of the locule, the tips of the polyads point directly into two cavities (asterisks), in which residual pollen adhesive is visible. Beyond of these cavities the central zone of the stomium, which is free of hairs (Eye; PT = parenchymatous tissue). – Scale bar = 100 μm in A, 10 μm in B.

4. Between the locules: Wrenching of the septum from the anther wall. This happens shortly before the anther opens, and initiates dehiscence of the stomium (Fig. 6C).

In parallel, the following changes on the anther's surface can be observed:

1. Formation of a distinct stomium along the borderlines of the locules. The cells of the stomium are small and lack wall thickenings (Figs. 2A, 3A-B, 6A-C).

2. Outgrowth of epidermal cells to hair cells along the margin of the stomium. These hair cells interlock along the stomium's margin, whereas the eye-like central part of the stomium is free of interlocking hairs (Figs. 3A-B, 5A-B, 6B-C).

3. Degeneration of the epidermal cells on the anther wall. This happens in parts complete, and in parts a degeneration to small lens shaped cells is discernible (Figs. 6A-C).

4. Differentiation of a massive connective, on which the two thecae are located separate from each other in an angle of about 65°. In the dorsal region of the connective distinct papillate epidermal cells are formed (Figs. 3A, 4, 5A, 6B-C).

All of these processes and changes are important in the course of pollen presentation and application of pollen adhesive, which will be dealt with in the two following chapters 4.2. and 4.3.

4.2. Production of Pollen Adhesive

Tapetal pollenkitt, which is the common case in angiosperms, is the least plausible. The tapetum is used up in parallel with the tectum formation, and it is soon no more discernible (Figs. 6A-C). Furthermore, in the case of tapetal origin an exact application on the tip of the polyads would be impossible and small amounts of sticky substances on the polyad's surface would inhibit their exact erection (see next chapter).

Fig. 6. *Calliandra angustifolia*, transverse anther sections. – A. Young anther showing pollen mother cells, which are separated in each theca by a usually four-layered septum. The pollen mother cells are enclosed by a one-layered tapetum and a two- to three-layered middle layer. Furthermore, the young endothecium and a one-layered epidermis is visible. – B. Older developmental stage after meiosis. The tapetum is used up completely and the middle layer (ML) is reduced. Within the endothecium cell walls are partially thickened, and the locular epidermis (Ep) is reduced to small lens shaped cells. Along the stomium epidermal cells enlarge to hair cells (Ep-Hair). – C. Anther out of an anthetic flower. The septum (Se) wrenches from the anther wall and thus the theca becomes unilocular. The stomium (St) dehisces, and the middle layer (ML) is widely reduced. – Scale bar = 50 μm in A-C.

