Evolutionary Tendencies in Flowers of *Marantaceae* with special reference to the Style Movement Mechanism

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Elke Pischtschan
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Summary of the thesis

One of the quickest plant movements ever known is made by the ´explosive´ style in *Marantaceae* in the service of secondary pollen presentation – herewith showing a striking apomorphy to the sister *Cannaceae* that might be of high evolutionary consequence. Though known already since the beginning of the 19th century the underlying mechanism of the movement has hitherto not been clarified. The present study reports about the biomechanics of the style-staminode complex and the hydraulic principles of the movement. For the first time it is shown by experiment that in *Maranta noctiflora* through longitudinal growth of the maturing style in the ´straitjacket´ of the hooded staminode both the hold of the style prior to its release and its tensioning for the movement are brought about. The longer the style grows in relation to the enclosing hooded staminode the more does its capacity for curling up for pollen transfer increase. Hereby I distinguish between the ´basic tension´ that a growing style builds up anyway, even when the hooded staminode is removed beforehand, and the ´induced tension´ which comes about only under the pressure of a ´too short´ hooded staminode and which enables the movement. The results of these investigations are discussed in view of previous interpretations ranging from possible biomechanical to electrophysiological mechanisms.

To understand furthermore by which means the style gives way to the strong bending movement without suffering outwardly visible damage I examined its anatomical structure in several genera for its mechanical and hydraulic properties and for the determination of the entire curvature after release. The actual bending part contains tubulate cells whose walls are extraordinarily porous and large longitudinal intercellular spaces. SEM indicates the starting points of cell-wall loosening in primary walls and lysis of middle lamellae - probably through an intense pectinase activity in the maturing style. Fluorescence pictures of macerated and living style-tissue confirm cell-wall perforations that do apparently connect neighbouring cells, which leads to an extremely permeable parenchyma. The ´water-body´ can be shifted from central to dorsal cell layers to support the bending. The geometrical form of the curvature is determined by the vascular bundles. I conclude that the style in *Marantaceae* contains no ´antagonistic´ motile tissues as in *Mimosa* or *Dionaea*. Instead, through self-maceration it develops to a ´hydraulic tissue´ which carries out an irreversible movement through a sudden reshaping.

To ascertain the evolutionary consequence of this apomorphic pollination mechanism the diversity and systematic value of hooded staminodes are examined. For this hooded staminodes of 24 genera are sorted according to a minimalistic selection of shape characters and eight morphological types are abstracted from the resulting groups. These types are mapped onto an already available maximally parsimonious tree comprising five major clades. An amazing correspondence is found between the morphological types and the clades; several sister-relationships are confirmed and in cases of uncertain position possible evolutionary pathways, such as convergence, dispersal or re-migration, are discussed, as well as the great evolutionary tendencies for the entire family in which – at least as regards the shape of hooded staminodes – there is obviously a tendency from complicated to strongly simplified forms. It suggests itself that such simplifying derivations may very likely have taken place as adaptations to pollinating animals about which at present too little is known. The value of morphological characters in relation to modern phylogenetic analysis is discussed and conditions for the selection of morphological characters valuable for a systematic grouping are proposed: they are to be characters of shape that are typical of a certain group. They shall be rather rare than frequent, rather discrete than continuous, and rather obligatory than facultative. From such characters as few as possible should be taken to carry out a grouping, contrary to previous methods of grouping that included numerous characters.

Altogether, in view of the evolutionary success of *Marantaceae* compared with *Cannaceae* the movement mechanism of the style-staminode complex can safely be considered a key innovation within the order Zingiberales.

1. General introduction

The plant family *Marantaceae* is considered to be one of the most advanced families within the order Zingiberales (Kress et al. 2001) and appears particularly appealing in view of its evolution. The genera show a number of synapomorphies that make them stand out from all other monocot families. So for instance in the vegetative parts there is a unique leaf venation which is not parallel as it is generally typical of monocots, but includes a midrib, sigmoid lateral veins and special transverse veinlets (Kennedy 1978, Andersson 1998). Likewise conspicuous is the ´pulvinus´, a thickening of the tissue at the leaf base which enables nyctinastic leaf movements (Guttenberg 1971). Only from these two characteristics are *Marantaceae* doubtlessly recognisable, even when they are not blooming. In the floral parts of *Marantaceae* a tendency which is frequent in Zingiberales, namely to a ´remodelling´ as well as to the loss of anthers, is reflected. In their flowers all anthers except for one fertile, monothecic anther are either lost (one to two in the outer androeceal whorl) or very strongly reshaped (two in the inner whorl). This results on one hand in an almost entire asymmetry of the flowers (as the remaining sepals and petals are small and inconspicuous) while the highly specialised inner staminodial whorl is in the service of a peculiar kind of secondary pollen presentation. The pollen transfer mechanism works extraordinarily precisely through an irreversible ´explosive´ movement of the style as we have never found it in any other family. The flowers of the sister group *Cannaceae* show a certain degree of specialisation and loss of anthers, too, if not to the same extent as in *Marantaceae,* and no style movement during pollination. From the fact that the monogeneric *Cannaceae* comprise only 10 species, *Marantaceae*, however, with 31 genera approximately 535 species (Prince and Kress 2006) we infer that the style movement mechanism may very likely be a key innovation that has afforded an enormous and probably ecologically adaptive radiation.

To clarify this, though, we still lack presently sufficient basic knowledge relating both to the emergence of the style movement itself and to the functional and ecologic relationship between the flowers and their pollinators that has developed in the course of evolution. In the present study we focus in our investigations first of all on two principal aspects; first, how is the style movement prepared for during development and how is it brought about in a biomechanical and hydraulic sense? And, secondly, which evolutionary tendencies and pathways can be deduced from the floral diversity?

For the biomechanical question we examine at first in which way and in which time of bud development the biomechanical potential for the style movement is built up and in a following examination we look into the question how the tissue of the style is ´designed´ to enable such an enormous movement. For the examination of the evolutionary pathways a single floral organ, namely the cucullate staminode of the inner androeceal whorl, is brought in because it has obviously an important share in the style movement and, moreover, it displays a conspicuous diversity and appears to be of high diagnostic value. The ´bauplan´ of the cucullate staminode is determined and its diversity is compared with an already available phylogenetic analysis (Prince and Kress 2006) and conceivable evolutionary pathways are deduced and discussed.

2. The setting-up of tension in the style of *Maranta noctiflora*

Abstract

Marantaceae stand out from other plant families through their unique ´style movement´ that is combined with a highly derived form of secondary pollen presentation. Though known for a long time the mechanism underlying the movement is not yet understood. In the present paper we report about an investigation into the biomechanical principles of the movement. For the first time we confirm by experiment that in the case of *Maranta noctiflora* longitudinal growth of the maturing style within the ´straitjacket´ of the hooded staminode involves both the arresting of the style before tripping and the building up of the potential for the movement. The longer the style grows in relation to the enclosing hooded staminode the more does its capacity for curling up increase. Hereby we distinguish between the ´basic tension´ that a growing style builds up anyway, even when the hooded staminode is removed beforehand, and the ´induced tension´ which comes about only under the pressure of a ´too short´ hooded staminode and which enables the movement. The results of our investigations are discussed in view of previous interpretations ranging from biomechanical to electrophysiological mechanisms.

2.1 Introduction

Marantaceae (31/535) are characterised by an explosive pollination mechanism which includes a unique ´style movement´ and a highly derived form of secondary pollen presentation (Kunze 1984, Claßen-Bockhoff 1991, Yeo 1993, Kennedy 2000, Locatelli et al. 2004). Though known for a long time the mechanism underlying the movement is not yet understood. In the present paper we report about an experimental investigation into the biomechanical principles of the movement.

2.1.1 The Flowers in *Marantaceae*

Flowers of *Marantaceae* are asymmetric (Fig. 2.1 A, B) and mostly arranged in pairs, being mirror images of each other (Kennedy 1978). The perianth is usually inconspicuous and the

showy parts of the flowers are staminodes. One or two outer staminodes are petaloid (Fig. 2.1 B: ost) while the inner androeceal whorl is differentiated into functionally modified structures: the fleshy staminode (staminodium callosum; Fig. 2.1 B: stcal), which forms the upper part of the androeceal tube and which bears a petaloid appendage in some species (Fig. 2.1 A: pa), the hooded staminode (staminodium cucullatum; Fig. 2.1: stcuc), which encloses the style, and the single stamen with its monothecic anther (Fig. 2.1 B: a) and petaloid appendage. Usually pollen is forced out of the anther before anthesis by the growing style (Kennedy 2000), hereby giving an example of extreme protandry (Heller 2003). It gets firmly attached to a small dorsal depression at the head of the style (Kennedy 1978, ´stamp´ of Andersson 1981; Fig. 2.2 B: d) by means of a pollen coat substance (´mucus´ of Gris 1859). At anthesis the style lies in the hooded staminode in a more or less ´overstretched´ way (Fig. 2.2 A). When an insect enters the flower it touches the so-called ´trigger´ (Kunze 1984, Claßen-Bockhoff 1991) or ´appendage´ (Andersson 1998) (Fig. 2.2 A: t) and the style curls up in adaxial (ventral) direction immediately (Fig. 2.2 B). In the course of this single movement the foreign pollen is scraped from the insect´s body and scooped into the stigmatic orifice (Locatelli et al. 2004). At the same time the self-pollen at the style head gets attached to the pollinator´s body by an adhesive secretion (Claßen-Bockhoff 1991, Yeo 1993). The pollen transfer is thus completed in a single and irreversible action and each flower has but one chance of being cross-pollinated (Kennedy 1978). The movement of the style goes very swiftly; slow motion pictures revealed that in *Maranta leuconeura* E. Morren the entire movement of the style was completed in 0,2 sec. (Kunze 1984). Also in a high frequency film it was shown that in *Thalia geniculata* L. the mere exchange of pollen was performed in a short space of even 0,03 sec. within the course of the complete style movement (Claßen-Bockhoff 1991).

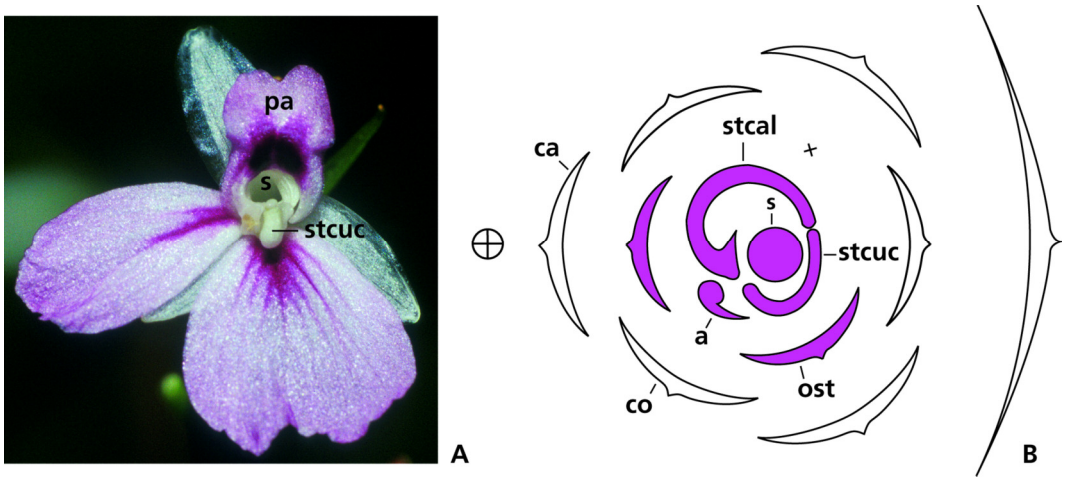

Fig. 2.1: *Maranta leuconeura* E. Morren var. *massangeana*: front view of a flower (A) and floral diagram (B). a = anther, ca = calyx, co = corolla, ost = outer staminodium, s = style, stcal = staminodium callosum or fleshy staminode, stcuc = staminodium cucullatum or hooded staminode (photo: M. Crone)

Though many interpretations of the style movement and assumptions have been made so far (e.g. Gris 1859, Delpino 1869, Schumann 1902, Kunze 1984, Claßen-Bockhoff 1991) only few attempts were made to clarify the actually underlying mechanism by experiment (Heller 2003). The present paper offers some first approaches and goes along with the following questions: What holds the style in its resting position before being tripped and what tensions the style for this irreversible and very swift movement? Regarding these two questions it appears crucial to find out in which relation to each other the style and hooded staminode grow together during the last hours of bud development and at which stage they come into so tight a contact that the critical tension for the movement can be built up. Whether the release of the movement is solely biomechanical, namely through a simple change of turgor, or whether it is supported by an additional conduction of action potentials is a question still unresolved and that we try at least to approach basically.

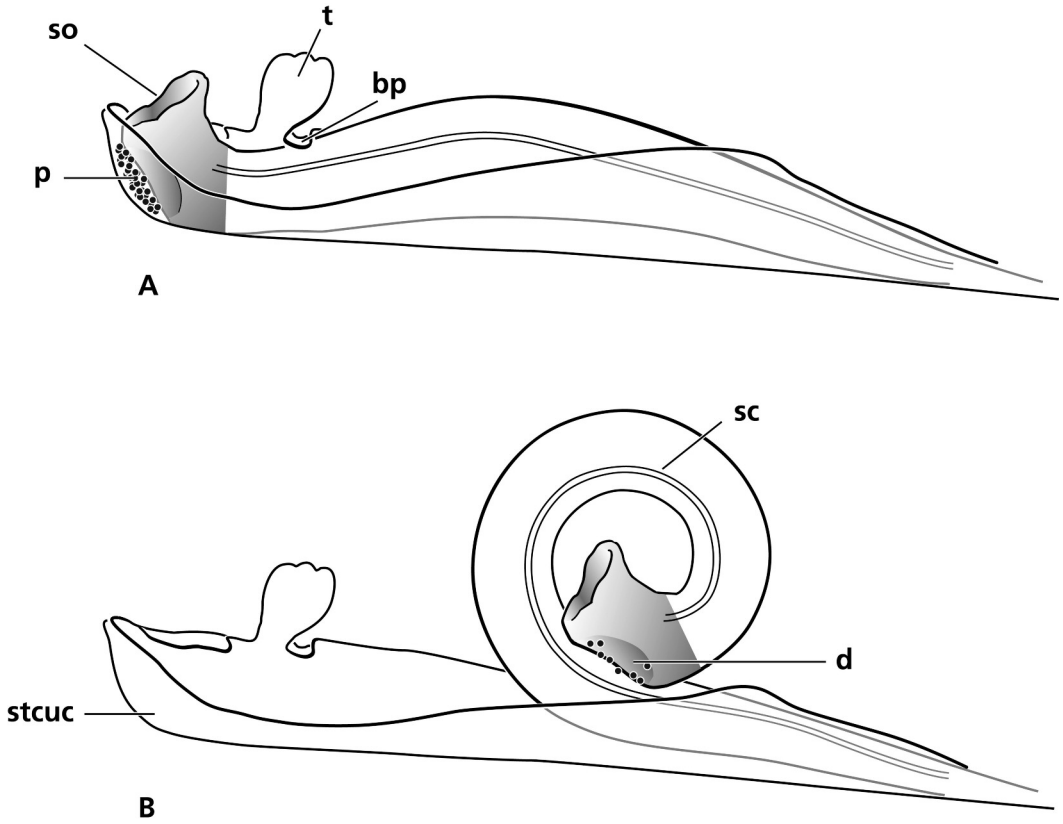

Fig. 2.2: *Maranta noctiflora*: style movement (compare with Claßen-Bockhoff 1991). A: the unreleased style lies in the hooded staminode (stcuc) in a slightly overstretched manner while self-pollen is already held to the style's ´head´. B: after release the style curves rapidly in adaxial direction. By this movement self-pollen is stuck onto the pollinator and foreign pollen is shovelled into the stigmatic orifice. bp = basal plate, d = depression (at the style head), p = pollen, sc = style canal (along with vascular bundles), so = stigmatic orifice, stcuc = staminodium cucullatum or hooded staminode, t = trigger

2.1.2 Historical outline of interpretations

The protandry in *Marantaceae* and the deposition of self-pollen on the style near the stigma (or even in the stigma in some cases) is firstly documented by Lindley (1819). Already a few years later does he describe the abrupt movement of the apex of the style, though without explaining it as a transfer of pollen (Lindley 1826 a, b). It is Gris (1859) who writes that the style comes into the hooded staminode by growth and that the ´touch of the flower´ releases the style movement. Delpino (1869) is the first to postulate that the inner tension of the style is built up during lengthening and that definitely the hooded staminode holds it in its over-stretched position before release. Relevant for the release is the touch of the trigger or ´dente´

by a pollinator. Hildebrand (1870) follows him in this opinion and adds that the pollen transfer is carried out during the released movement, hereby preventing autogamy. He interprets the bending of the style as a consequence of differential tensioning of cell-layers in both ´sides of the style´. Eichler (1884) makes even more differentiated observations: The style is enclosed in the hooded staminode and held in its resting position by the upper cap (i.e. the ´hood´) and is, as a consequence of stronger longitudinal growth at the dorsal side, arch-like tensioned. Touching the hook-like appendage loosens the hood and releases the movement. Eichler (1884) confirms Hildebrand´s (1870) opinion that autogamy is hindered by this method and cross pollination supported. The appendage serves the release of the movement, but also strong vibrations can release the style. Self-release is still doubted, but not absolutely excluded by Eichler (1884). Schumann (1902) bears Eichler out in all his observations relating to the inner tension and the hold of the style, but doubts plainly the importance of the appendage for the release mechanism because simply the touch of the edge of the hooded staminode or even just the tremor of the entire flower releases the style. In any case, an insect is believed to bring about the relief from the tautness. He also agrees that autogamy is prevented or at least impeded by this method of pollination. Furthermore, he says that no mechanical tissues can be found inside the style and that the strength of the style (as well as the fleshy staminode) is due only to the turgor of the parenchymatic tissues in these organs. According to Schumann (1902) the movement is made possible through a shift of water from inside the cells into the intercellular spaces. Costerus (1918) sees the style held in position by the hooded staminode and the adnate anther and the release brought about through either ´exciting the style´ or ´pressing the tooth to the other side of the hood´. Loesener (1930) comes to the conclusion that the style is in tension, being held in its position by the hooded staminode. Hereby the displacement of the ´little ear´ (i.e. the trigger, the authors) brings about the movement. Herein he is supported by Kennedy (1978). Petersen (1981) observes that an ´object´ entering the flower and touching the appendage loosens the hood and hereby releases the style. Andersson (1998) comes to the conclusion that the style is in tension due to ´differential lengthening´, being held in its backwards curved position by the ´hood-shaped´ staminodium cucullatum. Hereby the displacement of the ´appendage´ actually triggers off the pollination mechanism. Andersson´s notion of a differential lengthening is supported by Claßen-Bockhoff and Pischtschan (2000) who illustrate that the parenchymatic cells of the ventral and dorsal sides of the style alter their length and volume with the style movement. Kennedy (2000) speaks of a ´static equilibrium´ in which the tensioned style is held by the

hooded staminode and she supports the observation that the trigger or appendage releases the style movement. In contrast to suppositions of Hildebrand (1870) and Eichler (1884) she confirms autogamy in twelve genera. Arns et al. (2002) follow most previous authors in the sense that the style is in tension, being held in its position by the hooded staminode before visited by a pollinator. The touch of the ´apendice´ is the cause of the movement. Hereby the authors make more differentiated observations relating to the position of the appendage and the form of the curvature of the released style in different genera. Altogether, through observations of many authors up to now, a rather uniform picture comes about including an extremely close biomechanic connection between the style and the hooded staminode with the style being held in its position against its inner tension by the hooded staminode. Hereby either vibrations, an ´excitement´ or a displacement of the trigger bring about the release.

Entirely in contrast to all prior interpretations are the results of Kunze´s (1984) investigations of *Maranta leuconeura* and *Calathea undulata* Lind. et Aust., indicating that the hooded staminode cannot in any case be strong enough to hold the tensioned style, an opinion in which he is followed by Yeo (1993). Kunze (1984) postulates that the style only on being excited at the side leaning against the basal plate of the trigger builds up the tension which is then rapidly converted into the movement by an immediate change of turgor. This change of turgor is accompanied by a shift of water into the intercellular spaces, an opinion already given by Schumann (1902). The idea of a thigmonastic movement (see Costerus 1918) is strongly confirmed by observations of Claßen-Bockhoff (1991) at *Thalia geniculata* L.. She says that the inner tension of the style is built up during a phase of enormous lengthening inside the bud. The mature style is directly excitable in the area at which rests the basal plate of the trigger. She also confirms self-release in *Thalia geniculata*. The high speed of the style movement (Claßen-Bockhoff 1991) which is faster than mere diffusion may point to an electrophysiological reaction to trigger off the movement. An extraordinary interpretation of the style movement is given by Howell et al. (1993), namely that in the case of *Calathea zebrina* (Sims) Lindley the style can reset. This is a completely erroneous statement founded on a description of the pollination process given by Petersen (1981: 531) where it is said ".....in the course of its movement, the style curves back, blocking the way to the nectar.....". The expression ´curves back´ is here obviously misinterpreted in the sense of a reversibility of the style. In fact, it means exactly the regular curling-up movement in adaxial (or ventral) direction. Thorough investigations on our part have proven that there is no reset of the style in *Calathea zebrina*.

2.2 Materials and methods

2.2.1 Materials

Living plants of the neotropical species *Maranta depressa* E. Morren and *Maranta noctiflora* Koernicke were chosen for the experiments. Both are cultivated in the greenhouses of the Botanical Garden of the University of Mainz, Germany. Flowers open at 8°° and 20°°, respectively, and wilt after few hours. Buds develop almost exactly simultaneously what makes them very suitable for our experiments

2.2.2 Methods

The main growth phase of the buds was determined in *Maranta depressa*. A total of 23 equally old buds from different individuals were labelled at 14°°, i. e. eighteen hours before anthesis. Every two hours (14°° to 2°°) and every hour (2°° to 6°°) their length was measured from the base of the sepals (above the ovary) to the tip of the bud. The examinations were carried out at 32° Celsius.

The ´growth dynamics´ in *Maranta depressa* revealed that the strongest longitudinal growth took place at night during the last hours before anthesis. To have a more convenient test plant for the experiments we turned to *Maranta noctiflora* which regularly flowers at night and has provided us with a good number of simultaneously developing buds during the afternoon.
A total of 42 flowers were labelled at 13°°, i. e 7 hours before anthesis. In regular intervals of 90 minutes 6-8 fresh buds were picked from the plant to measure the length of the bud as in *M. depressa*. Then the styles were released by hand manipulation at the ´trigger´ and their length was measured by means of a thread going along with the entire dorsal line from the distal edge of the ovary to the rim of the stigmatic orifice as well as the length of the hooded staminodes in the corresponding places. The style´s increasing capacity for curling up at each time was determined by measuring the angle of curvature (see Fig. 2.3: α).