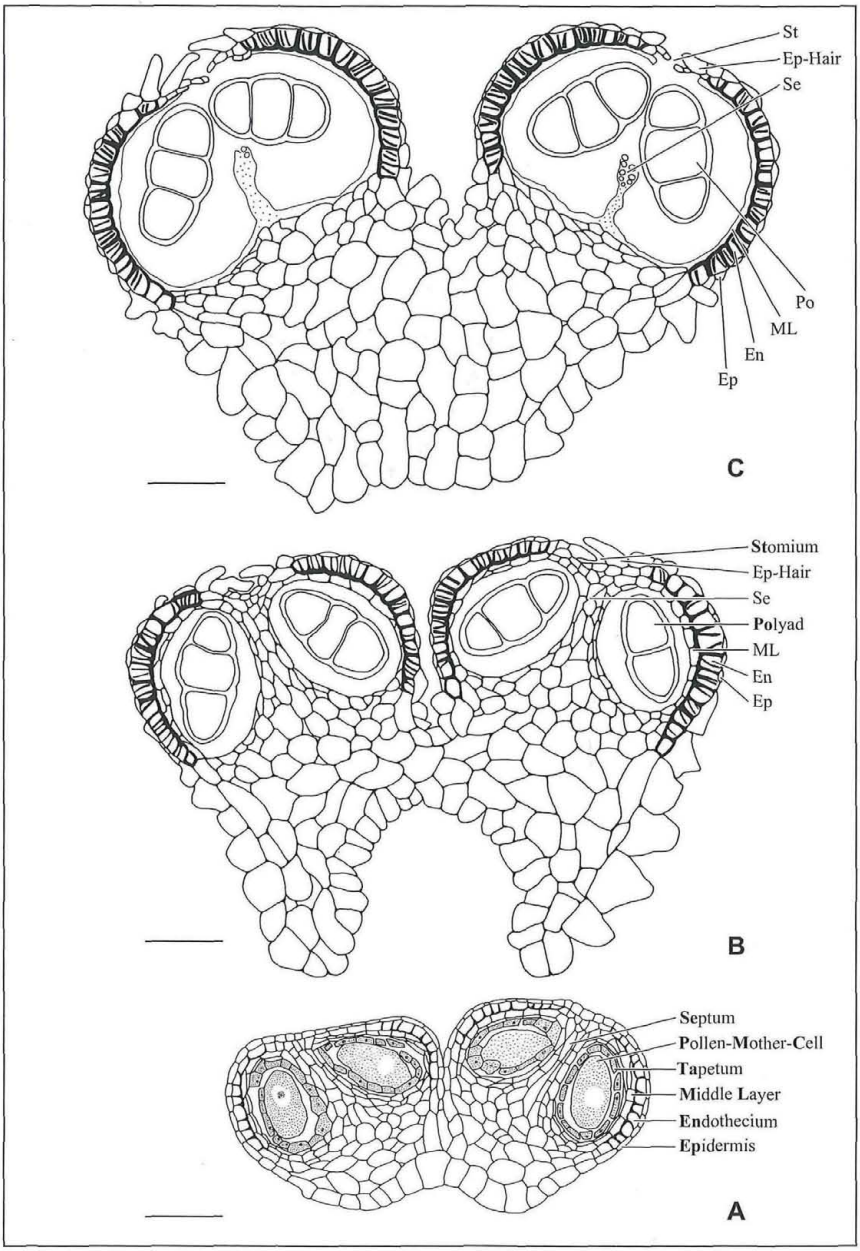

Fig 6.

No evidence for the production of pollen adhesive could be found in the hairs along the stomium. They are absent in the central part of the stomium, where the adhesive should be applicated (Fig. 3A, 5A-B). But the hairs play an important role in anther dehiscence and pollen presentation, which is shown in the next chapter.

Therefore, the small celled parenchymatous tissue which divides the locules, was observed in detail. In its range the septum is broadened, and the connective grows deeply in the locules (Figs. 4, 5A). In the region, where the tips of two polyads of neighbouring locules show against the parenchymatous tissue, small subepidermal cavities were found (Fig. 3B). The same cavities were also found in anthers, which were opened artificially (Figs. 5A-B). The tips of the polyads point directly into these cavities, where small amounts of pollen adhesive could be found (Fig. 5B). Because of these results, anther dehiscence was examined with particular attention to the centre of the thecae. By this, the assumption that the pollen adhesive is a product of the lysis of the parenchymatous tissue was confirmed. The adhesive is applicated exactly on the tips of the polyads, and no sticky substances contact other parts of the polyad's surface. Furthermore, the optical similarities of small amounts of pollen adhesive, which we found in artificially opened anthers and on adhering polyads confirm that it is a product of the partial lysis of the parenchymatous tissue (Figs. 3D-E, 5A-B).

Dissolubility tests showed that the adhesive is solvable neither in ethanol nor in formalindimethylacetal (used for SEM), whereas it is well solvable in the xylolsubstitute XEM 200 (used for LM). Hence the adhesive is found in SEM micrographs (Figs. 3D-E), while no remnants could be found in microtome sections, in which the pollen adhesive is washed out in the course of the preparation process (Fig. 3B).

4.3. Anther Dehiscence and Pollen Presentation

At a bud length of c. 6 mm, anther dehiscence is initiated by lysis of the parenchymatous tissue, which parts the locules (Figs. 3B, 5A). At the day of anthesis the septum between the two locules is redeemed from the anther wall, and the thecae become unilocular (Fig. 6C). Because of this, the thecae loose stability, and tensile stress by the endothecium acts entirely on the small-celled stomium. In this phase the interlocking hairs along the stomium's margin strengthen this region for a small amount. This firmness lacks in the centre of the stomium, where it is eye like broadened, and where the hairs are absent. Hence, the central part of the stomium is the most unstable part, where the dehiscence starts. From there the stomium dehisces towards the ends of the anther. Parallel to this, the anther walls move inwards glove-finger like from the centre towards the theca's ends. In this process adjacent polyads are successively pressed against