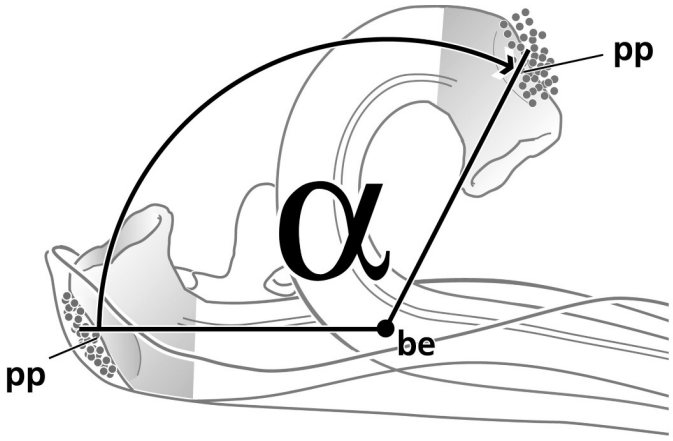

Fig. 2.3: Measurement of the style's movement. The angle of the curvature (α) is inferred from the distance between the positions of the style-head before and after the release. be = bending edge, pp = middle of the pollen plate

To determine the influence of the hooded staminode on the ´growing behaviour´ of the styles all staminodes and the perianth of a total of 25 flowers were bent away at 13°° to let the styles grow on freely – without restraint from any adjacent or enveloping floral organs. It turned out that at anthesis by 20°° a number of 10 flowers had completely withered due to the damage that had been done to the tissues through such a heavy hand manipulation. The remaining 15 flowers were used to measure the length of their styles in comparison to the length of regularly grown styles and to document their shape through the angle of the curvature.

To find out how far the style movement is supported by an alteration of membrane permeability or whether even the release of the movement might be triggered off by action potential some flowers were put under narcosis with diethyl-ether or chloroform (after Metzner 1982) to suppress the style movement to a certain extent. For this 20 flowers were taken at 19°° and set into a glass cuvette with the stems in a little ´vase´ to ensure water supply. A cotton-wool ball sprinkled with the pure narcotic was put beside the flowers and the cuvette was closed. After 10 minutes under ether or 30 minutes under chloroform almost no style movement could be produced for some time. It proved worthwhile to prefer the 10 minutes´ method to avoid wilting of the flowers. Nevertheless there was always an uncertain number of flowers which could not recover from the narcosis and whose styles could not be released any more. All styles of such flowers that had regained their ´excitability´ were

afterwards released by hand manipulation. Their length and the length of the hooded staminodes and the angle of the curvature were measured in the usual way.

2.3 Results

The increase in bud length in *Maranta depressa* was recorded during the last 18 hours before flowering (Table 2.1). Starting with a length of 6.20 mm ± 0,23 the buds elongated more or less continuously for the first 10 hours getting a length of 7.63 mm ± 0,39 at midnight. In the early morning hours growth increased exponentially (Fig. 2.4) reaching the final length of 12.60 mm at 8°° when the flowers usually open. Strongest longitudinal growth was observed between 2°° and 3°°, i. e. 5-6 hours before flowering. In the last hours before anthesis the style and hooded staminode elongated to different degrees. By growing faster than the hooded staminode the style grew against the ´hood´ of the hooded staminode and reached its slightly overstretched form (see Fig. 2.2 A).

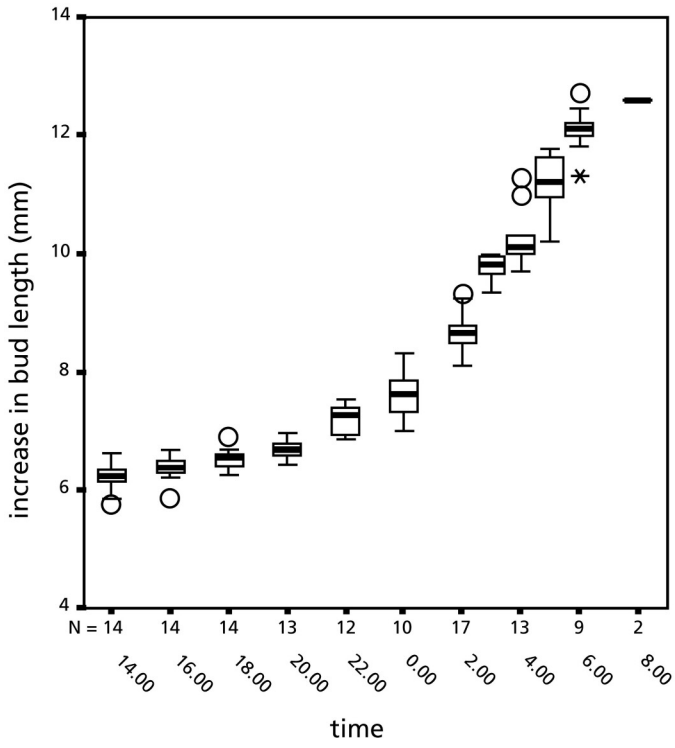

Fig. 2.4: *Maranta depressa*: increase in bud length during the last hours before anthesis. The boxplots cover 75% of the data each, n = number of buds.

Table 2.1: *Maranta depressa*: increase in length [mm] in 23 buds during the last hours before flowering, x = average, s = standard deviation, n = number of buds per time unit

	14°°	16°°	18°°	20°°	22°°	24°°	2°°	3°°	4°°	5°°	6°°	8°°
1	5.76	5.87	6.25									
2	6.21	6.39	6.41	6.42								
3	6.14	6.43	6.52	6.59	6.86							
4	6.16	6.51	6.57	6.92	7.27							
5	6.25	6.28	6.60	6.74	6.93	7.51						
6	6.24	6.32	6.47	6.60	6.94	7.33						
7	6.23	6.28	6.42	6.49	6.95	7.27	8.12					
8	6.18	6.23	6.40	6.66	7.25	7.46	8.92					
9	5.86	6.33	6.37	6.45	6.95	7.72	8.66		9.77			
10	6.62	6.68	6.68	6.80	7.26	7.82	8.52		9.73			
11	6.48	6.66	6.90	6.96	7.39	8.00	8.50		10.13		11.83	
12	6.38	6.51	6.60	6.70	7.50	7.01	8.66		10.33		12.14	
13	6.36	6.40	6.57	6.83	7.52	8.33	9.33		11.29		12.73	12.60
14	5.95	6.33	6.60	6.73	7.36	7.86	8.70		10.24		12.10	12.60
15							8.59	9.91	11.01	11.65	12.45	
16							9.24	9.80	10.12	11.40	11.33	
17							8.66	9.99	10.10	11.06	12.20	
18							8.71	9.87	10.32	10.20	12.21	
19							8.40	9.60	9.89	11.77	12.01	
20							9.01	10.00	10.04	10.97		
21							8.80	9.78	9.99			
22							8.45	9.34				
23							8.37					
x	6.20	6.37	6.53	6.68	7.18	7.63	8.68	9.79	10.23	11.18	12.11	12.60
s	± 0.23	± 0.20	± 0.16	± 0.17	± 0.24	± 0.39	± 0.31	± 0.22	± 0.45	± 0.57	± 0.39	± 0.00
n	14	14	14	13	12	10	17	8	13	6	9	2

Table 2.2: *Maranta noctiflora*: differential style elongation and growth of the hooded staminode during the last hours of bud development. n = number of buds, A = average length and standard deviation of buds [mm], B = average length and standard deviation of hooded staminodes (mm), C = increase in length of hooded staminodes compared with 13°° (%), D = average length and standard deviation of styles (mm), E = increase in length of styles compared with 13°° (%), F = relative length of hooded staminodes and styles

time	n	A	B	C	D	E	F
13°°	8	11.88 ± 0,23	10.75 ± 0,46	0	10.75 ± 0,31	0	1 : 1.00
14³°	6	13.00 ± 0.00	11.83 ± 0,26	10	11.83 ± 0,27	10	1 : 1.00
16°°	6	14.38 ± 0,32	13.57 ± 0,46	26	14.10 ± 0,46	31	1 : 1.03
17³°	6	15.05 ± 0,18	13.67 ± 0,77	27	14.50 ± 0,05	35	1 : 1.06
19°°	8	18.13 ± 0,71	16.31 ± 0,73	52	17.25 ± 0,52	60	1 : 1.06
20°°	8	20.75 ± 0,90	17.50 ± 0,85	63	20.20 ± 0,48	88	1 : 1.15

To test the hypothesis that the tension of the style is set up biomechanically by differential growth while being restricted within the ´straitjacket´ of the hooded staminode experiments were carried out using almost ripe buds of *Maranta noctiflora*. As in *M. depressa* strongest longitudinal growth took place shortly before anthesis between 17³° and 19°° (Table 2.2). At 13°° the style and hooded staminode were equally long and came into close contact for the first time (Fig. 2.5: A, Fig. 2.8: A, B). Self-pollen had already been forced out of the anther into the ´hood´ of the hooded staminode and got firmly attached to the style's dorsal depression under pressure and by means of an epidermal secretion. The style lay straightly in the hooded staminode and only its ´head´ was bent upwards. While the style was growing on tension was set up (Table 2.2). The style grew against the ´hood´ of the hooded staminode and became a little overstretched. At 14³° the style could already be released, but only rose by about 45° (Fig. 2.5: B, Fig. 2.8: C, D). When released in riper bud stages the ´bending

capacity´ of the style continuously increased (Fig. 2.5: C-E, Fig. 2.8: D, G). As in the experiment all outer floral organs had been removed the style could bend up to its maximum of 180° (Fig. 2.5: F, Fig. 2.8: G) whereas it is regularly restricted by the fleshy staminode to an angle of about 100° (see position at 17³°, Fig. 2.5: D). At anthesis a total length difference of 15% (1 : 1,15, Table 2.2) is found between the hooded staminode and the style. As a result of this the inner tension is obviously sufficient to transfer pollen by means of the style movement. The induced tension reflected by an increase in length which is by 15% higher in the style compared to the hooded staminode (Table 2.2) thus indicates the power with which the head of the style under live conditions hits on the fleshy staminode.

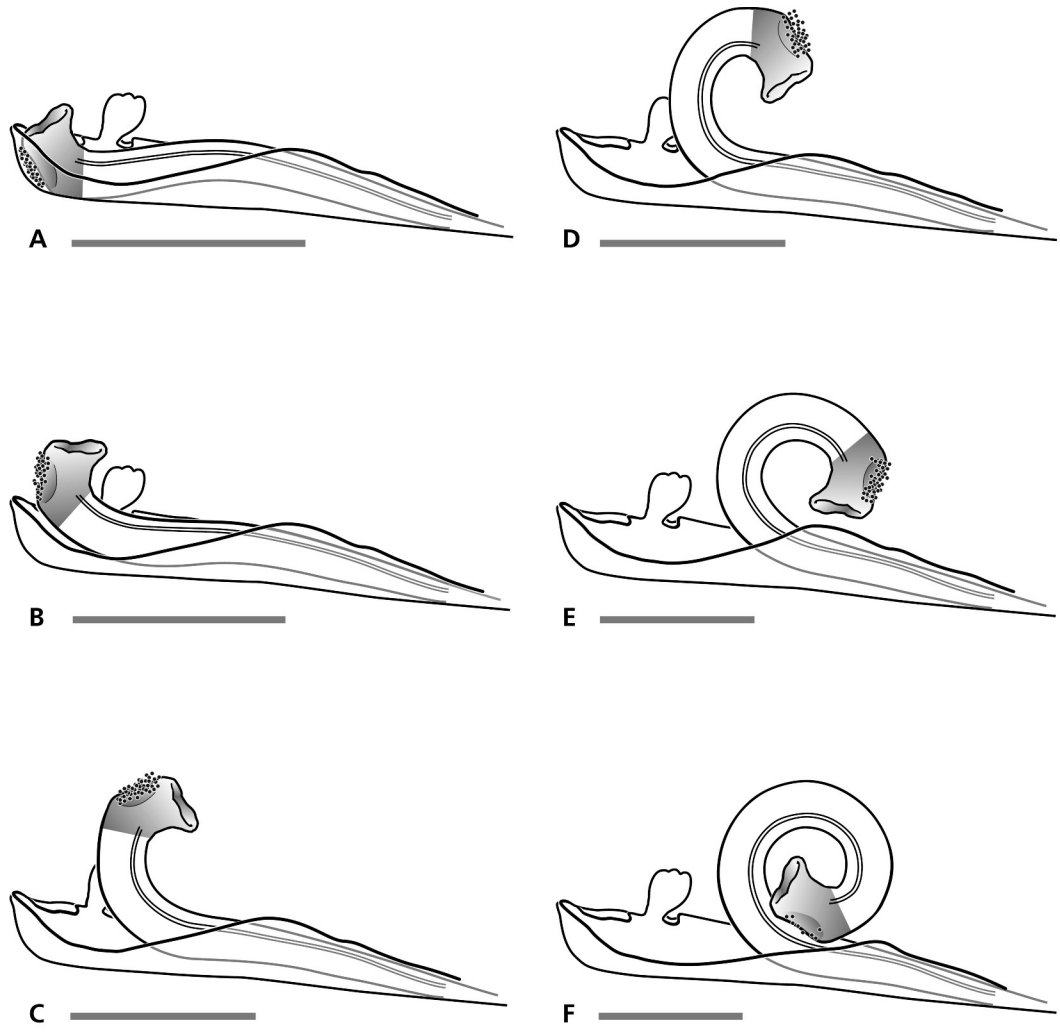

Fig. 2.5: *Maranta noctiflora*: increase in the capacity for curling up during the last hours before anthesis (see Table 2.2). Every 1.5 hours flowers were taken from the plant. Styles were released by hand manipulation and their lengths were compared to the ones of the hooded staminodes A 13°°, B 14³°, C 16°°, D 17³°, E 19°°, F 20°° (anthesis). Bar = 5 mm; note that the decreasing length of the bar indicates the growth of the styles and hooded staminodes.

To elucidate the influence of the hooded staminode on the setting up of tension styles were freed from their hooded staminodes 7 hours before anthesis. Again at 13°° the two organs were of equal length, but then again the style grew faster and longer than the hooded staminode. Without being restricted by the hooded staminode it grew in an almost straight way (Fig. 2.6, Fig. 2.8: H). It was stabilised by its turgor pressure that is here defined as ´basic tension´. Styles that had been allowed to grow freely could not be ´released´. They failed in building up the ´induced tension´ which is obviously necessary to develop the capacity for curling-up.

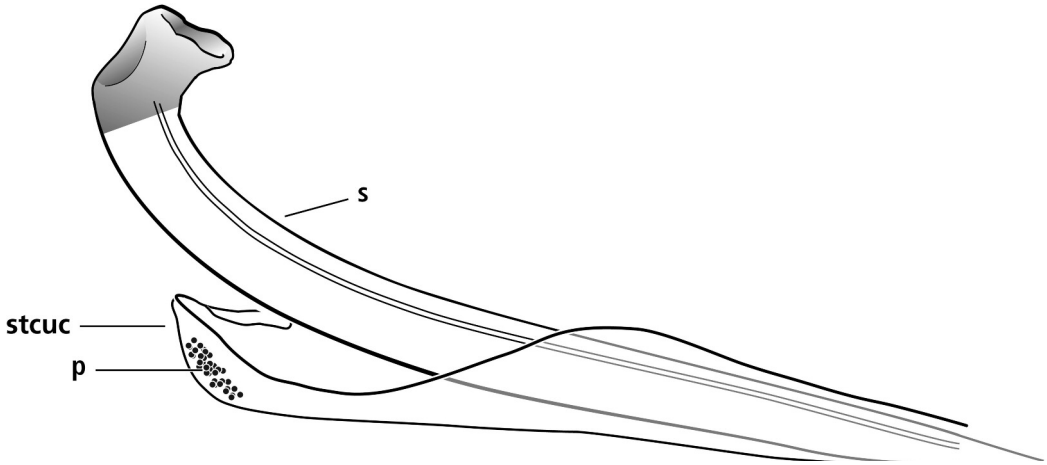

Fig. 2.6: *Maranta noctiflora*: capacity for curling up after growing outside the ´straitjacket´ of the hooded staminode. The style has increased its length and shows a slight curvature of its upper part which results from the growing process (´basic tension´) and the position of the vascular bundles. It has not built up an ´induced tension´ and thus cannot curl up any further. p = pollen, s = style, stcuc = staminodium cucullatum or hooded staminode.

Adult styles which had been ´narcotised´ for 10 minutes could either not recover from the narcosis or could be released after some time, but their capacity for curling up was irreversibly set back. They rose up to an extent as though they were by one-and-a-half hours

behind (see Fig. 2.7, Fig. 2.8: F), hereby suggesting that an ether narcosis may possibly have suppressed an involved alteration of the basic membrane potential.

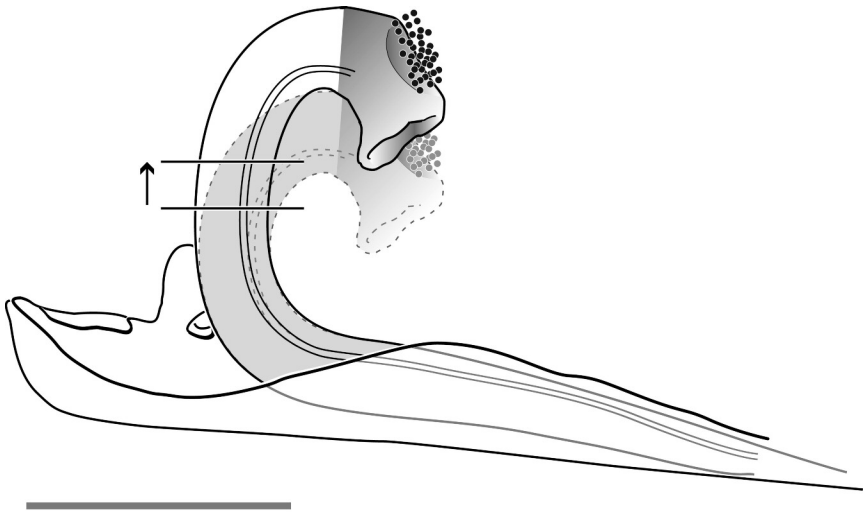

Fig. 2.7: *Maranta noctiflora*: reduction of the ´curling-up capacity´ after narcosis. At 19°° the style has developed its regular length but only an ability to curl up which is equivalent with a style at 17°°. The curling-up movement thus remains partly inhibited.

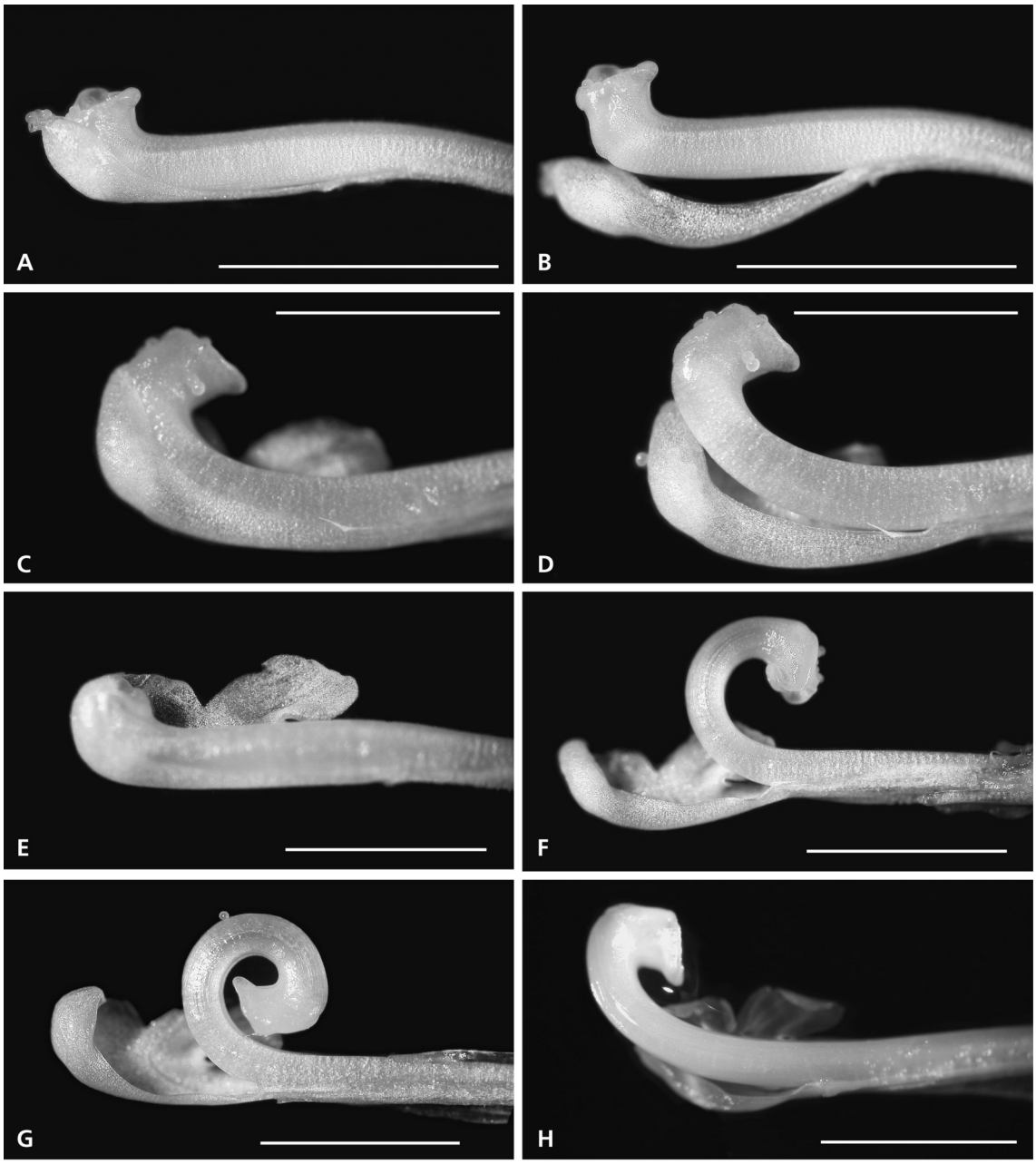

Fig. 2.8: *Maranta noctiflora*: length ratios between the style and hooded staminode in different phases of development. A, B: at the beginning of bud development; C, D: in the middle of bud development; E-G: at anthesis - with the mature style still arrested in the hooded staminode (E), ´narcotised´ style slightly curled up after release (F), strongly curled up under normal conditions (G). H: Great difference in length between the style and hooded staminode after the style has grown freely and in a sightly incurved way outside the hooded staminode.