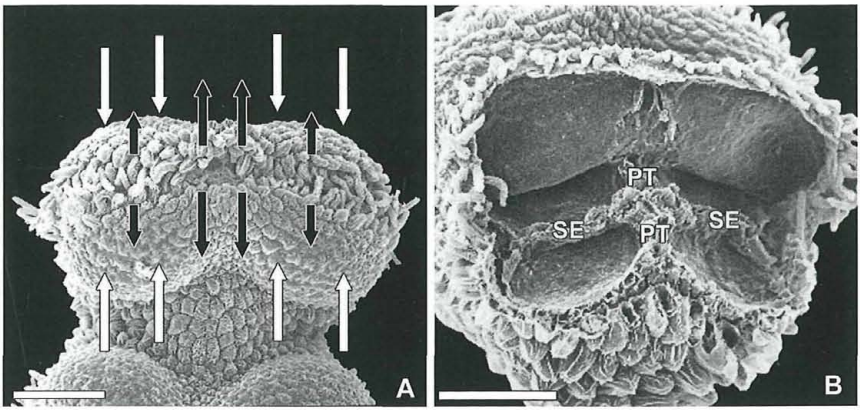

Fig. 7. *Calliandra angustifolia*, mature anther at the day of anthesis. – A. The black arrows show the mode of stomium dehiscence from the centre towards the margins of the theca. The white arrows mark the forces of the anther walls, which move inwards glove finger like from the centre towards the margins of the theca. – B. Opened theca [polyads and one (outer) theca valve removed]. Residuals of the septum (SE) and the parenchymatous transverse wall (PT) are visible, and act as guide rail and abutment, respectively, in the process of polyad erection. – Scale bar = 100 μm in A-B.

each other from their tips towards the rounded end. Furthermore, the anther wall in the centre is shuffled successively under the polyads (Fig. 7A), and the anther valves finally are bent to the dorsal side of the anther. In this way, the polyads lying parallel to the anther's longitudinal axis are forced upwards for 90°. Remnants of the septum and of the parenchymatous transverse wall assist this process by acting as guide rail (the septum) and as abutment (the transverse wall; Fig. 7B). They fix the polyads in a convenient position and allow no other movement of the polyads than a gradual erection.

5. Discussion

5.1. Microsporogenesis, Locule Septation, and its Biological Significance

The locules of *Calliandra angustifolia* are septated transversally by a parenchymatous tissue and not only by the tapetum. This fact was not entirely clarified by ENGLER 1876 (see also ENDRESS & STUMPF 1990). Parenchymatous septation is common in *Mimosoideae* and is found in *Albizzia, Archidendron, Dichrostachys, Parkia* and *Leucaena* (e.g., DNYANSAGAR 1954, ENDRESS & STUMPF 1990, 1991, JAIN & VIJAYARAGHAVAN 1992).

The transverse locule septation in *C. angustifolia* takes place in an early developmental stage, and goes along with the formation of two pollen mother cells per locule-half. The pollen mother cells undergo meiosis and eight-grained polyads are formed. The number of two pollen mother cells

per locule-half has already been emphasized by DNYANSAGAR 1955, and stands in contrast to CHEN 1973.

Multilocular anthers have been reported for 18 families of dicotyledons (cf. ENDRESS & STUMPF 1990). The biological significance of such septations is discussed controversial, and is not clarified sufficiently in most cases (cf. LERSTEN 1971: 494, ENDRESS & STUMPF 1990: 229, BAUMGRATZ & al. 1996). Concerning the functions of the septum in the course of pollen presentation and adhesive production in *C. angustifolia* (which will be discussed in the following chapters 5.2. and 5.3.), a correlation of the septation with pollination ecology is evident.

For *Calliandra* s.str., the discussed purpose of a better nourishment by tighter contact of the pollen grains with the tapetum is negligible, since the pollen grains lay in a single plane and a maximal contact is assured with and without septation. The tapetum's surface is also only marginally enlarged by transverse septation.