2.4 Discussion

Our examinations show that in *Maranta noctiflora* the style is able to build up two different sorts of tension: independently of any adjacent floral organ the growing style develops its regular turgor pressure which in connection with the stabilising vascular bundles we define as ´basic tension´. On the ventral und dorsal side the style is fully turgescent and both sides expand to such an extent as the tautness of the opposite side allows so that they are in an equilibrium. We believe that the style that has grown freely reflects the true properties of the tissue. Hereby the vascular bundles are in an acentric and ventral position and maybe a little shorter than the ´parenchymatic style´ (see chapter 3). Consequently, the ´free´ style grows in an almost straight, just slightly ventrally incurved way. On the other hand, the crucial ´induced tension´ which only makes the curling-up movement possible comes about under extraordinary growth conditions in the ´straitjacket´ of a ´too short´ enclosing staminode. The cells or vacuoles, respectively, are pumped up with water during an enormous elongational growth in the last hours and at the same time prevented from stretching in longitudinal direction, so that they get ´compressed´. We assume that the tissue of the style in its entirety is fully turgescent, but the tautness of the tissue on the ventral and dorsal sides develops differentially under straitjacket conditions. The pressure in longitudinal direction must be higher in the dorsal area than in the ventral because the ventral tissue can more easily expand in comparison to the dorsal. It can only be assumed that the place in which the head of the style is under maximum pressure inside the hood of the cucullate staminode is in its own dorsal portion. Consequently the style in *Maranta noctiflora* can become a little backwards overstretched when the ventral side builds up a slightly higher turgor pressure than the dorsal. The release of the style from the hooded staminode then leads to a sudden decompression of the tissue in the dorsal area followed by a maximum expansion of the dorsal cells. The final incurvation becomes stronger than in the equilibrium when the tension in the dorsal tissue is offset. Hereby the shape of the movement follows the course of the vascular bundles (see chapter 3). The longer the style has grown in relation to the hooded staminode the higher is its dorsal ´compression´ and herewith the ´induced tension´ that affords the capacity for strongly curling up. Comparative examinations of fully grown and released styles of *Ataenidia conferta* (Bentham) Milne-Redhead and *Donax cannaeformis* (Forster) K. Schumann and *Marantochloa purpurea* (Ridley) Milne-Redhead show that this principle applies to other genera, too (unpublished data): the relative proportion of the length of the style and the

hooded staminode is quite different among these species under greenhouse conditions – small in *Ataenidia*, but in *Donax* and *Marantochloa* much higher - and involves a very different capacity for curling-up. At the native habitat, however, the released style in *Ataenidia conferta* curls up very strongly (A. Ley, pers. com.) so that we conclude that the length difference between the hooded staminode and the style becomes distinctly higher under natural conditions than in the greenhouses.

Our results from the development of *Maranta noctiflora* clearly support the observations of all previous authors (except for Kunze 1984), namely that the style is held in its position by the hooded staminode alone and that it is tensioned during lengthening (see Delpino 1869). The hold of the style is due to differential growth (see Hildebrand 1870: ´differential tensioning´, Eichler 1884; Andersson 1998: ´differential lengthening´). The style growing freely outside the hooded staminode without developing an ability to curl up later makes it clearly evident that the inner tension and herewith the potential for the movement are built up before and not at the very moment of release. The hold alike the tensioning of the style within the hooded staminode are due to an extraordinarily close development and cooperation of these two floral organs.

Kunze´s (1984) interpretation was based on the observation in *Maranta leuconeura* and *Calathea undulata* that in his experiments it was possible to remove the hooded staminode from the style head without releasing the movement. A slight displacement of the basal plate alone caused release. This observation could not be absolutely confirmed by our experiments. Only in exceptional cases (e.g. only once with *Hylaeanthe hoffmannii*) we managed to free a style completely from its hooded staminode without the style curling up. The reason is not easily discernible. Probably the induced tension cannot always be built up. Physical conditions such as humidity and warmth surely have a share in it.

We have observed that in an exceptionally hot summer (2003, up to 40° C) the length difference between the style and the hooded staminode in *Maranta noctiflora* became decisively higher than under normal greenhouse conditions (32° C), presumably through a stronger enzymatic activitiy in the growing style (unpublished data). In fact, the ´robustness´ versus ´excitability´ of styles can also differ very much with the species, depending - among other things - on the time of the day (unpublished data). In *Pleiostachya pruinosa* Regel (K. Schumann), for instance, at the native habitat self-release in the morning is normal (Heller 2003) while in the greenhouses of the National Botanic Garden of Belgium according to our observations it always released itself by midday.

In any case it is not the regular, basic turgor in the style that enables the movement, but it is the ´induced tension´ gained under growth conditions of extraordinary pressure which makes the style ´releasable´ and may in some circumstances not be established so that even a fully turgescent, mature style can be freed from its hooded staminode without ´moving´.

As for the release, we expect an electrophysiological reaction to have a share in the movement in at least some species because in several cases there is evidence for thigmonasty (Gris 1859, Delpino 1869, Costerus 1918, Kunze 1984, Classen-Bockhoff 1991, Arns et al. 2002) and even seismonasty (Eichler 1884, Schumann 1902, Heller 2003). Also the fact that an ether narcosis works in such a way as hinders the release and sets back the capacity for curling up corresponding to a certain maturation-time indicates that there must be a change of membrane permeability involved in the movement (see Yang W., Lou Ch. 1994) which is a natural component of any turgor movement. In every species observed in this study it was possible to release the movement by simply displacing the hooded staminode. Therefore we believe that a purely biomechanical model based on differential growth and differential tensioning of the involved organs may explain the mechanism of the movement by principle and works in all species. In addition to that some genera, such as *Thalia* or *Hylaeanthe*, may have developed an ability to respond to an ´excitement´ with a full depolarisation of their membrane potential and possibly with the conduction of action potentials to release the movement of the style. Hereby it is still unclear whether these putative action potentials come about in the style or in the hooded staminode or even in both. Furthermore it appears that there are transitions between thigmonastic and seismonastic stimulations (regardless of any following growing processes which are sometimes included in the definition). Anyway, it appears that thigmonasty and seismonasty do not occur in all species and not under all circumstances. Solely the genus *Thalia* has always been extremely excitable in a seismonastic sense (releasable through tremor alone even without being touched) and reliably excitable under all tried circumstances (unpublished data). In other cases such an ´electrophysiological tension´ may only under the best conditions at the native habitat (humidity, warmth) be established and facilitate a much more ´sensitive´ and quicker release of the style.

Altogether, with respect to the size of the family and its high diversity and the diversity of its habitats it cannot be excluded that there may be various combinations of mechanical and physiological processes involved in the release of the movement.

Starting from these observations we have examined how the geometrical form and the ventral direction of the movement are determined and for the present we believe that they are due to

the acentric position and the relative shortness of the vascular bundles (see above). Furthermore, we know now, entirely in contrast to Schumann´s opinion (1902), that there are certain characteristics to the inner tissues of the style which allow the style to undergo such an enormous deformation without visible ruptures and which we call a ´hydraulic´ dorsal tissue. Here also the question about a shift of water from inside the cells into the intercellular spaces (Schumann 1902, Kunze 1984) has been examined by means of histological and anatomical research methods (see chapter 3).

3. A hydraulic model of the ´explosive´ style movement in *Marantaceae* – evidence from functional anatomic studies

Abstract

The ´explosive style´ in *Marantaceae* produces one of the quickest plant movements. We examined its anatomical structure in several genera for its mechanical and hydraulic features and for the determination of the entire curvature after release. The style consists of a ´head´, a middle part that carries out the actual bending movement, and a base that is regularly fused with the filament and the cucullate staminode. The central ´bending part´ contains tubulate cells whose walls are extraordinarily porous and large longitudinal intercellular spaces. SEM indicates the starting points of cell-wall loosening in primary walls and lysis of middle lamellae - probably through an intense pectinase activity in the maturing style. Fluorescence pictures of both macerated and living style-tissue confirm cell-wall perforations which do apparently connect neighbouring cells, which leads to an extremely permeable parenchyma. Dissolution of stabilising structures affords a pliability enabling the style to give way to the bending without outwardly visible damage. The ´water-body´ can be shifted from central to dorsal cell layers to support the bending. The geometrical shape of the curvature is determined by the vascular bundles.We conclude that the style in *Marantaceae* contains no antagonistic motile tissues. But through self-maceration it develops to a ´hydraulic tissue´ which brings about an irreversible movement through a sudden reshaping.

3.1 Introduction

After Skotheim and Mahadevan (2005) all plant movements – in contrast to muscular animal movements – are based on a mechanical fluid transport through plant tissue. Hereby the velocity is limited by the rate of water transport through a porous tissue of a certain thickness. The very rapid movements require either small size or special ´elastic instabilities´ of the tissue. A diagram of the authors in which the velocity of fluid transport is plotted versus the thickness of the parenchyma results in a classification of their 28 experimental plants in three natural groups of plant movements, namely swelling-shrinking, tearing and the so-called

´snap-buckling´ of the venus fly-trap. As the *Marantaceae* with their ´explosive´ style movement are not included in this study, we can already make assumptions about their putative position in this scheme. Considering the velocity of style movements measured in *Marantaceae*, namely 0,2 sec. in *Maranta leuconeura* (Kunze 1984) with a thickness of the style of about 7 mm and 0,03 sec. in *Thalia geniculata* (Classen-Bockhoff 1991) with a thickness of about 9 mm one can conclude that at least these two examples of the family may finally be positioned in the snap-buckling area just like *Dionaea*.

In *Marantaceae* the aforementioned style movement is a swift curling-up movement of the style in adaxial direction inside the flower in the service of an extremely precise pollination method (Kennedy 1978, Yeo 1993). In contrast to the trapping movement of the venus fly-trap it is an irreversible movement as a result of a single release of the style from the ´hooded staminode´ in which it has been tightly enclosed during bud development (Kennedy 2000). The question about the tensioning of the style for its rapid shooting up, its hold inside the hooded staminode and the mechanism of its actuation have long been discussed (see chapter 2). In the case of *Maranta noctiflora* Koernicke we could show by experiment that the tensioning of the style for the movement comes about through a peculiar kind of growth under ´straitjacket conditions´ inside the hooded staminode during a very close development of the two organs inside the bud. The longer the style grows in comparison to the length of the enclosing hooded staminode the more does its ability to curl up increase (see chapter 2). Assuming that a biomechanical principle as a result of differential growth rates and differential tissue tensioning may apply to other genera of *Marantaceae*, too, most prior authors from Delpino (1869) to Kennedy (1978) to Arns et al. (2002) could be borne out in their interpretation of the style movement.

However, concerning the internal processes that are actually going on in the tissue of the style during movement, there are only two amongst all previous authors who have offered sup-positions so far; Schumann (1902) writes that according to his observations at *Maranta bicolor* Ker and *Calathea grandiflora* (Roscoe) K. Schumann the style at anthesis is under tension inside the hooded staminode. The strength of the tensioned style is due solely to the turgor of the parenchymatic cells. The release comes about through the touch of the hem of the hooded staminode or even through a tremor of the flower. Hereby the appendage (´trigger´ of Kunze 1984, Classen-Bockhoff 1991) is irrelevant for the release. According to Schumann (1902) the style movement is accompanied by a water-outflow from inside the cells into the intercellular spaces. No specialised mechanical tissues can be detected in the style. Kunze

(1984) on the other hand says that according to his investigations of *Maranta leuconeura* E. Morren and *Calathea undulata* Linden et André the tautness of the style is built up not before release but only at the very moment of an ´excitement´ of the style through a slight displacement of the basal plate. Hereby the change of turgor comes about through a water-outflow from the cells into the intercellular spaces at the ´upper side´ (i.e. the ventral side; the authors) of the style and enables its shooting up. Only in this point, concerning a shift of water through the intercellular spaces, is Kunze (1984) in accordance with Schumann (1902).

Up to now no experimental investigation has been made to form an idea of the hydraulic principles of the movement and to make out the special properties of the style tissue that make such a strong curvature possible without outwardly visible ruptures. The only discernible difference detected so far between unreleased and released styles is that the length and volume of dorsal cells in *Maranta leuconeura* increase after the movement (Claßen-Bockhoff and Pischtschan 2000).

In this study we start from the assumption that there must be structures inside the style that determine the shape alike the adaxial direction of the movement. Furthermore, entirely in contrast to Schumann´s opinion (1902) that there exist no mechanical tissues, we expect to find at least certain characteristics to the inner tissues of the style because the demands on this tissue are extreme: to undergo such an enormous bending without visible damage the tissue needs an extraordinary pliabilty. The fact that the length and volume of dorsal cells in the style increase after release in *Maranta leuconeura* indicates that there is indeed a ´water body´ to be shifted - presumably from central to periferal (mainly dorsal) cell layers which requires an enhanced hydraulic permeability of the parenchyma.

We present investigations that we made to show by which means the style in several genera of *Marantaceae* meets these special mechanical and hydraulic requirements.

3.2 Materials and methods

3.2.1 Materials

The following species are chosen for the experiments: *Calathea veitchiana* Hook, f., *Donax cannaeformis* (G. Forster) K. Schumann, *Halopegia azurea* K. Schumann, *Maranta depressa* E. Morren, *Maranta leuconeura* E. Morren, *Maranta noctiflora* Koernicke, *Marantochloa*

purpurea (Ridley) Milne-Redhead, *Thalia geniculata* Linné. Furthermore, the following members of the order Zingiberales are selected for investigations: *Canna indica* Linné, *Costus pictus* G. Don, *Zingiber spectabile* Griffith. They are all cultivated in the greenhouses of the Botanical Garden of the University of Mainz, Germany.

3.2.2 Methods

3.2.2.1 Cellulose detection

To find out in advance which materials contribute to the solidity of the style or whether the parenchyma contains only primary or already secondary cell walls the Lugol test is performed on *Maranta leuconeura*. Tissue samples of fresh styles are put in a watch-glass with Lugol solution (2g KJ, 1g J, 100ml aqua destillata) for ten minutes. The pieces are then transferred to a slide with a drop of the same solution and covered with a cover slip. When some thinned sulfuric acid (H_2SO_4 : H_2O = 2:1) is drawn through the slide preparation cellulose turns blue whereas lignin, suberin and cutin turn yellow.

3.2.2.2 Histology

Styles of *Maranta leuconeura*, *Halopegia azurea* and *Thalia geniculata* and *Marantochloa purpurea* are selected for sectioning. Unreleased styles are obtained by applying a 10´ ether narcosis to the flowers immediately before fixation (see chapter 2). In the case of *Thalia geniculata*, as these styles are extremely easily released, a 60´ narcosis with chloroform is carried out. The released and unreleased styles are first fixed in a solution of 1 % glutaraldehyde, 4 % formaldehyde and 10 % ethanol in a potassium-sodium-phosphate-buffer for at least a week. After two days´ evacuation in an exsiccator, the tissue is gradually dehydrated in an alcohol sequence starting with 15 % and ascending in unusually small steps of 5 % (2 h each) in order to avoid artifacts in this very fragile tissue up to 96 % ethanol in which they stay over night. In the preinfiltration medium (ethanol 96 % and Historesin infiltration-medium Leica 1:1) the material remains for at least four days to prevent pieces of style tissue from falling out of the blocks during sectioning. In the pure infiltration medium (Historesin, Leica) the tissue rests for another two days. The samples are then embedded in

the embedding medium (Historesin infiltration-medium and Historesin hardener, Leica 15:1). In silicone-rubber moulds the objects are accurately positioned for sectioning and then dried for four days at 60° Celsius in the oven. Should they still be too soft and wet for sectioning after that, they may stay in an exsiccator for another day or two. Finally, they are cut in longitudinal and cross sections and tangential sections of 8 µm (*Maranta leuconeura* once 5 µm and *Thalia geniculata* longitudinally 6 µm) by means of a Leitz microtome (d-blade) and stretched on wet slides (in 15 % ethanol) at 80° Celsius on a slide warmer for eight hours. The samples are thereafter stained with toluidin blue 0.05 % for 5-10 minutes, then washed in distilled water, fixed in 0.1 % HCL for 5 minutes, washed again and fixed once more in an aqueous solution of ammonium-molybdate 5 % for 5 minutes, washed, dried and eventually enclosed in a mounting medium (Eukitt, Leica) and covered with a cover slip.

Buds of *Maranta leuconeura* two days before anthesis are fixed in ethanol 70 % for a week and after two days´ evacuation dehydrated in an alcohol sequence starting with 75 % up to 96 % and treated by the same method as above. The thickness of the longitudinal sections in this case is 10 µm.

Styles of *Marantochloa purpurea* are fixed in AFP (alcohol 70 %, formaldehyde, propionic acid in a volume ratio of 90:5:5) for a week and then, after another week in 70 % ethanol, evacuated and dehydrated as above, starting with ethanol 75 % up to 96 %. The sections of these specimen are 8 µm thick.

Furthermore, unreleased styles of *Maranta leuconeura* are fixed in pure methanol for a week and put into a preinfiltration medium (methanol and Historesin infiltration-medium Leica 1:1) for another week. In the pure infiltration medium (Historesin, Leica) the tissue rests for another week before being embedded and dried in the 60° C oven for five days. They are then cut in longitudinal sections of 6 µm and stained with toluidin-blue as described above.

3.2.2.3 Scanning electron microscopy

Experiments on a trial basis have shown that the tissue of the styles in *Marantaceae* is very soft and soaked with water and consequently very difficult to fix. Therefore, several different methods of chemical fixation are applied to produce the tissue at its best.

Fixation with glutaraldehyde

Released and unreleased styles of *Halopegia azurea*, *Maranta leuconeura*, *Maranta depressa* and *Thalia geniculata* and entire buds of *Maranta leuconeura* (two days before anthesis) are first fixed in a solution of 1 % glutaraldehyde, 4 % formaldehyde and 10 % ethanol in a potassium-sodium-phosphate-buffer for at least a week. After evacuation in an exsiccator the tissue is gradually dehydrated in an alcohol sequence starting with 15 % and ascending in small steps of 5 % (2 h each) in order to avoid tissue artifacts up to 96% ethanol in which they stay over night. Then, after 3 x 15 minutes in aceton they are all critical point dried at 37° Celsius with CO_2 (critical point dryer CPD 030, BAL-TEC). The dried styles of *Maranta leuconeura* and *Thalia geniculata* are then cut in longitudinal halves and crosswise in several thin pieces. The released styles of *Halopegia azurea* are once crosswise broken in two pieces exactly on the vertex of the incurvation. All these pieces are afterwards sputtered with gold (sputter coater SCD 005, BAL-TEC) to prepare them for SEM.

Fixation with 70 % ethanol

The experimental plants are here *Maranta noctiflora*, *Donax cannaeformis, Canna indica, Costus pictus* and *Zingiber spectabile*.

Unreleased and released styles of *Maranta noctiflora* and *Donax cannaeformis* and mature styles of all other species are fixed in 70 % ethanol for a week. The samples are then, after evacuation, dehydrated in an alcohol sequence starting with 75 % and ascending in small steps (as above) up to 96 % ethanol for one night. After 3 x 15 minutes in aceton, they are critical point dried and cut in two longitudinal pieces each and sputtered with gold for SEM as above.

Fixation with AFP

Released and unreleased styles and buds of *Calathea veitchiana* (one day before anthesis) are fixed in AFP (70 % ethanol, formaldehyde, propionic acid in a volume ratio of 90:5:5) for a week. The samples are then transferred to 70 % ethanol for at least 24 hours followed by the same treatment (evacuation, dehydration) as the alcohol material above.

Fixation with methanol

Released and unreleased styles of *Maranta leuconeura* and *Maranta noctiflora* and entire buds of *Maranta noctiflora* (one day before anthesis) are fixed in pure methanol for at least 24

hours and then transferred to aceton for 3 x 15 minutes. Hereafter they are immediately critical point dried, cut in longitudinal direction in halves and sputtered with gold to prepare them for SEM.

3.2.2.4 Fluorescence

The experimental samples for fluorescence detection are freshly picked flowers of *Maranta leuconeura* and *Thalia geniculata*. The styles are first macerated in 10 % and 1 % aqueous solutions of pectinase (Merck) and the solutions are left on a slide warmer for 1, 2 and 3 hours at 35° Celsius (pectinase should be warmed in a water bath to make it more easily soluble). Others are macerated by cooking them for 12 minutes in an aqueous solution of 0.5 % KOH. All styles are then torn apart, the cell layers ruffled to thin the tissue out. The remaining cell clusters are then stained with 0.5 % acridine-orange and immediately examined in a fluorescence microscope (Leitz Diaplan). To ensure that artifacts caused by possibly too aggressive chemical treatments can be entirely excluded we tear living styles of *Maranta leuconeura* in small pieces and layers, too, and stain them with 0.5 % acridine-orange as before and examine them by the fluorescence method.

3.2.2.5 Transmission electron microscopy

For TEM six open flowers and two buds (one day before anthesis) of *Maranta leuconeura* are selected. Half of the open flowers are put under narcosis before. The fixation medium is a solution of 3 % glutaraldehyde in a 0.1 molar cacodylate buffer. After prefixation for 0.5 hours the styles of open flowers and entire buds are dissected and then fixed in the same solution for another hour. Then follow three washing steps in cacodylate buffer at a pH-value of 7.3 to 7.4 and afterwards another fixation (extractor hood) for 1.5 h in a solution of 2 % osmium-tetroxide (OsO_4) in 0.1 molar cacodylate buffer, followed by triple washing in distilled water. Thereafter comes the ascending alcohol sequence up to the absolute alcohol for dehydration of the tissue (2 x in 30 % - 2 x in 50 % - 2 x in 70 % - 2 x in 96 % - 3 x in 99.5 % - each for 15 minutes). The absolute alcohol is then exchanged for propylene-oxide (= 1,2-epoxy propane, an intermediate solvent before araldite) in which the objects remain for 2 x 15 minutes. In a mixture of 50 % propylene-oxide and 50 % araldite the styles rest over night openly under the extractor hood for evaporation. Next day the objects are put in pure

araldite for another 24 hours under the extractor hood at room temperature. Semi-thin sections of 1 µm are made and stained on slides ´after Richardson´ (methylene blue/ azure-b) in order to find perforated patches in the cell walls of the ´bending portion´ of the styles which are suitable for TEM examination. Hence longitudinal sections of an unreleased and a released style are selected. The buds do not yet show any special structures in the putative bending portion and are no further prepared. The longitudinal sections of styles are correctly orientated and embedded in silicone-rubber moulds for polymerisation of the epoxy resin in the oven at 60-65° C for 48 hours. The blocks are eventually cut in sections of 50-70 ηm by means of an ultra-microtome (Reichert ultracut S). The sections are transferred to copper grids and stained with a solution of 2 % uranyl-acetate in 50 % ethanol for 10 minutes and in an aqueous solution of 1 % lead-citrate for 5 minutes. Finally they are viewed in a transmission-electron-microscope (Zeiss EM 900).

3.3 Results

3.3.1 Cellulose detection

The Lugol test for cellulose on living style tissue of *Maranta leuconeura* reveals through the blue colouring that cellulose is more strongly concentrated in the dorsal region of the style than in the ventral portion. No yellow colour does indicate the presence of suberin or cutin or lignin. We therefore assume that especially in the dorsal area of the parenchyma of the mature style more cell-wall material is incorporated - maybe even already some very thin secondary cell walls - and take this as a basis for other genera, too.

3.3.2 Histology and SEM

3.3.2.1 The suitability of methods of fixation

The different methods of fixation preparing for histological sectioning or for scanning electron microscopy (SEM) seem to be of varying reliability. Fixation with AFP for histological sections applied to styles of *Marantochloa purpurea* has caused that the tissue looks somewhat ´crumbly´, so as if deposits of damaged cellular components or proteins had

formed and spread over the picture. These ´dirty tissues´ are therefore left out for the evaluation. The same goes for the fixation of styles of *Halopegia azurea* and *Maranta depressa* in glutaraldehyde. On the other hand we have obtained clear enough histological sections of *Maranta leuconeura* and *Thalia geniculata* in glutaraldehyde. After methanol fixation the histological cutting and staining can be a little difficult due to the resulting brittleness of the blocks as we have found in *Maranta leuconeura*. Nevertheless the sections can still be interpreted and the preparation for SEM is not disturbed. After preparation for SEM we have obtained very clean pictures of the dorsal tissue of styles in buds of *Calathea veitchiana* in AFP as well as clear enough SEM pictures of *Maranta leuconeura*, *Thalia geniculata* and *Halopegia azurea* in glutaraldehyde. Fixations with ethanol and methanol have almost always resulted in very clean histological and SEM pictures. Especially the water-free methanol fixation generally has the advantage that the tissue is fixed in a seemingly fully turgescent condition which makes some interpretations safer. Solutions that still contain a certain percentage of water, such as 70 % ethanol, always lead to an inevitable shrinking of the tissue which one must take into consideration (H. Edelmann, pers. com.). The pectinase maceration of styles of *Maranta leuconeura* and *Thalia geniculata* and in the case of *Thalia geniculata* even the maceration with KOH for some unclear reasons have lead to similarly ´dirty´ tissues as in the case of *Marantochloa purpurea* in AFP and *Halopegia azurea* and *Maranta depressa* in glutaraldehyde (see above) and must therefore be left out for the evaluation, too.

3.3.2.2 Histology and SEM – first impressions

Longitudinal histological sections of mature styles in glutaraldehyde result in a schematic division of the style tissue in three parts (Fig. 3.1 A): the head of the style, the bending portion in the middle and the base of the style. The three vascular bundles are in a ventral position accompanying the style canal so that the dorsal region of the style is much thicker and contains many more cell layers than the ventral (Fig. 3.1 A, B). The head of the style, which is slightly bent upwards, includes the stigmatic orifice with three stigmatic lobes and on its dorsal side a depression on which the own pollen is placed during secondary presentation, the pollen plate.

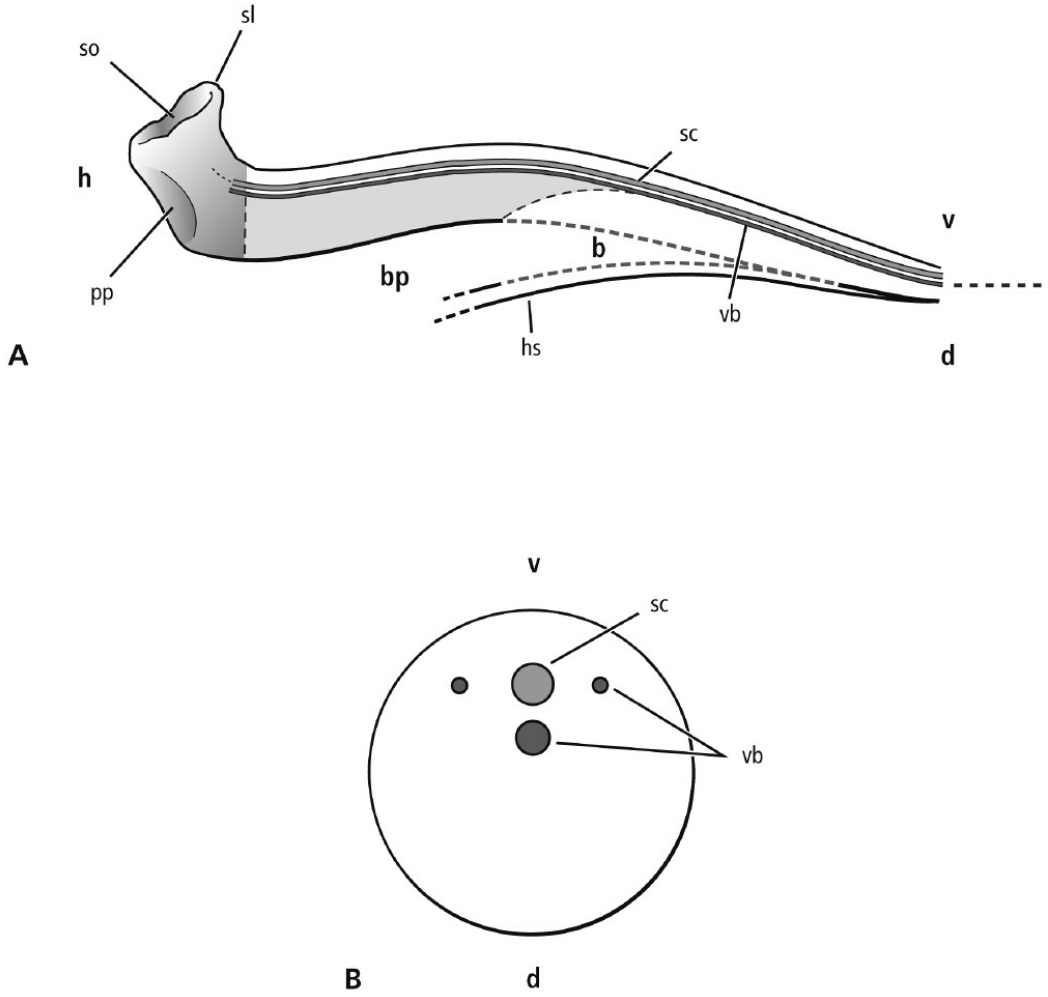

Fig. 3.1: Schematic drawing of an entire style of *Maranta leuconeura*: unreleased style with a part of a loosened hooded staminode in longitudinal side view (A) and cross view (B). b = base of the style, bp = bending portion of the style (light grey), d = dorsal, h = head of the style (dark grey), hs = hooded staminode, pp = pollen plate, sc = style canal, sl = stigmatic lobe, so = stigmatic orifice, v = ventral, vb = the vascular bundles

The style head shows a dense tissue of polygonal cells with no considerable intercellular spaces between them. (Fig. 3.2 A, B). The base of the style is regularly in its entirety fused with the hooded staminode and the filament. Herein the tissue looks much ´loosened´ with cells lying isolated from each other without discernible middle lamellae between them which appears a little like in a lenticel (Fig. 3.2 A, B). Most important is the region in the middle of the style which passes through the strongest bending after release: this part in its dorsal area

consists of longitudinal cell-layers lying parallel just like hoses or tubes. The tubulate cells are separated from each other by even longer intercellular spaces where no middle lamellae can be detected and the cell walls are conspicuously porous (Fig. 3.2 C). The contents of these mature cells consists only of very large vacuoles and a thin cytoplasmatic ´wall covering´ including a nucleus on the wall (Fig. 3.2 D) – in sharp contrast to the short and highly plasma-filled cells in styles of buds of *Maranta leuconeura* fixed in ethanol two days before anthesis (Fig. 3.2 F-J). Tangential histological sections from the same dorsal region in styles of *Maranta leuconeura* - fixed in glutaraldehyde - show a similar pattern (Fig. 3.2 E) which proves that this porosity reaches clearly into the dorsal region and all lateral directions inside the tissue.

Many of these longitudinal cell walls in mature styles are conspicuously porous – a great number of very large holes lying close together catch the eye, here in a dorsal tissue of a style of *Maranta leuconeura* (Fig. 3.2 C) and *Thalia geniculata* (Fig. 3.2 K-N), both fixed in glutaraldehyde. A comparison of histological sections of glutaraldehyde-fixed tissue samples of unreleased (Fig. 3.2 K, L) and released (Fig. 3.2 M, N) styles of *Thalia geniculata* also confirms that these holes are present already in the dorsal parenchyma of unreleased styles. The histological experiment with *Maranta leuconeura* after fixation in methanol shows that the sections are not very strongly or easily stained but the holes are clearly discernible here, too (Fig. 3.2 O, P). It is obvious from these histological sections that neither the different fixations nor the released or unreleased condition of the styles make any difference for the interpretation of this porosity in the dorsal cell walls. The cross section of the bending portion of a style of *Maranta leuconeura,* that was before fixed in glutaraldehyde, confirms that the longitudinal cells are highly turgescent with wide intercellular spaces between them (Fig. 3.2 Q, R).

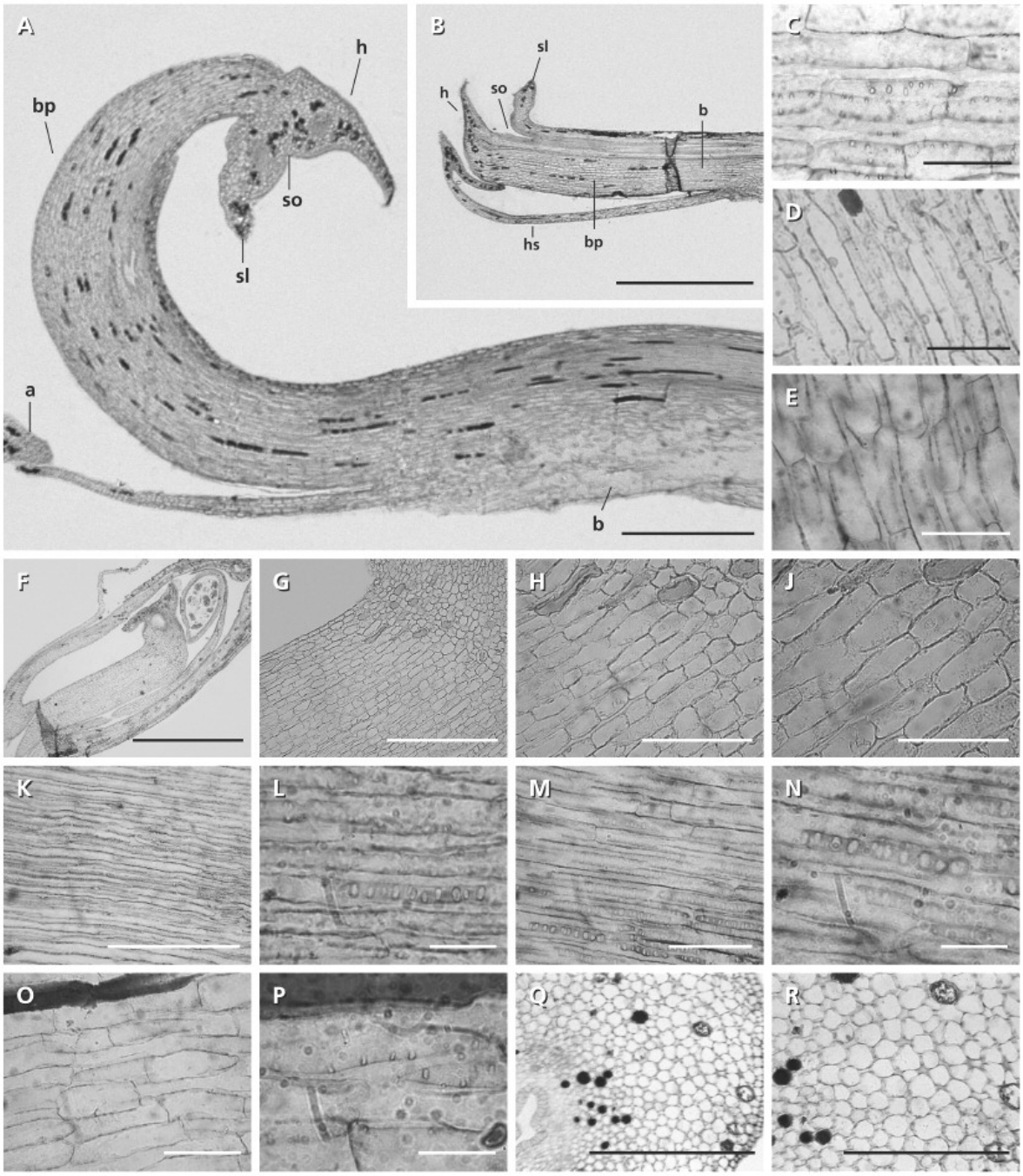

Fig. 3.2: Histological sections of styles of different species and after different fixations:
A-E: Styles of *Maranta leuconeura* after fixation in glutaraldehyde, A-D in longitudinal sections; released (A) and unreleased (B), thickness in both 8 µm. The division of the style in three parts is discernible. a = anther, b = base of the style, bp = bending portion of the style, h = head of the style, hs = hooded staminode, sl = stigmatic lobe, so = stigmatic orifice
The long, porous cell walls and long intercellular spaces are visible in the tissue of the bending portion of an unreleased style (C), thickness 5 µm. The nuclei on the cell walls are discernible, proving that mature cells are filled with large vacuoles in the tissue of the bending portion of an unreleased style (D), thickness 8 µm. E: Tangential section of the tissue of the bending portion of an unreleased style of *Maranta leuconeura* , thickness 8 µm. Slits in the

cell walls are visible, proving that they reach in all directions. F-J: Bud of *Maranta leuconeura* after fixation in 70 % ethanol two days before anthesis in longitudinal sections, thickness in all 10 µm. Juvenile cells are short and plasma-filled. K-N: Tissue of the bending portion of a style of *Thalia geniculata* after glutaraldehyde-fixation in longitudinal section, unreleased (K, L), and released (M, N), thickness in all 6 µm. These pictures show that there is no difference about these holes in cell walls of unreleased and released styles. O, P: Tissue of the bending portion of an unreleased style of *Maranta leuconeura* after methanol-fixation in longitudinal section, thickness in both 6 µm. The porous cell walls are discernible also after a different chemical fixation. Q, R: Tissue of the bending portion of an unreleased style of *Maranta leuconeura* after glutaraldehyde fixation in cross section, thickness in both 8 µm. The longitudinal cells are highly turgescent with large intercellular spaces between them. Bar in A: 1 mm, B: 2 mm, C: 40 µm, D: 45 µm, E: 50 µm, F: 1,5 mm, G: 6 mm, H: 15 mm, J: 24 mm, K: 12,5 µm, L: 25 µm, M: 12,5 µm, N: 25 µm, O: 50 µm, P: 125 µm, Q: 230 µm, R: 380 µm

Cross sections of the bending portion of the styles of *Maranta leuconeura* (see above, Fig. 3.2 Q, R) have shown that in the parenchyma the rounded, highly turgescent cells have tight contact areas and – alternating - large intercellular spaces between them. This is supported by SEM pictures of a released style of *Halopegia azurea* that was crosswise cut after glutaraldehyde-fixation (Fig. 3.3 A, B). Unreleased styles of *Maranta leuconeura* prepared by the same method (Fig. 3.3 C, D) and *Thalia geniculata* (Fig. 3.3 E, F) also support this. Tissue from a released style of *Thalia geniculata* after glutaraldehyde fixation cut in a transverse direction (Fig. 3.3 G) also supports the impression of a parenchyma in which long, tubulate cells are parallel with wide intercellular spaces between them.

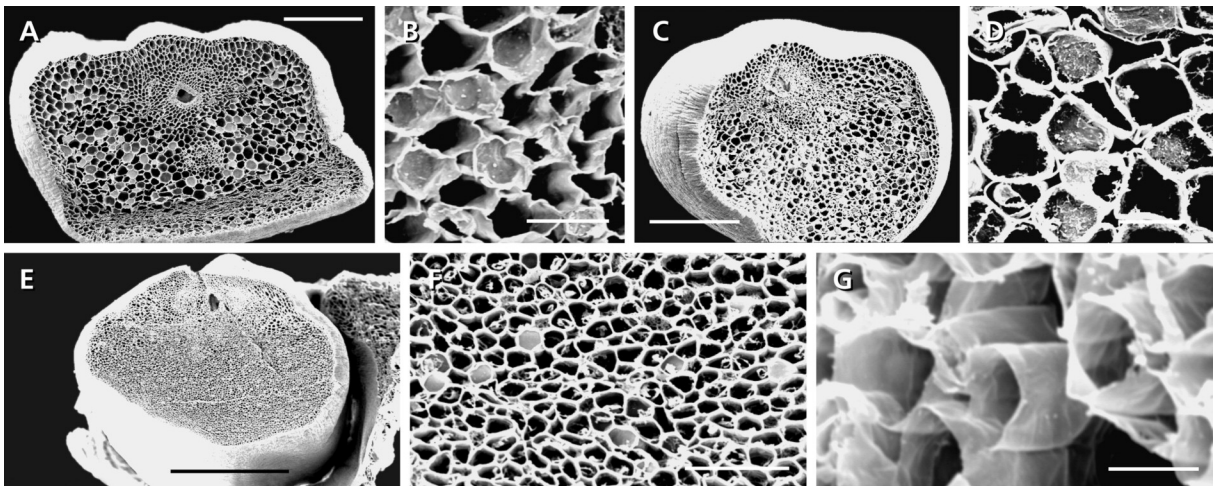

Fig. 3.3: SEM-pictures of intercellular spaces in crosswise cut styles of different species of *Marantaceae* fixed in glutaraldehyde: A-G: Rounded and highly turgescent cells and wide intercellular spaces in a released style of *Halopegia azurea* (A, B) and an unreleased style of *Maranta leuconeura* (C, D), in an unreleased style of *Thalia geniculata* in cross section (E, F) and in a released style of *Thalia geniculata* in transverse section (G). They all show how much thicker the dorsal side of the style is in comparison with the ventral part. Bar in A: 200 µm, B: 50 µm, C: 200 µm, D: 20 µm, E: 500 µm, F: 50 µm, G: 10 µm

3.3.2.3 SEM – texture and surfaces

The tissue of the ´bending portion´ of an unreleased style of *Maranta noctiflora* fixed in methanol and seen with SEM more thoroughly (Fig. 3.4 A, B) shows the same pattern of long tubulate cells with long intercellular spaces as in the histological sections of *Maranta leuconeura* (see above, Fig. 3.2 C). At certain breaks in an unreleased style in ethanol (Fig. 3.4 E-G) and in a released style in methanol (Fig. 3.4 L-N) it becomes clear that the aforementioned pattern indeed is only in that portion of the style parenchyma that undergoes the strongest bending during the style movement: long tubulate cells with long intercellular spaces between them and whose cell walls are unusually porous. In a style of *Maranta noctiflora* that was first put under an ether narcosis for 10 minutes and then fixed in methanol (Fig. 3.4 C, D) the very long and large intercellular spaces are quite easily seen with SEM as well as in an unreleased style of the same species that was fixed in methanol, too (Fig. 3.4 H-K). Very conspicuous are the clear differences in the outer texture of cell walls which at a larger magnification come to light. The cell walls of these long, tubulate cells have two different surfaces, one of which looks smooth and glossy, and another surface where a fine, slightly rough texture is discernible (Fig. 3.4 G). Only in the smooth and glossy surfaces is the conspicuous porosity discernible. Herein a pattern of minute spots and thin slits (Fig. 3.4 B) indicates the starting points of putative cell wall loosening and a number of spots which are already open - leading to a conspicuous amount of holes which are unequally and irregularly enlarged (Fig. 3.4 G).

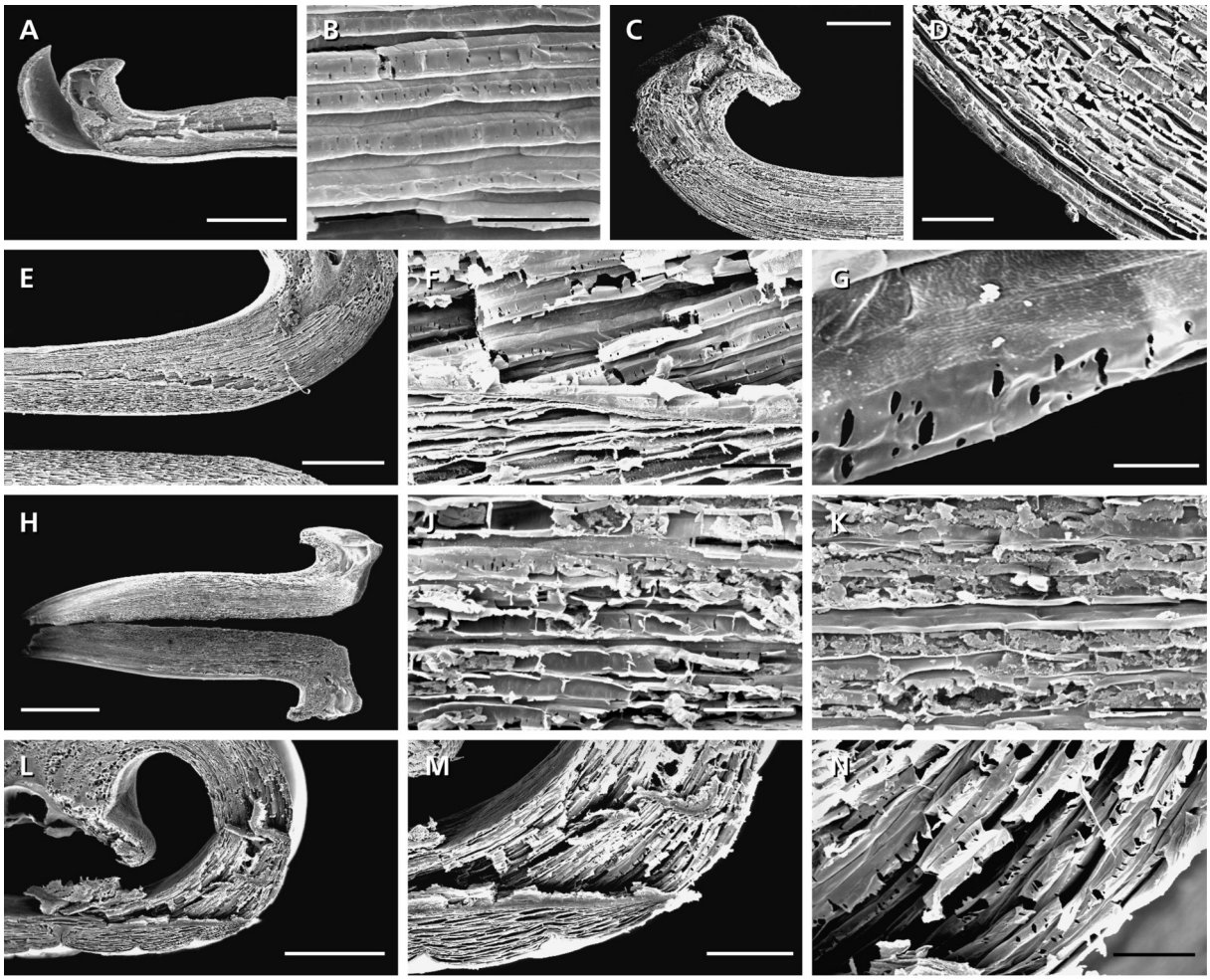

Fig. 3.4: SEM-pictures of the texture and surface of longitudinal dorsal cells in styles of *Maranta noctiflora* after fixation in different solutions:A, B: parallel cell layers looking like tubes and thin slits in the cell walls in an unreleased style, fixed in methanol; C, D: very long intercellular spaces in a ´half-released´ style after narcosis, fixed in methanol; E-G: the break showing that porous cell walls are only in the most bending portion of the style (F) and the different surfaces of the cell wall (G) in an unreleased style, fixed in ethanol; H-K: very long intercellular spaces in an unreleased style, fixed in methanol; L-N: porous cell walls are only in the most bending part of the style (M, N) in a released style, fixed in methanol. Bar in A: 1 mm, B: 50 µm, C: 500 µm, D: 100 µm, E: 500 µm, F: 50 µm, G: 10 µm, H: 1 mm, J: 50 µm, K: 50 µm, L: 500 µm, M: 200 µm, N: 50 µm

The positioning of these different surfaces of longitudinal cell walls is lengthwise-parallel and alternate (Fig. 3.4 G, Fig. 3.5 B, C, G). Between the mat and the glossy surfaces there is allways a clear marking of the margin which looks like a fine and slightly lighter bulge in the SEM pictures, (Fig. 3.5 K, L). Again, solely in the smooth and glossy surfaces is the aforementioned porosity discernible. Here, too, in a pattern of minute spots and thin slits the

starting points of putative cell wall loosening and a number of spots which are already open are obvious. As in histological sections (see above) it is obvious that neither the fixations in ethanol or methanol nor the state of the style in the sense of being released or unreleased play any role for the appearance of these slits.