According to GUINET 1981, *Calliandra* s. str. is the sole genus of *Ingeae* with only eight grains per polyad, which differs from the 16 grains per polyad in the segregated genus *Zapoteca* (GUINET & HERNÁNDEZ 1989). Within the other tribes of *Mimosoideae*, polyads with eight grains are found in 12 genera of *Mimoseae* and in species of the subtribe *Heterophyllum* of *Acacieae*. Particularly these taxa are discussed in connection with the genus *Calliandra* s. str., because the pollen grains also lay in a single plane (GUINET 1981: 846). But GUINET 1990 mentions that the pollen differences between *Acacia* s. l. and *Calliandra* s. str. do not suggest a close relationship between the two genera. Furthermore, NIEZGODA & al. 1983 suggest that the calymmate condition among *Calliandra* s. str. has probably been independently derived as it is associated with pollen characters unique within *Ingeae*, and in the case of the eight-grained species, unique within mimosoids.

5.2. Proof of Pollen Adhesive in *Mimosoideae*

Although the sticky substance on the apex of the drop-shaped polyads in *Calliandra* s. str. is known since MOHL 1834, details of its ontogeny were still unknown and erroneous or false interpretations are found in the recent literature. HERNÁNDEZ 1986 mentions a "viscid appendage in basal grain", which better should be quoted "ON the APICAL grain" in respect of the final position. BERNHARDT 1996 cites this statement and mentions that "glues may be manufactured exogenously by the basal cells of *Calliandra*", which is incorrect too, as shown in the present paper.

We could rather show that the sticky substance is a resolvent product of the parenchymatous tissue, which divides the locules. Hence it differs clearly from pollenkitt, which is defined as a complex mixture of lipid viscous substances, produced by the plastids of the anther tapetum (HESSE

1981, PACINI & HESSE 2005). To emphasize the non tapetal provenience of the substance, the term extra-tapetal pollen adhesive as used by VOGEL 2002 is seen as appropriate; but it is abbreviated here as "pollen adhesive" because this is sufficient as a contrary to pollenkitt (for definitions see chapter 3.5.). This is the first report of pollen adhesive in *Mimosoideae*. Within *Leguminosae* pollen adhesive is found only once in the caesalpinioid *Tylosema esculentum* (BURCH.) A. SCHREIBER (DE FREY & al. 1992).

VOGEL 2002 lists three possible origins of pollen adhesives which are (1) stigmatic products, (2) extra-locular glands of the anther, or (3) sticky substances produced by the corolla wall. The pollen adhesive in *Calliandra angustifolia* (and most likely pollen adhesives in the whole genus *Calliandra* s.str.) can be seen as a fourth kind of pollen adhesive, which is a lysigenous product of the parenchymatous septation within the locules. According to VOGEL 2002 conditions increasing the risk of pollen to be lost before reaching a stigma are (1) few grains produced per flower, (2) big and heavy grains or polyads, (3) friction between narrow corolla tubes and pollen-carrying tongues of insects, (4) attachment on rapidly moving wings, or (5) too smooth surfaces of vectors (see also VOGEL 1981, MOYANO & al. 2003). In these cases pollen adhesive would assist or replace pollenkitt in attaching pollen to animal vectors. *C. angustifolia* fits quite well onto numbers (1), (2) and (4) of this scheme. There are only few grains per flower produced (c. 600), which are compound to big eight-celled polyads (c. 140 x 75 µm), and the flowers are visited in the night by moths (HT, pers. observation).

In *Tylosema esculentum (Caesalpinioideae)* a kind of pollen adhesive or "anther mucilage" is produced by the anther connective tissue (DE FREY & al. 1992). But this taxon has up to 14,500 small pollen grains per anther, and the authors state that the substance serves not only in sticking the pollen grains to the pollinator. Sticking pollen grains against each other as well as on the stigma, and serving as protection against running dry and against radiation are further functions, which are discussed by the authors. Finally, DE FREY & al. 1992 suggest a possible benefit in germination on the stigma. HARTLEY & al. 2002 found that the anther mucilage dried up by the end of the first day of anthesis. They suggest that this reduces the likelihood of pollen adhering to potential pollinators and that "the time available for pollen transfer is likely to be short".