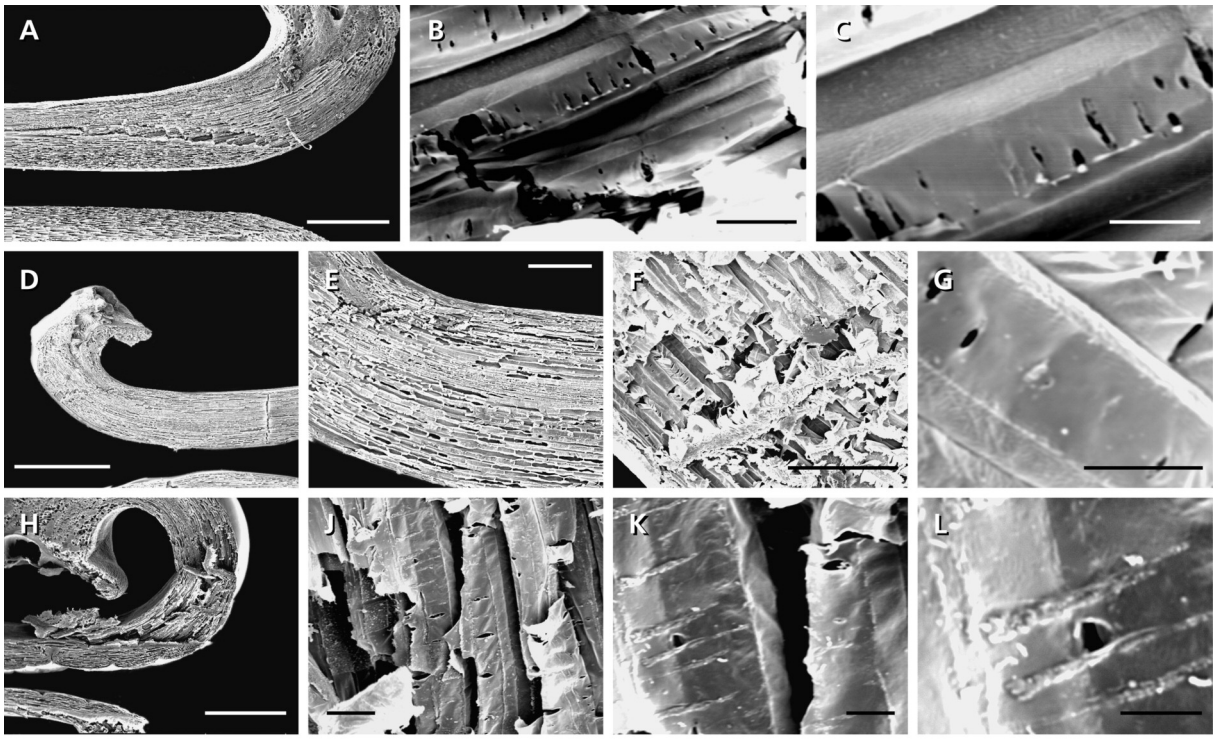

Fig. 3.5: SEM-pictures of the porosity and starting spots of lytical holes in longitudinal dorsal cells of styles of *Maranta noctiflora* fixed in ethanol: A-C: starting points of cell wall loosening and irregularly enlarged holes (B, C) in an unreleased style; D-G: different surfaces of longitudinal cell walls in lengthwise-parallel formation (G) with a clear margin between them in the bending part of an unreleased style; H-L: the break in the most bending portion of a released style shows that the porous cell walls are only here (K, L). Starting points of cell wall loosening are visible. Bar in A: 500 µm, B: 20 µm, C: 10 µm, D: 1 mm, E: 200 µm, F: 100 µm, G: 10 µm, H: 500 µm, J: 20 µm, K: 10 µm, L: 5 µm

3.3.3 The genuinity of the porosity

3.3.3.1 Fluorescence

A comparison with macerated material of *Maranta leuconeura* and *Thalia geniculata* leads to the following results: The enzymatic maceration by pectinase has lead to ´dirty´ tissues (see

above) in both genera and is therefore left out entirely. The same goes for KOH-macerated tissue of the style of *Thalia geniculata*. KOH-macerated tissue of the style of *Maranta leuconeura*, which was afterwards stained with acridine-orange, shows very clearly that cell walls are extraordinarily porous, even ´riddled with holes´ (Fig. 3.6 A) .Margins between areas with and without holes are as obvious as in SEM pictures, whereas the different cell wall textures remain invisible by this method. Single cell strands lying on top of one another with holes in the area where they appear to be directly in touch support what we have seen in histological sections and SEM pictures. Moreover, here the perforated areas evidently seem to be mutual contact areas between adjacent cells (Fig. 3.6 B). The fluorescence picture of living tissue of *Maranta leuconeura* stained with acridine-orange shows the same pattern of an extremely porous tissue (Fig. 3.6 C). By this method we can safely exclude that the porosity of cell walls might have been an artifact caused by too aggressive chemical treatments such as methanol or glutaraldehyde. On the contrary - this occurs also with other, ´softer´ treatments, such as maceration, and even in living and not pre-treated tissues.

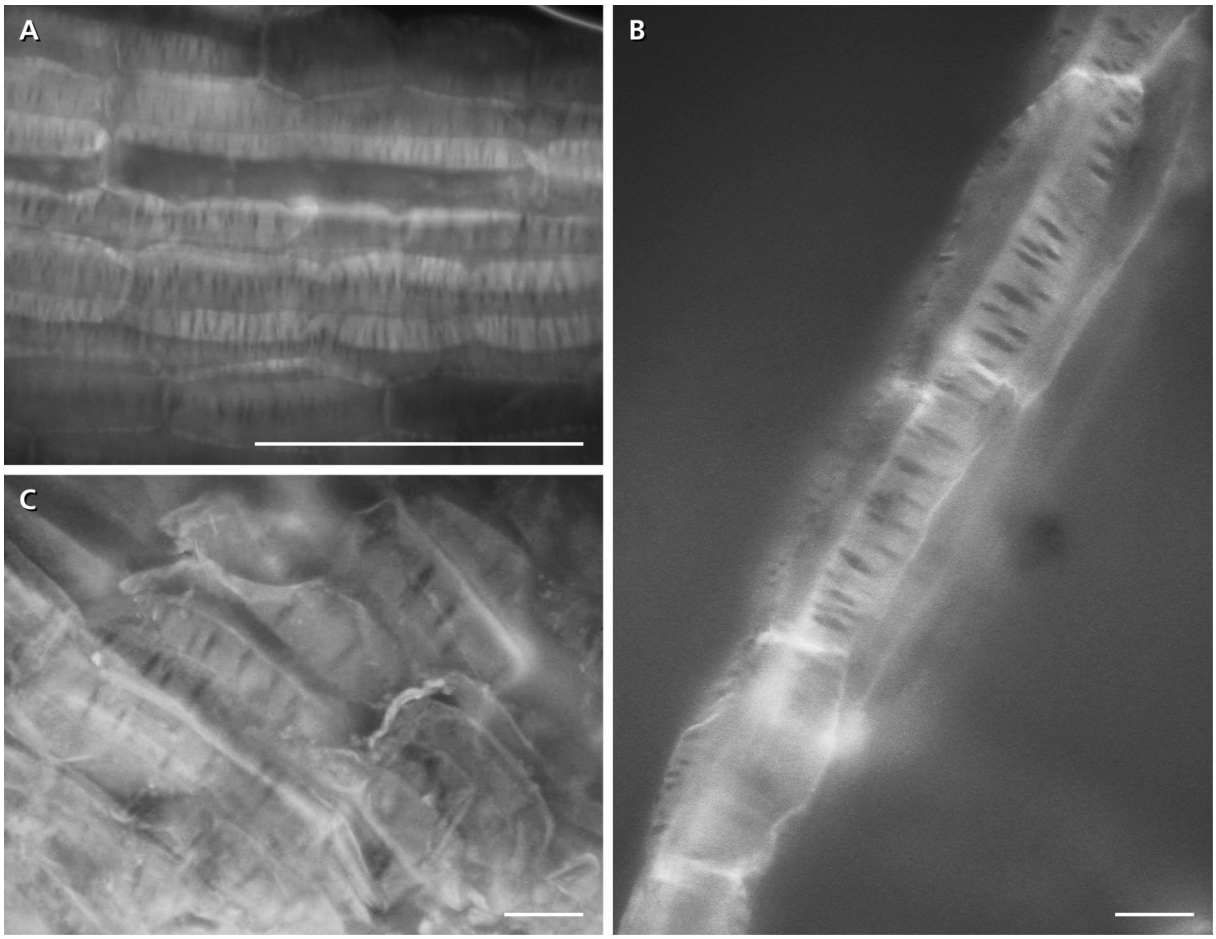

Fig. 3.6: Fluorescence pictures of styles of *Maranta leuconeura* macerated in different ways and stained with acridine-orange – to show the genuineness of the perforation: A, B: After maceration in KOH: the tissue is extremely perforated (A); Two single cell strands lying on top of one another indicating that these holes are in the contact area between adjacent cells (B); C: Tissue of a living style proving that these holes in the cell walls are no artifact caused by chemicals. Bar in A: 100 µm, B: 10 µm, C: 10 µm

3.3.3.2 TEM

By transmission electron microscopy we have made just a trial experiment in the hope of seeing the true texture of these extremely perforated cell wall regions much more thoroughly and have obviously found one of these particular slits or holes. What one can see definitely, is that there are only primary cell walls with a middle lamella between them. A thin plasmatic layer on the cell walls on either side is discernible (Fig. 3.7 A). Also a ´thin spot´ in the cell wall can be seen in top view, which is far too big for a plasmodesm (Fig. 3.7 B), and in side view in a longitudinally cut section (Fig. 3.7 C) where it is either not exactly cut through the middle, as there is no ´plasma-bridge´ discernible, or not yet entirely ´eaten through´.

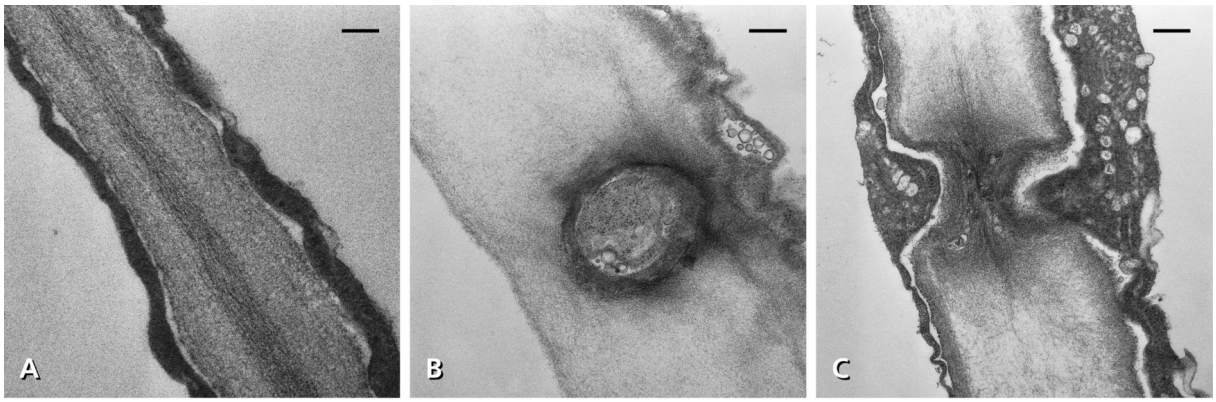

Fig. 3.7: TEM-pictures of cell walls of longitudinal cells in the bending portion of styles of *Maranta leuconeura*: A: Longitudinal section of two parallel primary cell walls with a middle lamella between them and a thin plasmatic ´wall covering´ on either side; B: Top view of a lytical hole and C: the same in longitudinal section. Bar in A: 0,15 µm, B: 0,4 µm, C: 0,4 µm

3.3.4 The time of emergence of the porosity

As for the time of emergence of these holes, we see in buds of *Maranta leuconeura* two days before anthesis that they are not yet visible (Fig. 3.8 A, B). But they occur already in buds of *Calathea veitchiana* one day before anthesis (Fig. 3.8 E-G) and in buds of *Maranta noctiflora* one day before anthesis (Fig. 3.8 C, D) where they can be seen from inside the empty cells (Fig. 3.8 D) and in a similar preparation (Fig. 3.8 H-K) where this porosity of cell walls can be seen from the outer surface of the cells (Fig. 3.8 J, K). The aforementioned margin between the smooth and glossy and porous cell walls and the other surface without any of these holes is also very clear in the tissue of the juvenile style of *Maranta noctiflora* (Fig. 3.8 J, K). In any case, the release of the style movement does not cause the emergence of these holes, because styles that are still in bud cannot yet be released. Thus, the holes emerge already during the development of the style in the last 24 hours before anthesis.

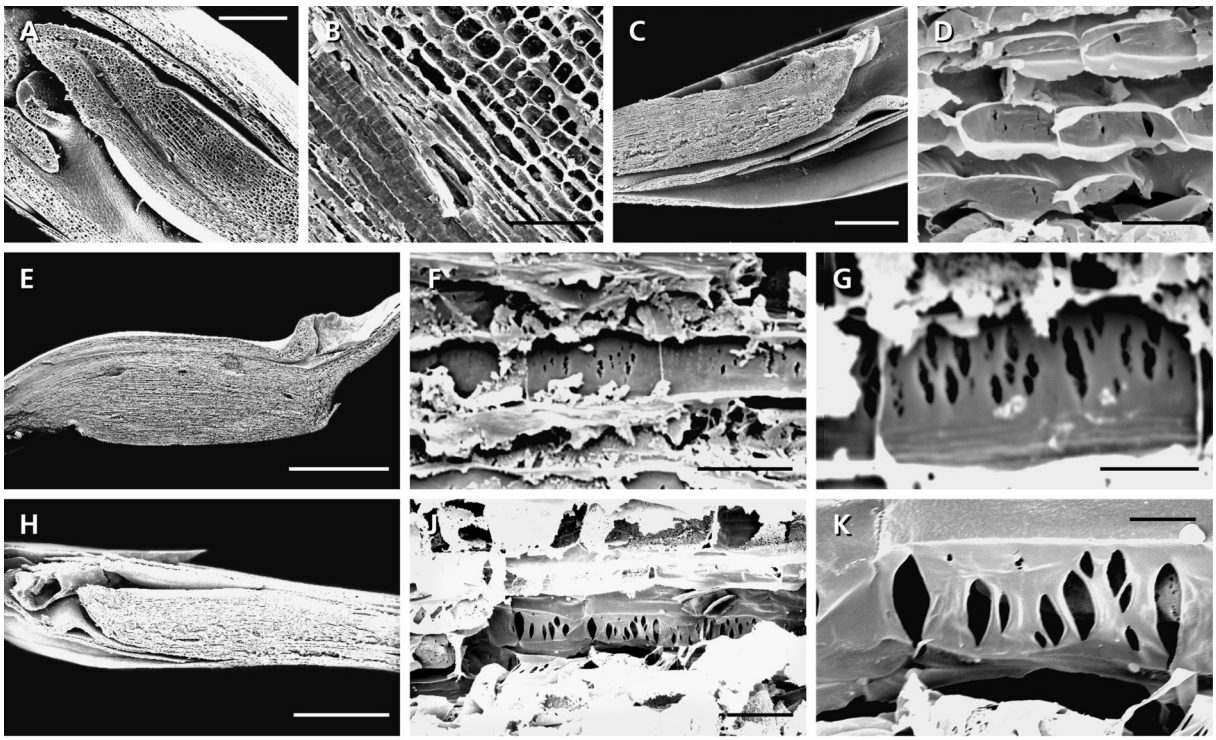

Fig. 3.8: SEM-pictures of buds of different species of *Marantaceae* fixed in different solutions: A, B: two days before anthesis: short cells without any perforation in a bud of *Maranta leuconeura* after fixation in glutaraldehyde; C-K: all one day before anthesis: porous cell walls seen from the inner side of the cells in a bud of *Maranta noctiflora* fixed in methanol (C, D); developed holes or slits in cell walls like in mature styles in a bud of *Calathea veitchiana* after AFP fixation (E-G); irregularly enlarged holes and a clear margin between the different surfaces of the cell walls in a bud of *Maranta noctiflora* after methanol-

fixation (H-K). Bar in A: 200 µm, B: 50 µm, C: 50 µm, D: 20 µm, E: 1 mm, F: 20 µm, G: 7 µm, H: 1 mm, J: 20 µm, K: 5 µm

3.3.5 Other members of the order

3.3.5.1 Comparison of different *Marantaceae*

Except in these four species examined so far we find in an unreleased style of *Donax cannaeformis* similar holes by SEM (Fig. 3.9 A-C). The same applies to style tissue of *Halopegia azurea* that we prepared by two different methods: an unreleased style was once longitudinally cut in the normal way (Fig. 3.9 D-F). A released style on the other hand was once broken crosswise on the vertex of the incurvation and shows that the holes reach in all directions inside the bending tissue (Fig. 3.9 G-K). Here, again, the different solutions for fixation and the released or unreleased condition of the styles make no difference for the interpretation.

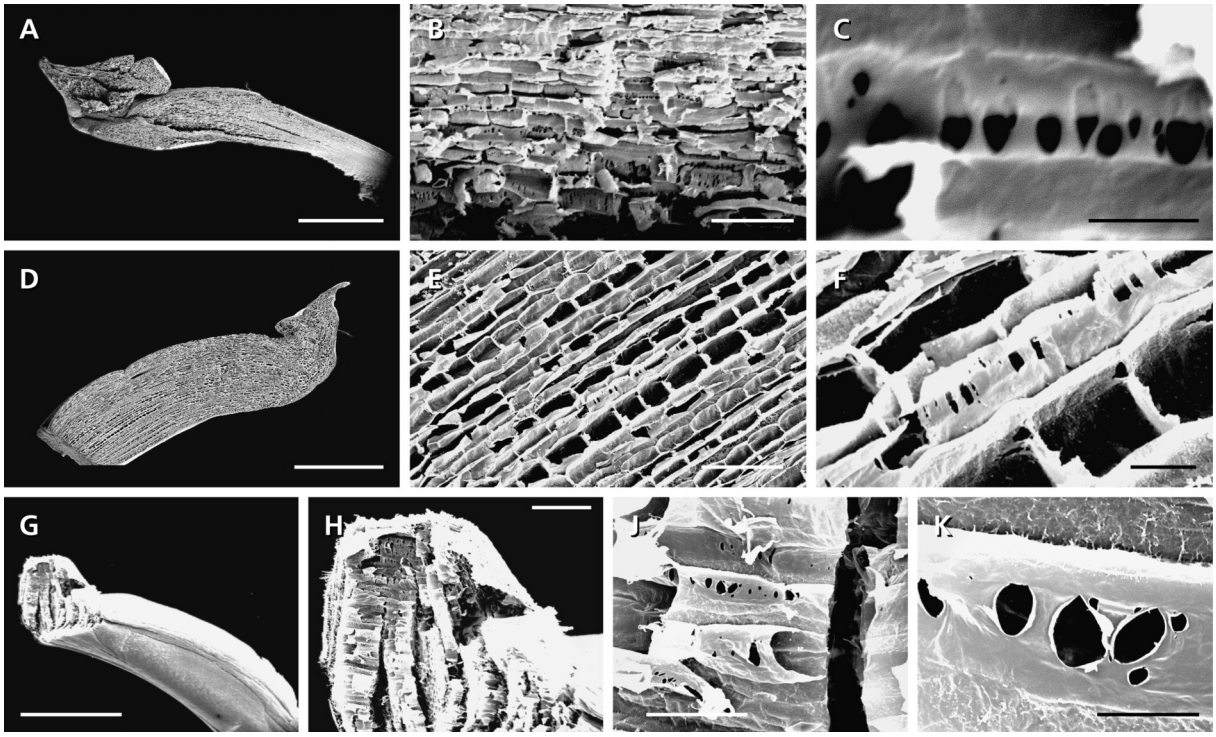

Fig. 3.9: SEM-pictures of styles of different species of *Marantaceae* fixed in different solutions: A-C: porous cell walls in the bending portion of an unreleased style of *Donax cannaeformis* after fixation in 70 % ethanol; D-F: perforated cell walls in an unreleased style of *Halopegia azurea*; G-K: perforated cell wall structures reach in all directions in the tissue

of a released and crosswise broken style of *Halopegia azurea*, both after fixation in glutaraldehyde. Bar in A: 1 mm, B: 50 µm, C: 5 µm, D: 1 mm, E: 70 µm, F: 20 µm, G: 1 mm, H: 200 µm, J: 50 µm, K: 7 µm

3.3.5.2 Comparison of different Zingiberales

In the case of other representatives of the order Zingiberales whose styles we fixed in 70 % ethanol and examined with SEM on a trial basis we find that in the tissue of the styles of *Canna indica* and *Costus pictus* similar holes are evident, too, if in a much smaller concentration than in *Marantaceae* (Fig. 3.10 C, F). Naturally, the styles in other Zingiberales´ flowers are much thinner and consist of less cell layers than in *Marantaceae*. Here, only *Zingiber spectabile* does not show any developed holes or slits of this kind (Fig. 3.10 G-J) which does not prove that it is impossible for the genus *Zingiber* to develop them - maybe when the flowers are older.

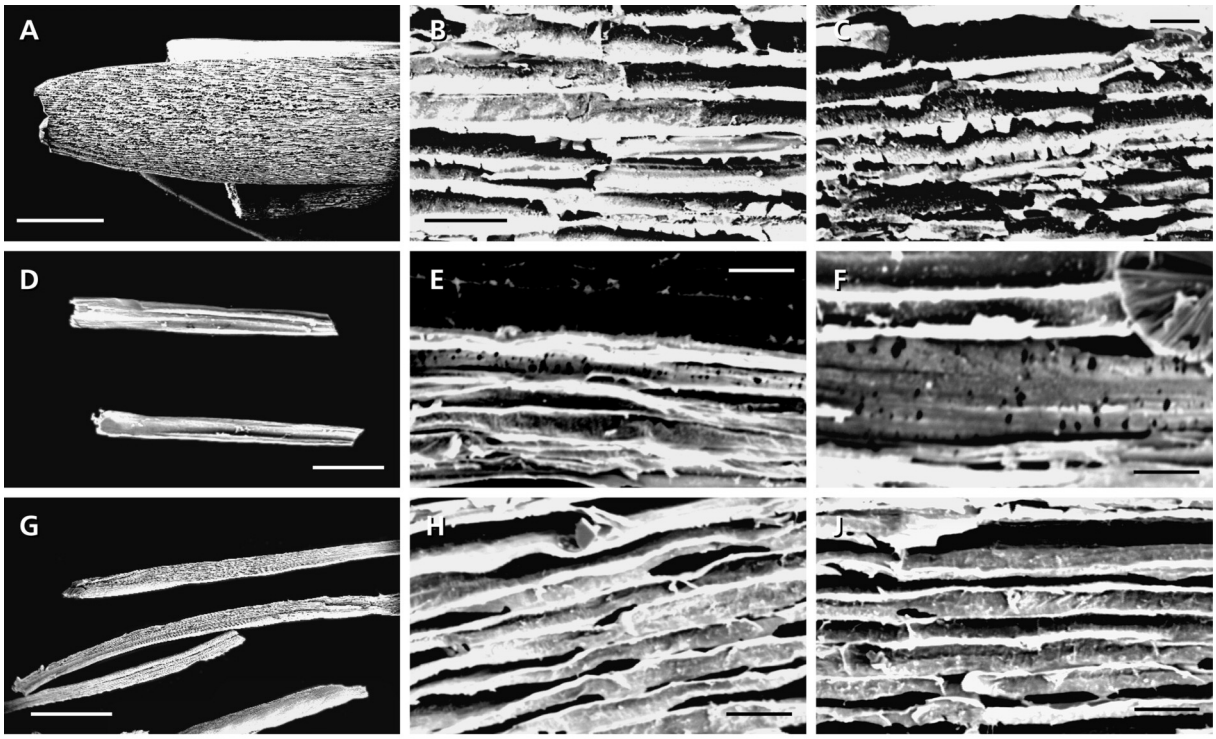

Fig. 3.10: SEM-pictures of styles of different species of Zingiberales fixed in 70 % ethanol: A-F: a small concentration of probably lytical holes in the cell walls of styles of *Canna indica* (A-C) and *Costus pictus* (D-F); G-J: Styles of *Zingiber spectabile* showing no similar structures. Bar in A: 1 mm, B: 50 µm, C: 20 µm, D: 1 mm, E: 20 µm, F: 7 µm, G: 1 mm, H: 20 µm, J: 20 µm

3.3.6 Summary of the results

We can safely conclude that these interesting holes and slits do not occur through the release of the style. They are discernible in unreleased as well as released styles. They are present in buds one day before anthesis. They can be found in the tissue of living styles and in tissues of styles after four different chemical fixations and in macerated tissue. We have found them in all examined 5 genera (6 species) of *Marantaceae* and even in representatives of two other families of Zingiberales. They appear to be only in the thin, probably primary, cell walls and between adjacent cells. We never found them in the thicker and more strongly textured cell walls. It appears that they are no regular pits, but emerge through cell wall loosening.

3.4 Discussion

All results that we have to hand about differential growth rates and differential tissue tension (see chapter 2) and anatomical and histological structures meet in the following interpretation of both the development and function of the style: the maturing style is actively (i.e. energy-demanding) ´pumped up´ with water under a strong osmotic potential during an enormous elongational growth in the last hours before anthesis. This leads to the aforementioned ´straightjacket´ syndrome with the style being extremely turgescent and actually longer than the hooded staminode in which it is jammed. The pumping-up process produces an elongational pressure with largely extended cells on the ventral side. The shorter hooded staminode on the other hand causes a counterpressure in the opposite direction. Hereby the style becomes a little overstretched with ´compressed´ dorsal cells that are actually hindered from expansion. The result is a differential turgor on the ventral and dorsal side (Fig. 3.11 A).

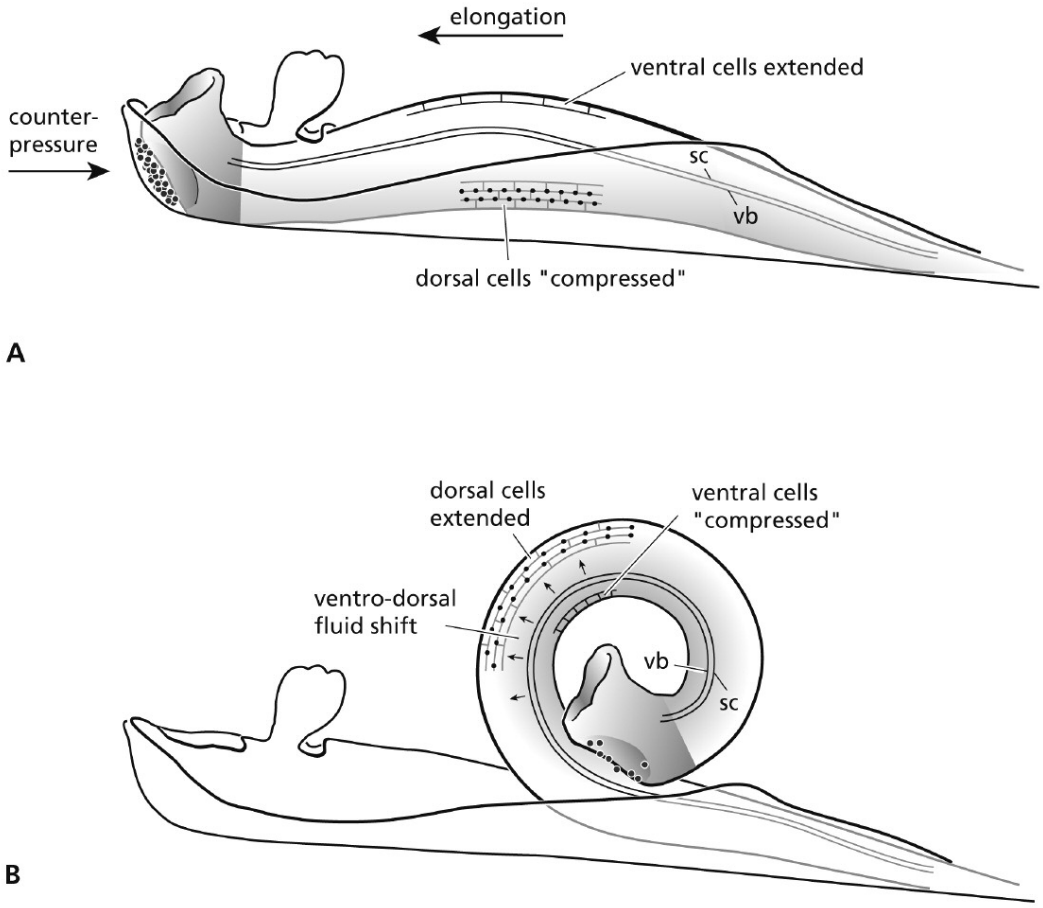

Fig. 3.11: Depiction of a style model (following the scheme of *Maranta leuconeura)*: A: Side view of an unreleased style with shorter dorsal and longer ventral cells. The elongational pressure during development versus counterpressure from the hooded staminode produce a ventro-dorsal turgor gradient in the style; B: Side view of a released style with longer dorsal and shorter ventral cells. The ventro-dorsal fluid shift supports the release of the movement and offsets the turgor gradient. Arrows indicate the direction of the supposed shift of the ´water–body´ from central to periferal cell layers. vb = vascular bundles, sc = style canal

During the pumping-up process the ventro-dorsal turgor gradient increases and the vacuoles become so large that there remains just a very thin layer of cytoplasma on the inside of the cell walls. At the same time a self-maceration takes place – presumably through an intense pectinase activity. In the ´bending portion´ many of the longitudinal middle lamellae are dissolved so that even longer intercellular spaces just like channels come about - with the effect that in case of a release the longitudinal cell layers in the dorsal region are enabled to glide alongside each other to a certain extent and hereby to give way to the bending. In the

contact areas between adjacent longitudinal cells an extreme porosity of probably primary cell walls is brought about through cell wall loosening - also through pectinase activity. The question is whether these holes or slits derive either from primary pit fields in the juvenile cell walls while in the course of maturation the individual plasmodesma are secondarily enlarged to slits in the service of a quick water flow or whether they are just ´lytical holes´ that develop only in a late phase of bud growth due to an intense pectinase activity. This cannot be answered absolutely. The unusually great number of holes and slits that we have found concurrently in the dorsal bending portion of different genera does not let us believe in normal pits although especially in tissues where neighbouring cells must allow a mass flow (e.g. in phloem-bundles, Strasburger 1999) they can appear with strongly increased denseness. Nevertheless, as they are, first, only discernible in buds one day before anthesis and, secondly, very irregularly shaped (see Fig. 3.4) we rather assume that the lysis through pectinase does not necessarily begin with juvenile primary pit fields, but that in riper buds there may be extra preformed spots in the cell walls (an idea also of H. Edelmann, pers. com.) in the contact area of adjacent cells which are ´eaten through´ by pectinase. It appears that primary cell walls are preserved in such places where cell-wall loosening is meant to start and that they may be strengthened with secondary wall material (mainly cellulose) only in such areas where these holes shall not emerge. Differential strengthening of cell walls may hereby develop in a similar fashion as in a lamellar collenchyma. The style appears to make a compromise between on one hand stability – to maintain an extremely high and even differential turgor – and, on the other hand, pliability through differential cell-wall strengthening to endure the bending without rupture.

Anyway, this unusual porosity may also support the litheness of the region. But the main function of the perforation surely is not in first place pliability, but permeability. The theory of a sudden loss (or change) of turgor through a runoff of water into the intercellular spaces (Schumann 1902, Kunze 1984) in our opinion cannot explain the unusually high speed of the movement sufficiently. Diffusion may have a share in the whole process but differential turgor surely does not produce the movement alone. Our findings indicate that this unusual perforation is in primary cell walls and between neighbouring cells (see Fig. 3.12).

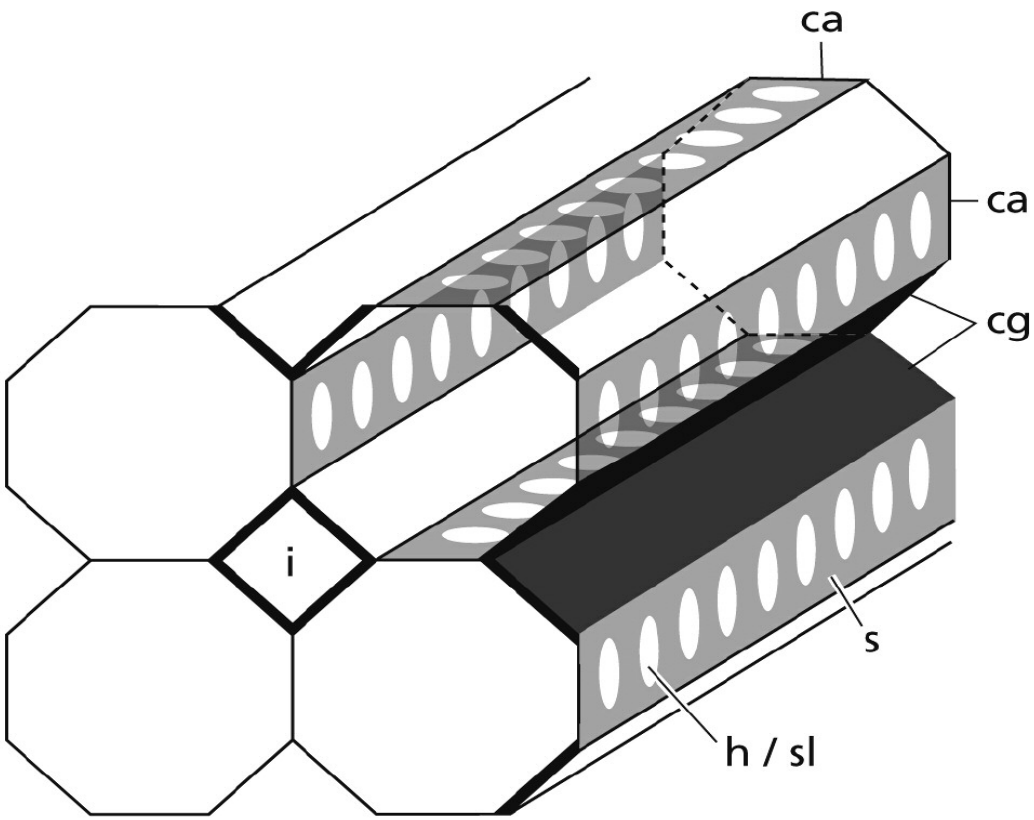

Fig. 3.12: Model of a group of cells isolated from the dorsal bending portion of a style in oblique view: Long tubulate cells lying parallel with large intercellular spaces. The cells have different surfaces in lengthwise parallel formation. In the contact areas the cell walls are porous and have a smooth surface. The cell walls leading to the intercellular spaces are strengthened with a cross grain. ca = contact area, cg = cross grain, h = hole, i = intercellular space, s = smooth (surface), sl = slit

We believe that for the release it can be assumed that first, a simple displacement of the hooded staminode or – in special cases – even conducted action potentials after an ´excitement´ can release the style movement. Both cases are accompanied by a change of membrane potential so that the plasmalemma is no longer semipermeable. Thus both osmotic potential and turgor break down immediately. This is very likely accompanied by a water outflow into these large intercellular spaces just as Schumann (1902) and Kunze (1984), H. Edelmann (pers. com.) have suspected. On top of that, as the movement goes so quickly, we

think it even possible that, as an additional effect, the vacuoles in the region may break down. The effect that we assume is that in one complete and very sudden shift of fluid from central to periferal cell layers the entire ´water-body´ sweeps through and comes to a standstill (Fig. 3.11 B). This vast - and fast - ventro-dorsal shift of fluid goes through the whole symplastic body in the middle of the style and outdoes the runoff of water through intercellular spaces by far and may hereby explain the particularly high speed of the style movement. As the water flow goes from central into the periferal (i.e. dorsal) cell layers and not, as assumed by Kunze (1984), in the ventral side of the style the dorsal longitudinal cells eventually become longer, but not thinner which means that they increase their volume (Classen-Bockhoff and Pischtschan 2000). This, again, explains the irreversibility of the movement. Consequently the assumed break down of vacuoles in the middle part involves at least a partial dying of the parenchyma of the style. This indeed would do no further harm because the pollen only needs to migrate through the pollen canal in the next hours before the style withers completely. There is an interesting observation at the genus *Calathea* described by Hildebrand (1870) and Kennedy (2000) whereafter the style already during movement first turns brownish and later violet-black. Both authors have assumed that as a result of this discolouring the insects recognise that the respective flower is already done. One might perhaps assume that it is a necrosis of the parenchyma as a result of the complete break down of the vacuoles in that middle area of the style. Meanwhile, Hildebrand emphasises that the style when it dies unreleased also discolours in such a way.

Concerning the strength or stability of the style we agree with Schumann (see chapter 2) that the turgor is already built up before and not, as Kunze says, only at the moment of an ´excitement´ of the style via the basal plate.

The geometrical shape of the complete curvature after release is in all probability determined by the course and position of the only supporting elements the style has, namely the three vascular bundles. It may even be assumed – but still unproven by our methods - that these vascular bundles during the ´pumping-up´ process in the last hours remain a little shorter in comparison to the water-filled ´parenchymatic style´ in order to support and stabilise the incurved form through their tension. In this sense we agree with Schumann (1902) that there are no mechanical (i.e. antagonistic) tissues as they can be found in leaf bases of *Dionaea* and *Mimosa* which explains again why in *Marantaceae* there is no reset of the movement. Nevertheless, in the bending portion there is a tissue specialised in the service of elasticity and permeability - a motile tissue producing a movement and which emerges in the last hours of

maturation through self-maceration. This may not be called a mechanical tissue, too, but perhaps or at least a ´hydraulic tissue´. The style ´movement´ is thus facilitated once by differential growth rates and differential tissue tensioning during development (see chapter 2) and at the same time by differential cell-wall strengthening followed by enzymatic dissolution of stabilising and damming structures in the parenchyma. In this sense also the demand of Skotheim and Mahadevan (2005) for ´elastic instability´ is here not only fulfilled but absolutely carried to extremes. It seems, as the investigation of other members of Zingiberales has shown, that a certain predisposition for such a tissue instability may already be laid out in the order, though being still functionless in the other families. Maybe that only *Marantaceae* are able to take advantage of this ´design´ – particularly as their styles are much more voluminous and consist of many more cell layers than those of the other families of Zingiberales. The potential to make use of such a feature is anyway higher in *Marantacea*e due to the fleshy consistency of the style. Altogether, even if we take the term of the ´elastic instability´ as a basis, it must here be modified in the sense that the release of the style indeed results not in an elastic and herewith reversible movement, but in a plastic and irreversible reshaping of the organ. It is remarkable that the flowers of *Marantaceae* in the service of an extremely precise pollination method obviously take advantage of self-maceration which is a natural process related to ageing.

4. Diversity and systematic value of the cucullate staminode in *Marantaceae*

Abstract

The cucullate staminode in *Marantaceae* plays a critical role in the pollination process and shows a remarkable morphological diversity. Cucullate staminodes of 24 genera of *Marantaceae* are sorted according to a minimalistic selection of shape characters such as the trigger-appendage and few others. Eight distinct morphological types are abstracted from the resulting groups and mapped onto a maximally parsimonious tree by Prince and Kress (2006) containing five major clades. An amazing correspondence is found between the morphological types and the clades; especially the given Calathea-clade and the Sarcophrynium-clade are absolutely consistent with the respective American and African types of hooded staminodes. Furthermore, several sister-relationships are confirmed and for some uncertain positions possible evolutionary pathways are discussed, as well as for the entire family. The value of morphological characters in relation to the modern phylogenetical analysis is discussed and conditions for the selection of morphological characters most meaningful for a systematic grouping are proposed.

4.1 Introduction

While actually trying to clarify the mechanisms underlying the ´explosive´ style movement in *Marantaceae* (see chapters 2 and 3) in years of flower preparation an intriguing ´side-observation´ was made that eventually led to the impression that the hooded (cucullate) staminodes in this family bear conspicuously diverse appendages and other morphological features that might be of high systematic value. Expressed in simplified terms: only from a hooded staminode alone one might make out which continent a specimen of *Marantaceae* comes from and which systematic group it belongs to without even needing to investigate into other floral or vegetative or reproductive characters – hereby being provided with a handy and quick morphological key to the genera and their geographic position.

Why of all floral parts in *Marantaceae* the cucullate staminode is so conspicuously divers that it alone may be of diagnostic value - about this we hypothesise as follows: the flowers and the

pollination mechanism in *Marantaceae* are unique to the family and the cucullate staminode has an essential share in it (see chapter 2). Therefore, a fundamental notion of an adaptive relation between flowers and their pollinators seems to suggest itself. It is likely that these flowers can only be pollinated by insects or other animals that can cope with the biomechanical properties of flowers of a certain genus or species, depending on their size, shape, physical force and maybe even weight. It is also possible that these flowers are more exclusively specialised in their pollinators than the other way round so that a certain selective pressure may be supposed which acts more stringently on the plants than on their pollinators. This might explain why in particular the plant family has developed such specialisations and not the euglossine bees that are the principal pollinators of *Marantaceae* in the New World (Kennedy 1978). However, to make a more reliable statement about the adaptive effect of these divers structures extensive observations of pollinators and their physical ´pollinating-behaviour´ (see Kennedy 1978) - especially in connection with the mechanical properties of the triggers (see below) - at natural habitats in the Old World are necessary. As ecological investigations are still in progress at present, the question of the adaptive value and the processes selective on these structures must remain open here. Furthermore, the varied modifications of the cucullate staminodes might also provide much phylogenetic information, so affording a better knowledge about the evolutionary paths of the family and the degree of relationship amongst them.

The hooded staminode in *Marantaceae* is nowadays agreed to be an element of the epipetal, inner androeceal whorl (Eichler 1884, Schumann 1902, Loesener 1930, Kunze 1984), together with the anther and the callose staminode. Its function is the hold of the ´explosive´ style prior to the visit of a pollinator alike the setting-up of the potential for the style movement after release through a pollinator (Eichler 1884, Costerus 1918, Loesener 1930, Kennedy 1978; see chapter 2) The release of the movement is predominantly believed to be caused by the touch of the ´trigger´ which is a lateral appendage at the margin of the hooded staminode while the ´basal plate´, a swelling at the base of the trigger, transmits the stimulus from the trigger to the style (Kunze 1984, Claßen-Bockhoff 1991, Yeo 1993). About the appendages of the hooded staminodes there are already some early observations: Koernicke (1859) observed the diversity of trigger-appendages and he was the first to describe the possible presence of a second appendage or ´bulge´ in examples of *Calathea*, *´Monostiche´* and *Ischnosiphon*. On top of that he considered the trigger in *Thalia* to be one appendage splitted in two ´ribbons´

contrary to Andersson (1998) who saw *Thalia* with two appendages. For the development of a hooded staminode there are first interpretations from Eichler (1883): one margin of the staminode develops to the hooded part of it and the other margin forms 1-2 appendages (in: Costerus 1918). Hereby in *Maranta sanguinea* (Eichler 1884) the margin of the staminode elongates to a ´tooth-shaped appendage´ that later ´moves down´ (in the direction of the leaf base, the authors). Kunze (1984) carried out ontogenetic investigations at hooded staminodes of *Maranta leuconeura*, *Ataenidia conferta* and *Calathea vaginata* with special focus on the course of the medianus or the midrib, respectively. His conclusions support the observations of Eichler (1883) that the two main portions that are divided by the medianus eventually are the hood and the trigger and that they start early to develop very differently. The early formed indentation between the hood and the trigger-appendage is the actual leaf apex. Through allometric growth on the trigger-side there develops a fold and at its base the basal plate. In many species of *Calathea* and in *Ataenidia* he observed a swelling of the tissue in a place proximal to the trigger and treats them as equivalent, at least as regards their function (Kunze 1984). For this the medianus takes a turn ´into the trigger´.

The first systematic subdivision of the family was carried out by Petersen (1889) dividing the family into two tribes, namely the tribe *Maranteae* with one fertile locule in the 3-carpellate ovary and the tribe *Phryniae* with three fertile locules in the ovary, hereby following Koernicke´s (1858) first key to the genera. This subdivision was accepted by Schumann (1902) and Loesener (1930) and others, but was criticised as artificial by Andersson (1981) who presented a subdivision of the genera in five ´informal´ groups (1998), based on a great number of morphological and reproductive characters relating to the entire plants, such as branchings in the vegetative and floral part, metric data like the length of the corolla tube, numeric data like the number of outer staminodes or fertile locules, fruit dehiscence, the form and position of the trigger-appendages and many more. This grouping of the family by Andersson (1998) must now be called into question, too. Andersson and Chase (2001) presented a phylogenetic analysis based on combined morphological and molecular data, hereby using plastid-DNA sequence data (rps 16 intron) representing 21 genera and resulting in a reordering of the family. There was no support for the ´antique´ division in *Phryniae* and *Maranteae* (Petersen 1889). Anderssons (1998) informal groups were only partly supported, e.g. the monophyletic Calathea group including the genera *Calathea*, *Pleiostachya*, *Ischnosiphon* and *Monotagma*. The newest phylogenetic analysis of the family was recently

presented by Prince and Kress (2006) using plastid DNA sequence data (matK coding and 3´ intergenic spacer region and the trnl-F intergenic spacer region) representing 27 genera and considering also some of the morphological characters used by Andersson and Chase (2001), such as bracteoles, shape of the corolla tube, outer staminodes, appendages of the cucullate staminode and fruit. This investigation resulted in five major clades, namely a Calathea-clade, a Sarcophrynium-clade, a Donax-clade, a Maranta-clade and a Stachyphrynium-clade. Herein, besides *Calathea*, the genera *Schumannianthus*, *Phrynium*, and *Marantochloa* turned out to be non-monophyletic.