5.3. Anther Dehiscence and Polyad Presentation

Observations of the anther dehiscence show that the unique process of pollen presentation is a complex interaction of anther morphology and anatomy, of lysigenous processes in and between the locules, and of polyad morphology (Chapters 4.2. and 4.3.). All mentioned components stand in a strong interaction, and enable the exact application of the pollen adhesive

on the tips of the polyads as well as the exact erection of the polyads. Hence statements of RICHTER 1929: 159 are specified respectively emended. Suggestions of NEVLING & ELIAS 1971 that the massive connective plays a role in pollen presentation cannot be proved. We could not find parallels with the opening process of *Leucaena leucocephala* (LAM.) DE WIT, in which pollen grains are released through a longitudinal slit-like opening in the anther (JAIN & VIJAYARAGHAVAN 1992). HUGHES 1997 reports for *L. macrophylla* that the tapetum remains intact until after dehiscence of the anther and that it forms a "sac" which holds the whole locular contents together as a unit. According to HUGHES 1997, the function of the intact tapetal membranes surrounding the locules remains unknown and it is unclear at what stage the tapetal membrane breaks up after dehiscence of the anther.

KENRICK & KNOX 1979, 1989 show open anthers of *Acacia subulata* BONPL., presenting the 16-celled discoidal polyads with their edges pointed upwards. In contrast to this, GUINET & HERNÁNDEZ 1989 and HERNÁNDEZ 1990 showed open anthers of *Zapoteca* with the polyads lying plain on the inside of the thecal valves (see also TEPPNER 1998 for *Inga feuillei* DC.). GUINET & HERNÁNDEZ 1989 highlight an "inner-outer polyad disymmetry" of the four central pollen grains in *Zapoteca*, with only one side showing lens-shaped thickened areas (see also HERNÁNDEZ 1989). After anther dehiscence the exposed sides of the polyads are those bearing these lens-shaped areas (see also HERNÁNDEZ 1990). The authors mention that "inner-outer polyad disymmetry" can also be found in several other genera of *Ingeae* such as *Abarema, Albizia* (Sect. *Zygia*), *Lysiloma* (GUINET 1990) and *Inga* (HOC 1985), while it appears to be absent in the other *Mimosoideae* tribes.

5.4. Systematic Conclusions

Because of its particular pollen morphology, the isolated position of *Calliandra* s. str. with eight-celled and drop-shaped polyads was highlighted several times (e.g., GUINET 1965, SORSA 1969, GUINET & FERGUSON 1989), and finally the genus *Zapoteca* with 16-celled polyads was segregated (HERNÁNDEZ 1986, HERNÁNDEZ 1989, GUINET & HERNÁNDEZ 1989).

A close relationship of the two genera, as suggested by GRIMES 1995, 1999 could neither be proved by molecular data (MILLER & al. 2003, LUCKOW & al. 2003) nor by morphological observations (PRENNER 2004, present study). However, both, MILLER & al. 2003 and LUCKOW & al. 2003 could not solve the problem of accurately placing the genus *Calliandra* s. str., and for *Ingeae* as a whole, LEWIS & RICO ARCE 2005: 193 mention that "clarification of generic relationships within tribe *Ingeae* still suffers from a paucity of molecular data".

LAVIN & al. 2005 show that *Calliandra* s.str. apparently form a relatively long branch within node 12 of the mimosoid crown node which suggests a more recent diversification of the genus. Together with the unique patterns of early floral development (reversed unidirectional sepal initiation, cochlear descending sepal aestivation and helical tendencies in the androecium; PRENNER 2004), the clarification of the particular mode of pollen presentation and the finding of extra-tapetal pollen adhesive (present study) give further evidence for the isolated position of the genus *Calliandra* s. str. within *Mimosoideae*.

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7. References

BARNEBY R. C. 1998. Silk tree, Guanacaste, Monkey's Earring, 3, *Calliandra*. – Mem. New York bot. Garden 74: 1–223.

BAUMGRATZ J. F. A., SOUZA M. L. D., WOODGYER E. M. & NIC LUGHADHA E. M. 1996. Polysporangiate anthers: described for the first time in *Melastomataceae*. – Kew Bull. 51: 133–144.

BERNHARDT P. 1996. Anther adaptation in animal pollination. – In: D'ARCY W. G. & KEATING R. C. (Eds.), The anther: form, function and phylogeny, p. 192–220. – Cambridge University Press, Cambridge.

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

D'ARCY W. G. 1996. Anthers and stamens and what they do. – In: D'ARCY W. G. & KEATING R. C. (Eds.), The anther: form, function and phylogeny, p. 1–24. – Cambridge University Press, Cambridge.