Although flowers in *Marantaceae* are on the whole morphologically very uniform and easy to recognise (Kennedy 2000) this shows how difficult it is to make out the value of single morphological characters and to decide which of them to use for a grouping. Starting from the aforementioned impression of the conspicuous diversity of hooded staminodes we bring up in this study the following three hypotheses:

- Characters of form and shape of the hooded staminode may be more meaningful for the systematic grouping than vegetative, metric or numerical characters which were predominantly used in previous subdivisions of the family (Petersen 1889, Andersson 1998).

- Rare characters are of higher significance than frequent characters.

- If the most suitable floral organs and characters are selected, even purely morphological features are of a very high systematic value.

In the present study we make an attempt, only based on a small number of morphological characters of hooded staminodes, to subdivide the *Marantaceae* into few types. These types are then compared with the results of the two recent phylogenetic analyses by Prince and Kress (2006) in first place, Andersson and Chase (2001) and Andersson´s informal grouping (1998). Based on these comparisons we are going to judge the weight of our own morphological characters for the systematics of the family.

4.2 Materials and methods

4.2.1 Materials

Hooded staminodes of 40 species from 24 genera are investigated, whereas each genus is represented by at least one species; in greater genera, such as *Calathea*, *Ctenanthe*, *Marantochloa* and *Maranta* we view several species to capture with a high degree of certainty the typical generic characters of their hooded staminodes. Alcohol material is taken from the following species:

Calathea microcephala (Poeppig & Endlicher) Koernicke *Calathea picturata* (Linden) Koch et Linden *Calathea rufibarba* Fenzl *Calathea veitchiana* Veitch ex Hook. f. *Ctenanthe lubbersiana* (E. Morren) Eichler ex Petersen *Ctenanthe oppenheimiana* (E. Morren) K. Schumann *Maranta leuconeura* E. Morren *Maranta noctiflora* Koernicke *Marantochloa leucantha* (K. Schumann) Milne-Redhead *Thalia geniculata* Linné	Botanical Gardens of the University of Mainz
Donax cannaeformis (G. Forster) K. Schumann	University of Bayreuth
Afrocalathea rhizantha K. Schumann *Haumannia danckelmanniana* (J. Braun et K. Schumann) Milne-Redhead *Hypselodelphys scandens* Louis et Mullenders	sampled in Gabon
Hylaeanthe hoffmannii (K. Schumann) Jonker et Jonker	Biological Station Esquinas Rainforest, Costa Rica

Ischnosiphon heleniae Koernicke *Marantochloa mannii* (Bentham) Milne-Redhead *Sarcophrynium brachystachyum* (Bentham) K.Schumann *Thaumatococcus daniellii* (Bennet) Bentham	University of München
Marantochloa purpurea (Ridley) Milne-Redhead	University of Bochum
Myrosma setosum Bentham	University of Greifswald
Schumannianthus dichotomus (Roxburgh) Gagnepain *Phrynium obscurum* Willdenow	University of Aarhus
Pleiostachya pruinosa (Regel) K. Schumann	University of Bonn
Stachyphrynium jagorianum (K. Koch) K. Schumann	Royal Botanic Gardens Kew
Stromanthe sanguinea (Hooker) Sonder *Calathea cylindrica* (Roscoe & K. Koch) K. Schumann	Palmengarten Frankfurt
Ataenidia conferta (Bentham) Milne-Readhead *Calathea albertii* (Pynaert & Van Geert) L.H. Bailey *Calathea crotalifera* S. Watson *Calathea lietzei* E. Morren *Ctenanthe burle-marxii* H.A. Kennedy *Halopegia azurea* (K. Schumann) K. Schumann *Marantochloa congensis* (K.Schumann) J. Léonard et Mullenders *Pleiostachya porphyrocaulis* (W. Bull) H.A. Kennedy *Megaphrynium macrostachyum* (Bentham) Milne-Redhead *Stromanthe porteana* Grisebach *Stromanthe tonckat* (Aublet) Eichler *Trachyphrynium brauneanum* (K. Schumann) Baker	National Botanic Garden of Belgium
Pressed herbarium flowers of *Koernickanthe orbiculata* (Koernicke) L. Andersson	University of Ulm

4.2.2 Methods

Selection of characters:

Vegetative characters like those that Andersson (1998) took as a basis for his grouping are entirely avoided. Metric floral characters such as the size of a flower, length and width of the corolla tube, distances between single markings and all other floral parts like callose staminodes, anthers a. s. o. are also excluded from the study. Numerical characters like the number of outer staminodes or the number of ovules are avoided, too. All these ´traditional´ characters have proven to lead to an unsatisfying grouping which is little consistent with the results of modern phylogenetical analysis. We are going to work by a minimalistic method based only on hooded staminodes to fathom the diagnostic value of our own characters in relation to genetic phylogeny. Functional features of hooded staminodes relating to the pollination mechanism and the mutual dependence of flowers and their pollinators must be disregarded here because we have only alcohol material or herbarium material to hand. The only characters that we share with Andersson (1998) as well as Andersson and Chase (2001) and Prince and Kress (2006) are the position and shape of the trigger-appendages. Consequently, a very small number of distinctive characters of form and shape only at the hooded staminodes are taken as a basis for the sorting. The most important characters for our purpose are

- the presence of one or two lateral appendages on the trigger-side
- the presence or absence of a basal plate (sensu Kunze 1984) and its strength
- the basal plate that may be elongated in distal direction like a ´flag´
- the form of the appendages with swellings or callose thickenings
- the form of the appendages that may be rolled up or elongated
- the course of the midrib
- the area of the midrib that may have ledges or swellings
- the degree of support of the marginal and laminal growth of the hood

Pressed herbarium flowers of *Koernickanthe orbiculata* are first cooked in water at 80° C for 7 minutes and further put into 70 % alcohol for a day and eventually treated by the same method as all others: the hooded staminodes of the pickled flowers are removed and stretched on a black microscope slide so that the ´inner side´ of the hooded staminode (adaxial in

relation to the axis of origin of the single flower) lies uppermost and affords a clear view of the basal plate and trigger appendage. Only the hooded staminode of *Phrynium obscurum* is not removed, but just bent away from the entire flower. They are photographed by means of binoculars and a digital camera (Nikon coolpix 995). A selection of these photographs are then drawn by hand in a slightly simplified way according to the abovementioned list of characters, but still following the natural proportions, and then touched up by means of an Illustrator 7 programme so that finally every genus in this study is represented by at least one specimen and one sketch. Drawings are made of those that appear very typical of the respective genus. As we have only alcohol material to hand, the positioning of these drawings must be independent of the actual position and function of hooded staminodes in living plants in order to secure an exclusively morphological observation and assessment uninfluenced by any knowledge of real processes during pollination. These drawings of the hooded staminodes are then sorted ´without prejudice´ according to the already mentioned characters into groups. Finally a small number of types are abstracted from the groups and this entirely morphological sorting is compared with the recent phylogenetic trees and discussed.

4.3 Results

In all hooded staminodes of the 24 genera investigated it is the midrib that does not take a straight course to the leaf tip as in ´normal´ leaves (Fig. 4.1). Instead, it takes a turn of about 90°. The resulting leaf apex is in a lateral position and is clearly discernible because the main vascular bundle is the thickest. Herewith the hooded staminode is generally divided into two different ´halves´. On the adaxial side of the leaf (relating to the main axis of a pair of flowers) there is the bigger ´half´ that forms the actual ´hood´ of the cucullate staminode. The growth of the margin of the hood is always more or less supported. The hood can therefore cover or even envelope the stigmatic orifice in some species or leave it entirely open in other species.

The smaller, abaxial half forms the 1-2 appendages, one of which is considered the ´trigger´ for the release of the style movement. In the place where the midrib ends it reaches into a small indentation between the hood and the first appendage – seen from the tip of the flower. Here the appendage ´rises´ from the leaf margin of the cucullate staminode. In the place where the appendage ´reenters´ the leaf margin there is often a fold that can have a callose

thickening or even a stiffening to its base, the ´basal plate´ which is believed to have an important share in the release mechanism of the style movement. In proximal position to the first appendage there can be a second appendage in some genera, e.g. *Calathea*. These appendages are very divers in their shaping. They appear flat, cushion-like swollen or very long extended and lengthwise rolled up (Fig. 4.1).

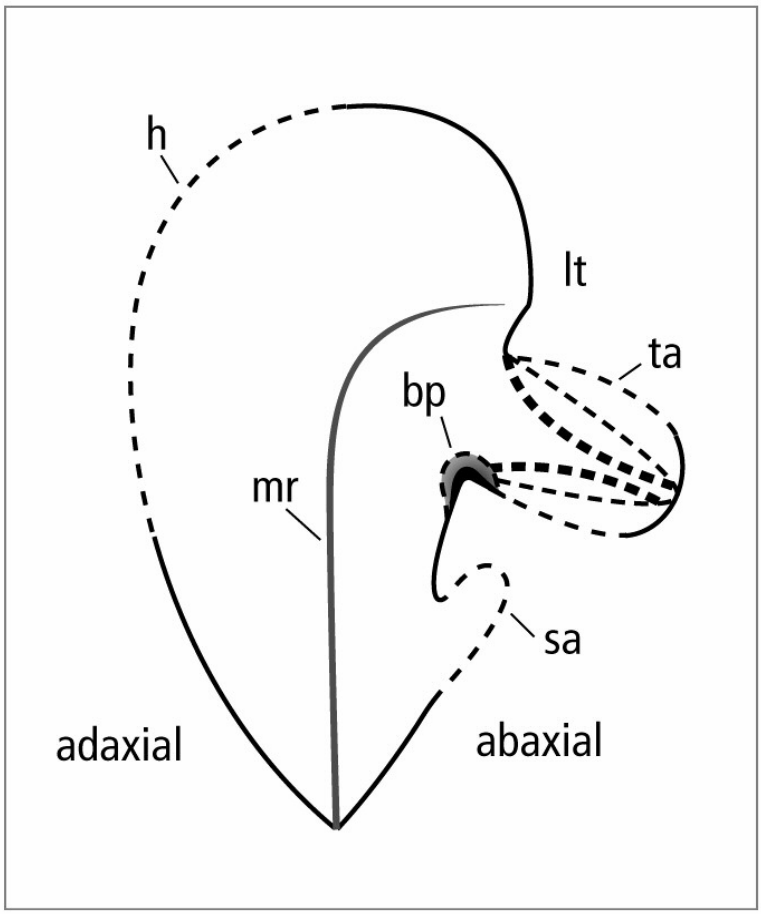

Fig. 4.1: Model of a hooded staminode in *Marantaceae* in general: bp = basal plate, h = hooded half of the staminode with dotted lines indicating the differently supported growth of the leaf margin, lt = the approximate position of the actual leaf tip, mr = midrib taking a turn, sa = second appendage with dotted lines indicating its possible presence or absence, ta = trigger-appendage with dotted lines showing the different lengths and shapes of it

The sorting of the 24 genera according to these morphological aspects leads to the abstraction of 8 distinct form types, of which two genera, namely *Haumannia* and *Thalia* (Fig. 4.5 and Fig. 4.9) both create a type of their own.

Type **Asia** (Fig. 4.2)

The first five genera/species whose hooded staminodes fit very beautifully together, are *Schumannianthus dichotomus*, *Stachyphrynium jagorianum*, *Donax cannaeformis* and *Phrynium obscurum* and *Afrocalathea rhizantha* (Fig. 4.2). There is only one appendage into whose distal portion the midrib leads. *Donax*, *Stachyphrynium* and *Afrocalathea* have in this place a slight indentation. In *Phrynium* and S*chumannianthus* there is no indentation; instead the leaf margin merges gradually into the appendage. The appendages are small and always flat and soft. In all five in the place where the appendage ´reenters´ the margin of the staminode the leaf margin is folded and the fold is slightly stiffened, forming a small, but distinctive basal plate. There are no further swellings or stiffenings in these staminodes, even not alongside the midrib. The ´hooded half´ of the staminodium is barely supported in its marginal growth so that the stigmatic orifice is probably always uncovered.

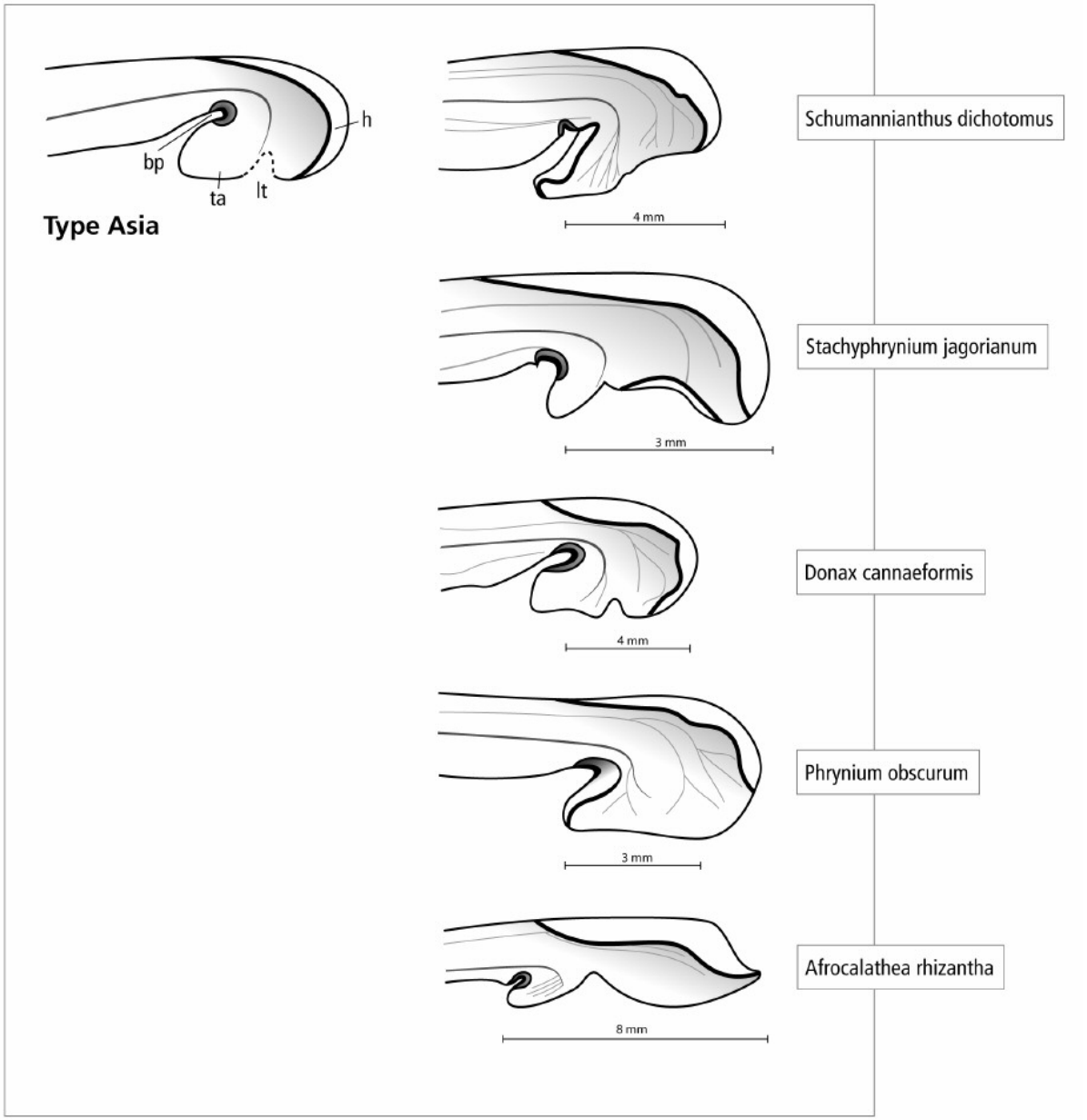

Fig. 4.2: Type Asia represented by *Schumannianthus*, *Stachyphrynium*, *Donax, Phrynium* and *Afrocalathea* with a simple and flat trigger-appendage and a small basal plate, in *Stachyphrynium* and *Donax* and *Afrocalathea* with an indentation in the place where the midrib ends. Dotted lines in the type indicate the possible presence or absence of this indentation.

Type **Africa I** (Fig. 4.3)

Another very uniform group can be derived from the genera/species *Ataenidia conferta*, *Marantochloa mannii*, *M. congensis*, *M. leucantha*, *M. purpurea* and *Halopegia azurea*. Here the two latter fit extremely well together due to the length of their hooded staminodes. All the others are shorter. Nevertheless, the group in its entirety is characterised by a single

appendage which is always cushion-like swollen and has neither in its proximal portion a fold nor a distinct basal plate. Generally the appendage lies in a very proximal position inside the flower tube, more conspicuous in the two abovementioned longer flowers. The hood of the cucullate staminode in its laminal and marginal growth is barely supported. The midrib runs also into the place where the appendage starts to rise from the leaf margin of the hooded staminode and has no other swellings.

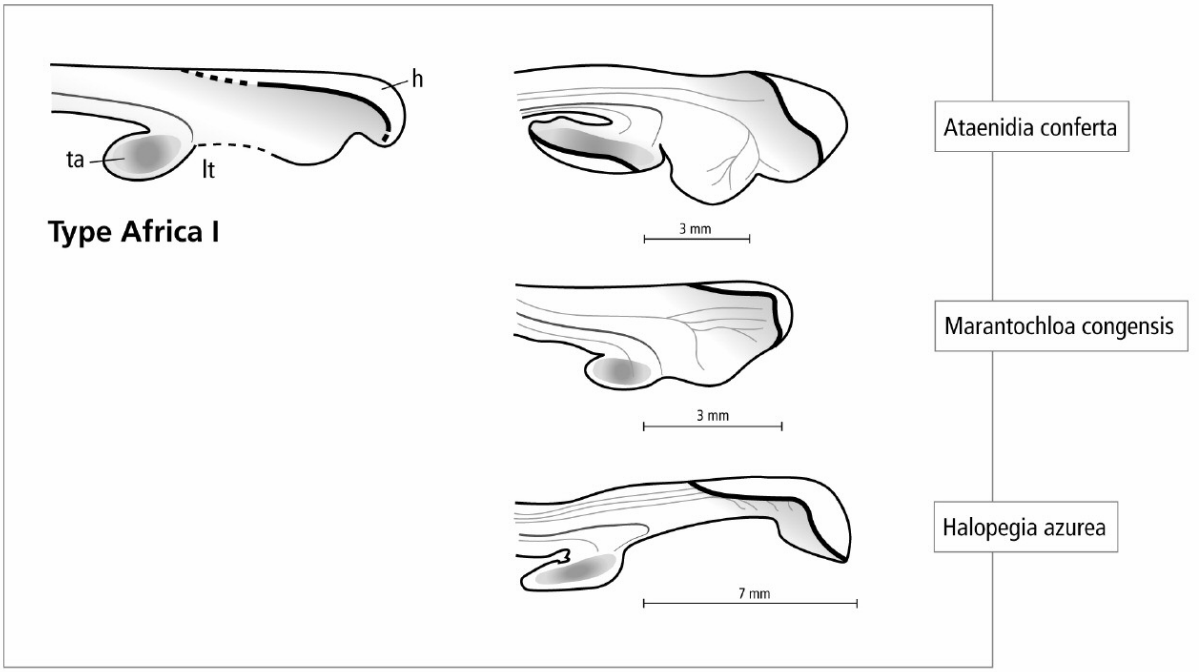

Fig. 4.3: Type Africa I represented by *Ataenidia*, *Marantochloa* and *Halopegia* with a trigger-appendage that is cushion-like swollen and without a basal plate. Dotted lines in the type indicate the possibly different lengths of the hooded staminode.

Type **Africa II** (Fig. 4.4)

A more complicated looking, though uniform appearing group of cucullate staminodes can be derived from the genera/species *Trachyphrynium brauneanum*, *Hypselodelphys scandens, Megaphrynium macrostachyum*, *Thaumatococcus daniellii* and *Sarcophrynium brachy-stachyum*. All have a single appendage that can be extremely elongated (especially in *Hypselodelphys*) and lengthwise rolled up. In the place where the ´trigger´ reenters the leaf margin of the staminode it is stiffend in such a way that the resulting basal plate is formed like the brim of a hat which is put on-end .This ´brim of a hat´ exists in no other group and elongates in distal direction into the shape of a thin membraneous ´flag´. This flag covers the

stigmatic orifice of the respective style as the marginal growth of the hooded half of the staminodium is only slightly supported. Two of them, namely *Trachyphrynium brauneanum* and *Hypselodelphys scandens,* bear a stiffening of the tissue along the midrib while *Megaphrynium macrostachyum* has a swelling aside the midrib at the trigger-side. *Megaphrynium* and *Thaumatococcus* have shorter appendages that are lengthwise rolled up, too, and an almost identical course of vascular bundles. *Trachyphrynium* and *Sarcophrynium* have the biggest hoods in this group.