DE FREY H. M., COETZER L. A. & ROBBERTSE P. J. 1992. A unique anther mucilage in the pollination biology of *Tylosema esculentum*. – Sex. Plant Reprod. 5: 298–303.

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

— 1955. Embryological features and formula and taxonomy of the *Mimosaceae*. – J. indian bot. Soc. 34: 362–374.

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

ENDLICHER St. & UNGER F. 1843. Grundzüge der Botanik, Wien.

ENDRESS P. K. & STUMPF S. 1990. Non-tetrasporangiate stamens in the angiosperms: structure, systematic distribution and evolutionary aspects. – Bot. Jahrb. Syst. 112: 193–240.

— & — 1991. The diversity of stamen structure in "lower" *Rosidae (Rosales, Fabales, Proteales, Sapindales)*. – Bot. J. linn. Soc. 107: 217–293.

ENGLER A. 1876. Beiträge zur Kenntniss der Antherenbildung der Metaspermen. – Jahrb. wiss. Bot. 10: 275–321.

GOEBEL K. 1923. Organographie der Pflanzen, Teil 3, 2. Auflage. – Gustav Fischer, Jena.

GREEN J. W. 1980. A revised terminology for the spore-containing parts of anthers. – New Phytol. 84: 401–406.

GRIMES J. 1995. Generic relationships of *Mimosoideae* tribe *Ingeae*, with emphasis on the New World *Pithecellobium*-Complex. - In: CRISP M. & DOYLE J. J. (Eds.), Advances in legume systematics 7: 101–121. – Royal Botanic Gardens, Kew.

— 1999. Inflorescence morphology, heterochrony, and phylogeny in the mimosoid tribes *Ingeae* and *Acacieae (Leguminosae: Mimosoideae)*. – Bot. Rev. 65: 317–347.

GUINET PH. 1965 Études des caractères du pollen dans le genre *Calliandra (Mimosaceae)*. – Pollen et Spores 7: 157–173.

— 1981. *Mimosoideae*: the characters of their pollen grains. – In: POLHILL R. M. & RAVEN P. H. (Eds.), Advances in legume systematics 1: 835–857. – Royal Botanic Gardens, Kew.

— 1990. The genus *Acacia (Leguminosae, Mimosoideae)*: its affinities as borne out by its pollen characters. – Plant Syst. Evol. (Suppl. 5): 81–90.

— & FERGUSON I. K. 1989. Structure, evolution and biology of pollen in *Leguminosae*. – In: STIRTON C. H. & ZARUCCHI J. L. (Eds.) Advances in legume biology. – Monogr. syst. Bot. Missouri bot. Gard. 29: 77–103.

— & HERNÁNDEZ H. M. 1989. Pollen characters in the genera *Zapoteca* and *Calliandra (Leguminosae, Mimosoideae)*. Their systematic and phylogenetic relevance. – Pollen & Spores 31: 5–22.

HARTLEY M. L., TSHAMEKENG E. & THOMAS S. M. 2002. Functional heterostyly in *Tylosema esculentum (Caesalpinioideae)*. – Ann. Bot. 89: 67–76.

HERNÁNDEZ H. M. 1986. *Zapoteca*: a new genus of neotropical *Mimosoideae*. – Ann. Missouri bot. Gard. 73: 755–763.

— 1989. Systematics of *Zapoteca (Leguminosae)*. – Ann. Missouri bot. Gard. 76: 781–862.

— 1990. A new subgenus and a new species of *Zapoteca*. – Syst. Bot. 15: 226–230.

HESSE M. 1981. Pollenkitt and viscin threads: their role in cementing pollen grains. – Grana 20: 145–152.

HOC P.S. 1985. *Inga* MILL. in Argentina: a palynological study. – Bull. int. Group Stud. Mim. 13: 70–86.

HUGHES C. E. 1997. Variation in anther and pollen morphology in *Leucaena* BENTH. *(Leguminosae-Mimosoideae)*. – Bot. J. linn. Soc. 123: 177–196.

JAIN S. & VIJAYARAGHAVAN M. R. 1992. Mechanism of anther dehiscence in *Leucaena leucocephala (Leguminosae, Mimosoideae)*. – Phyton (Horn, Austria) 32: 103–109.

KENRICK J. & KNOX R. B. 1979. Pollen development and cytochemistry in some Australian species of *Acacia*. – Aust. 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. Gard. 29: 127–156.

LAVIN M., HERENDEEN P. S. & WOJCIECHOWSKI M. F. 2005. Evolutionary rates analysis of *Leguminosae* implicates a rapid diversification of lineages during the Tertiary. – Syst. Bot. 54: 530–549.

LERSTEN N. R. 1971. A review of septate microsporangia in vascular plants. – Iowa State J. Sci. 45: 487–497.

LEWIS G. P. & RICO ARCE L. 2005. *Ingeae*. – In: LEWIS G., SCHRIRE B., MACKINDER B. & LOCK M. (Eds.), Legumes of the World. – Royal Botanic Gardens, Kew.

LUCKOW M., MILLER J. T., MURPHY D. J. & LIVSHULTZ T. 2003. A phylogenetic analysis of the *Mimosoideae (Leguminosae)* based on chloroplast DNA sequence data. – In: KLITGAARD B. B. & BRUNEAU A. (Eds), Advances in legume systematics 10: 197–220. – Royal Botanic Gardens, Kew.

LUDWIG F. 1897. Ueber das Leben und die botanische Thätigkeit Dr. Fritz MÜLLER'S. – Bot. Centralbl. 71: 347–363.

MACQUEEN D. J., NORTON B. W. & STEWART J. L. 2001. Chapter 5. Use and management. – In: CHAMBERLAIN J. R. (Ed.) *Calliandra calothyrsus*, an agroforestry tree for the humid tropics. – Tropical Forestry Papers 40: 36–51. – Oxford Forestry Institute, Dep. Plant Sci., Univ. Oxford.

MILLER J. T., GRIMES J. W., MURPHY D. J., RANDALL J. B. & LADIGES P. Y. 2003. A phylogenetic analysis of the *Acacieae* and *Ingeae (Mimosoideae: Fabaceae)* based on *trn*K, *mat*K, *psb*A-*trn*H, and *trn*L/*trn*F sequence data. – Syst. Bot. 28: 558–566.

MOHL H. v. 1834. Über den Bau und die Formen der Pollenkörner. – Beiträge zur Anatomie und Physiologie der Gewächse, Erstes Heft, 130 Seiten, mit VI lithographierten Tafeln. – Fischer und Comp., Bern.

MOYANO F., COCUCCI A. & SÉRSIC A. 2003. Accessory pollen adhesive from glandular trichomes on the anthers of *Leonurus sibiricus* L. *(Lamiaceae)*. Plant Biol. 5: 411–418.

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

NIEZGODA C. J., FEUER S. M. & NEVLING L. I. 1983. Pollen ultrastructure of the tribe *Ingeae (Mimosoideae: Leguminosae)*. – Amer. J. Bot. 70: 650–667.

PACINI E. & HESSE M. 2005. Pollenkitt – its composition, forms and functions. – Flora 200: 399–415.

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

RICHTER S. 1929. Über den Öffnungsmechanismus der Antheren bei einigen Vertretern der Angiospermen. – Planta 8: 154–184.

ROSANOFF S. 1865. Zur Kenntnis des Baues und der Entwicklungsgeschichte des Pollens der *Mimoseae*. – Jahrb. wiss. Bot. 4: 441–452.

SORSA P. 1969. Pollen morphological studies on the *Mimosaceae*. – Ann. bot. fenn. 6: 1–34.

TEPPNER H. 1983. Review of VAN STEENIS 1981, Rheophytes of the world. – Phyton (Horn, Austria) 23(2): 323–324.

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

TOBE H. & RAVEN P. H. 1986. Evolution of polysporangiate anthers in *Onagraceae*. – Amer. J. Bot. 73: 475–488.

VOGEL S. 1981. Die Klebstoffhaare an den Antheren von *Cyclanthera pedata (Cucurbitaceae)*. – Plant Syst. Evol. 137: 291–316.

— 2002. Extra-tapetal pollen adhesives: where they occur and how they function. – Flowers: Diversity, development and evolution. – Symposium at the Institute of Systematic Botany, Zürich (Abstract volume), p. 48.

WAGENITZ G. 1996. Wörterbuch der Botanik. – Gustav Fischer, Jena.