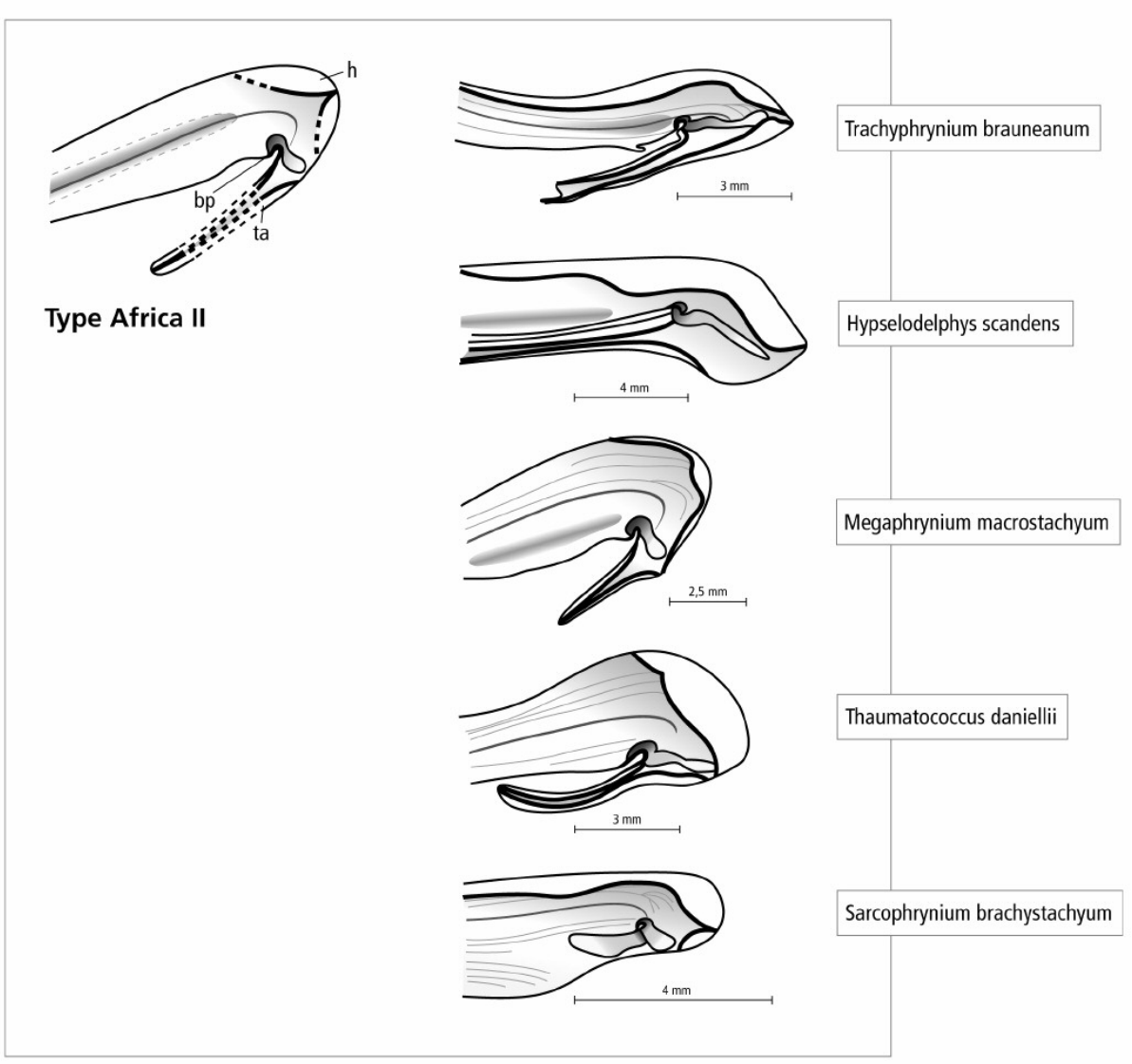

Fig. 4.4: Type Africa II represented by *Trachyphrynium*, *Hypselodelphys*, *Megaphrynium*, *Thaumatococcus* and *Sarcophrynium* with elongated trigger-appendages, the shortest in *Sarcophrynium*, the longest in *Hypselodelphys*, that are always lengthwise rolled up. The basal plates are very stiff and put on-end and extended into a ´flag´ that can cover the

stigmatic orifice. Dotted lines in the type reflect the different lengths of the triggers and the different dimensions of the hood in this group.

Type Africa **III** (Fig. 4.5)

The genus *Haumannia*, here represented by the species *Haumannia danckelmanniana*, is characterised by some unique morphological features and cannot be assigned to any of the previous types, but creates a type of its own. The midrib runs into a thick first appendage that is – contrary to all other genera investigated – distally directed, i. e. towards the entrance of the corolla tube and which has to its base a cushion-like swelling instead of a basal plate. In proximal position to the first appendage there is a very tiny second one. The hood of the cucullate staminode on the adaxial side (see Fig. 4.1) is barely supported, but in the place where the hood merges into the trigger-appendage there is a double fold so that finally the trigger is in crosswise position in the corolla tube, so closing the entrance of the flower (A. Ley, pers. com.).

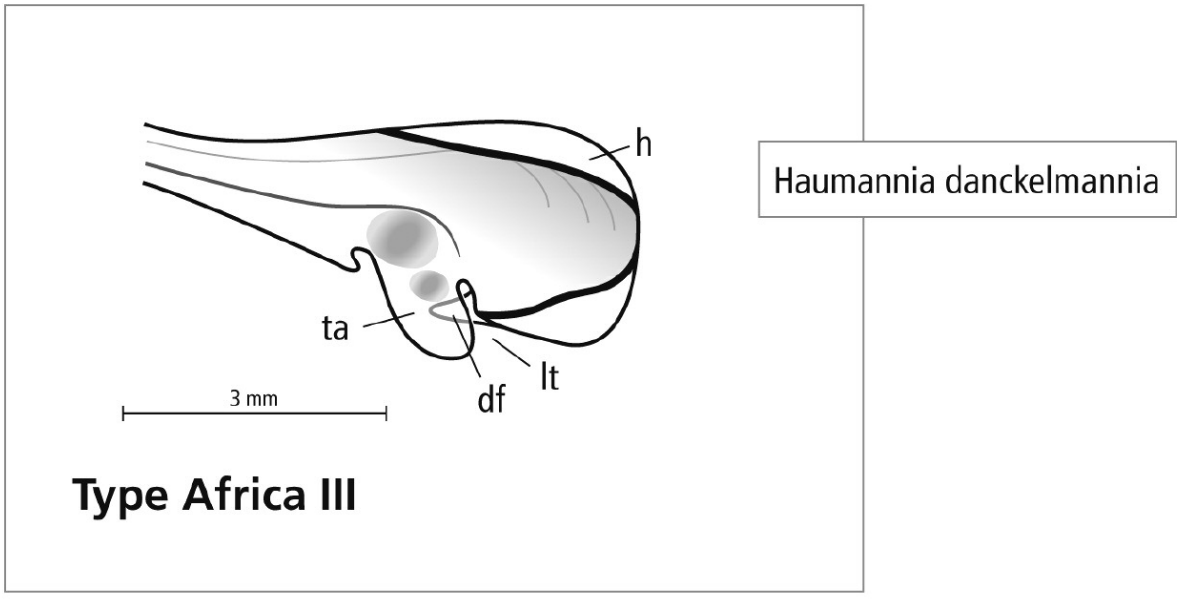

Fig. 4.5: Type Africa III represented only by *Haumannia*. The trigger is, when spread out flat, directed in distal direction and doubly folded, with a cushion-like swelling to its base.

Type **America Ia** (Fig. 4.6)

A slightly varied group includes the genera/species *Ctenanthe oppenheimiana, Ctenanthe lubbersiana*, *Ctenanthe burle-marxii*, *Myrosma setosum* and *Stromanthe tonckat*, *Stromanthe*

porteana and *Stromanthe sanguinea* from which nevertheless a common type can be derived. Some of these cucullate staminodes bear two appendages. The first or ´trigger-appendages´ are relatively small and tend to be slightly rolled up, in contrast to another group where the rolling-up is very distinctive (see Fig. 4.8). A second appendage in proximal position to the first is present in *Ctenanthe burle-marxii,* as well as in *Stromanthe tonckat* and *Stromanthe porteana*, if very small in both. But it is missing in *Ctenanthe oppenheimiana* and in *Myrosma setosum.* In *Ctenanthe lubbersiana* and in *Stromanthe sanguinea* it is only faintly suggested. The midrib leads as always to the base of the first, the trigger-appendage, and there is always a basal plate to the fold, if in some species very small and faint as e.g. in *Myrosma* and likewise in *Ctenanthe lubbersiana* and *Stromanthe sanguinea.* In these three the trigger-appendage is also not rolled up, but flat. There are no swellings of the tissue, neither to the midrib nor to the one or two appendages.

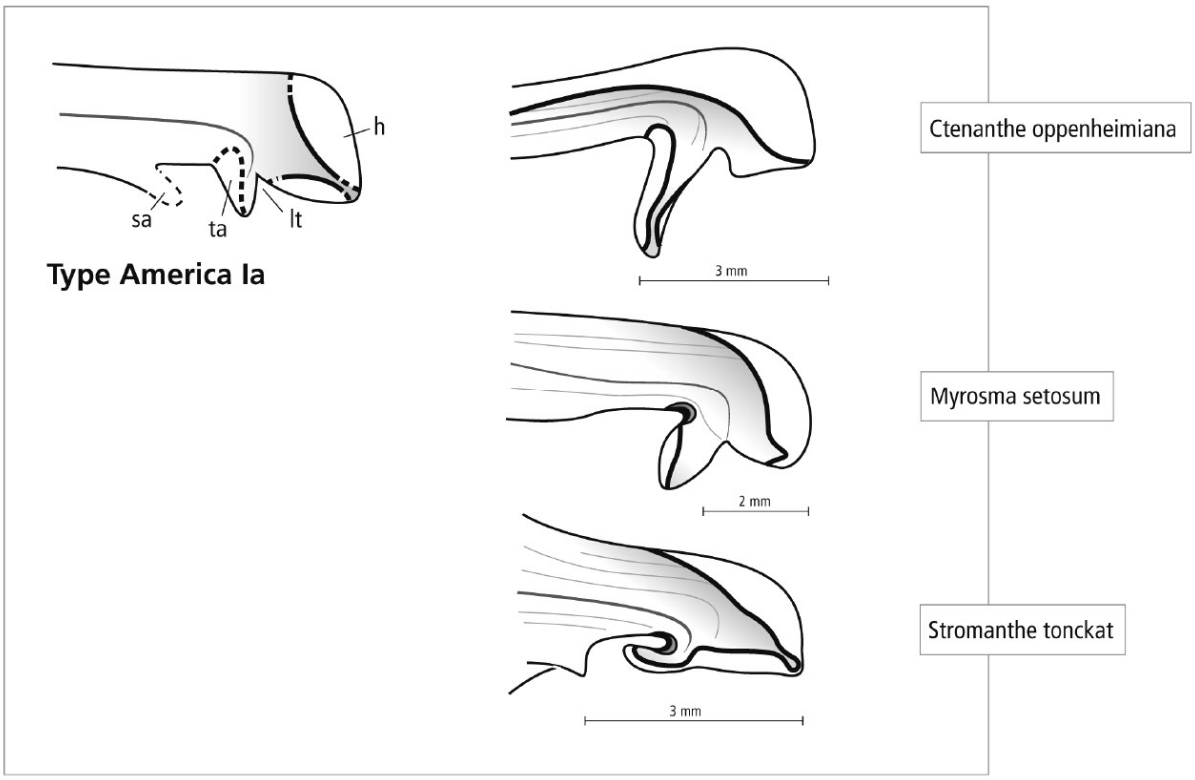

Fig. 4.6: Type America Ia represented by *Ctenanthe*, *Myrosma* and *Stromanthe*, all with more or less rolled-up trigger-appendages and in *Stromanthe* a faint second bulge. Dotted lines in the type show that the trigger can be more or less rolled up and that there can be a second appendage.

Type **America Ib** (Fig. 4.7)

Another group with simple and very uniform features includes the genera/species *Koernickanthe orbiculata*, *Maranta noctiflora*, *Mar. leuconeura*, *Mar. depressa* and *Hylaeanthe hoffmannii*. *Koernickanthe* is a little more difficult to examine as there is only herbarium material available and few vascular bundles can be discerned. But it is clear that all three genera have only one appendage that is short and flat and has - except for *Hylaeanthe* - a small stiffened basal plate in the place where the appendage reenters the leaf margin. *Hylaeanthe* is an exception as the trigger is in a more proximal position in comparison to the other two genera and has sometimes a slight swelling at the base of the trigger (´ledge´ sensu Heller, 2003). The midrib also in these three leads exactly to the point where the appendage starts rising from the leaf margin. Hereby, *Maranta noctiflora* and *Hylaeanthe hoffmannii* show a conspicuously similar branching of the midrib in the tissue of the appendage. The hooded portion of the cucullate staminode is moderately supported.

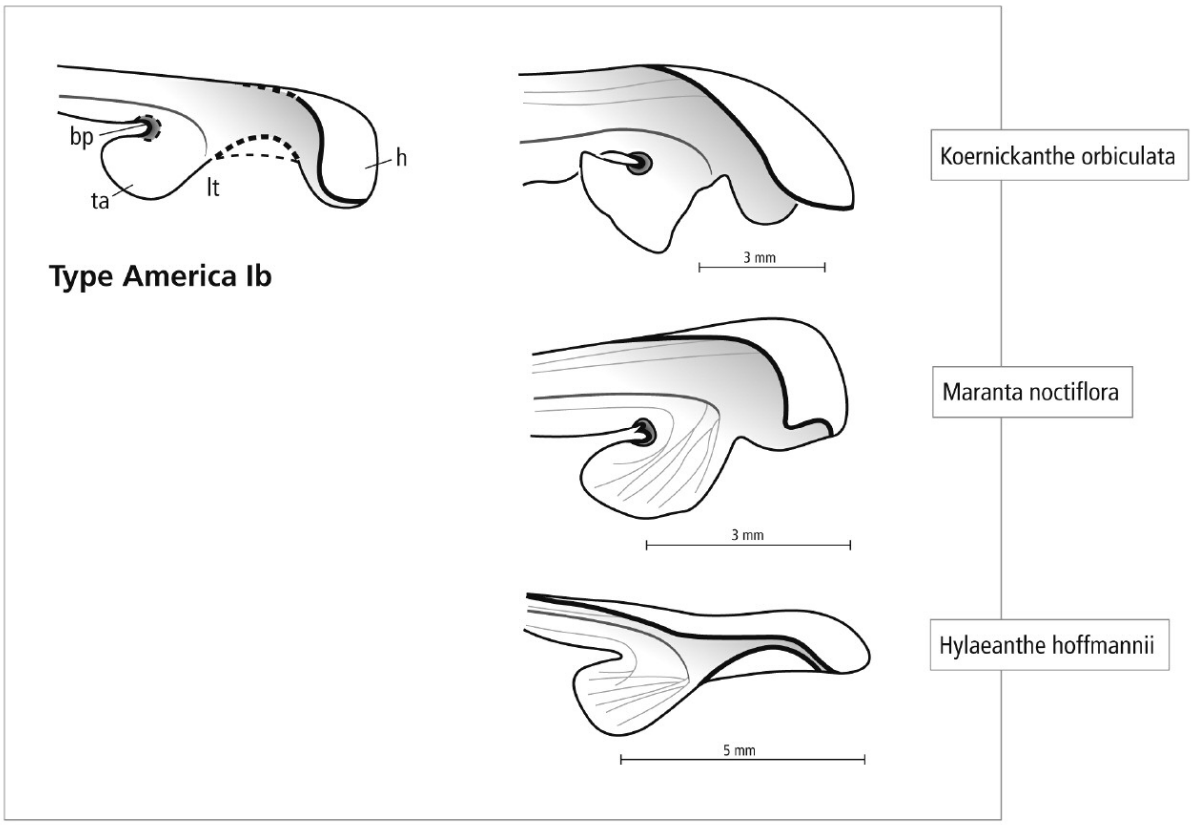

Fig. 4.7: Type America Ib represented by *Koernickanthe*, *Maranta* and *Hylaeanthe*, all with flat and short trigger-appendages and small basal plates – in *Hylaeanthe* missing. Dotted lines

in the type indicate the different lengths of the hooded staminodes and the basal plate that can be missing.

Type **America II** (Fig. 4.8)

In the three genera/species *Ischnosiphon heleniae*, *Pleiostachya pruinosa* and *Pl. porphyrocaulis* and *Calathea veitchiana*, *C. crotalifera*, *C. picturata*, *C. microcephala*, *C. albertii*, *C. lietzei*, *C. cylindrica* the characters seen in **America Ib** (Fig. 4.7) appear more elaborate. The hooded staminodes all have two appendages so that in this group – similar to America I a - there is a second bulge or appendage proximal to the first, the actual trigger. This bulge can be cushion-like thickened in those species that have a thickening alongside the midrib, too, namely in *C. crotalifera*, *C. veitchiana*, *Pleiostachya pruinosa*, *Pl. porphyrocaulis* and *Ischnosiphon heleniae*. Hereby the first appendage is relatively short and very strongly lengthwise rolled up. Its base is always strongly stiffened to form a basal plate. The hooded part in this group is much supported so that the hoods are almost formed like shovels and the stigmatic orifices of the styles can be enveloped.

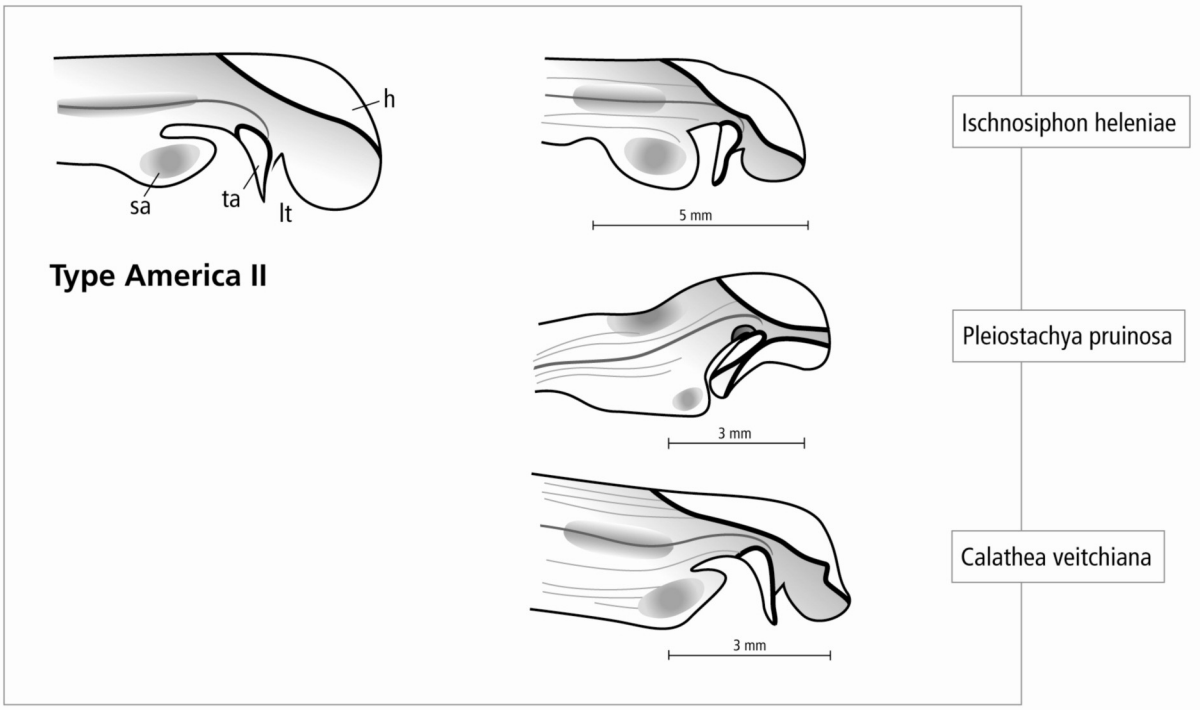

Fig. 4.8: Type America II represented by *Ischnosiphon*, *Pleiostachya*, and *Calathea* with two appendages each, the first of which is very strongly rolled up. The second appendage is cushion-like swollen. The basal plate is behind the rolled up trigger and therefore invisible.

Type **America III** (Fig. 4.9)

The genus *Thalia*, here represented by the species *Thalia geniculata*, is characterised by some very unique morphological features and cannot be associated with any of the previous types, but creates a type of its own. The midrib runs into a very long and thin first appendage which has to its base neither a fold nor a basal plate. In proximal position to the first appendage there is a second one, even longer than the first, that has to its base – in the place where it reenters the leaf margin - the small but strongly stiffened basal plate that is supposed to convey the stimulus caused by a pollinating insect to the style. The hood of the cucullate staminode is extremely supported, so that it looks like a shovel.

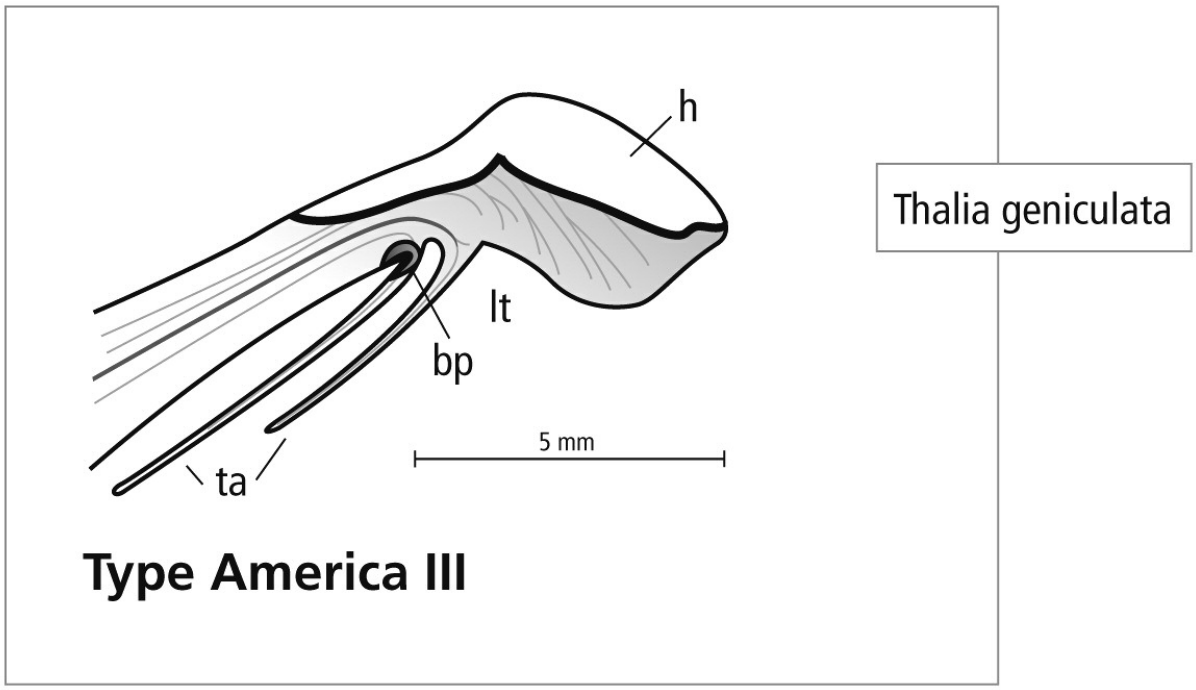

Fig. 4.9: Type America III represented only by *Thalia* with a very large hood and two very long appendages, the second of which bears the basal plate.

The mapping of the types (Fig. 4.10)

Eight morphological types of cucullate staminodes mapped onto the phylogenetic tree by Prince and Kress (2006) lead to a large degree of correspondence between the types and the clades; Type Asia (orange) is distributed over the Donax- and Stachyphrynium-clade and

Africa I (green) over the Stachyphrynium- and Maranta-clade. America I a (dark blue) and America I b (yellow) are consistent with the tree except for *Halopegia*. America II (lilac) is consistent with the Calathea-II-clade as well as Africa II (light blue) with the Sarcophrynium-clade. *Thalia* (America III, violet) is in a basal position in the Donax-clade and *Haumannia* (Africa III, dark orange) in a basal position of the Calathea-clade. The genera *Saranthe*, *Monotagma* and *Schumannianthus virgatus* are missing in our investigation and all specimen of the Calathea-I-clade, and *Myrosma* and *Koernickanthe* are not represented in the tree.

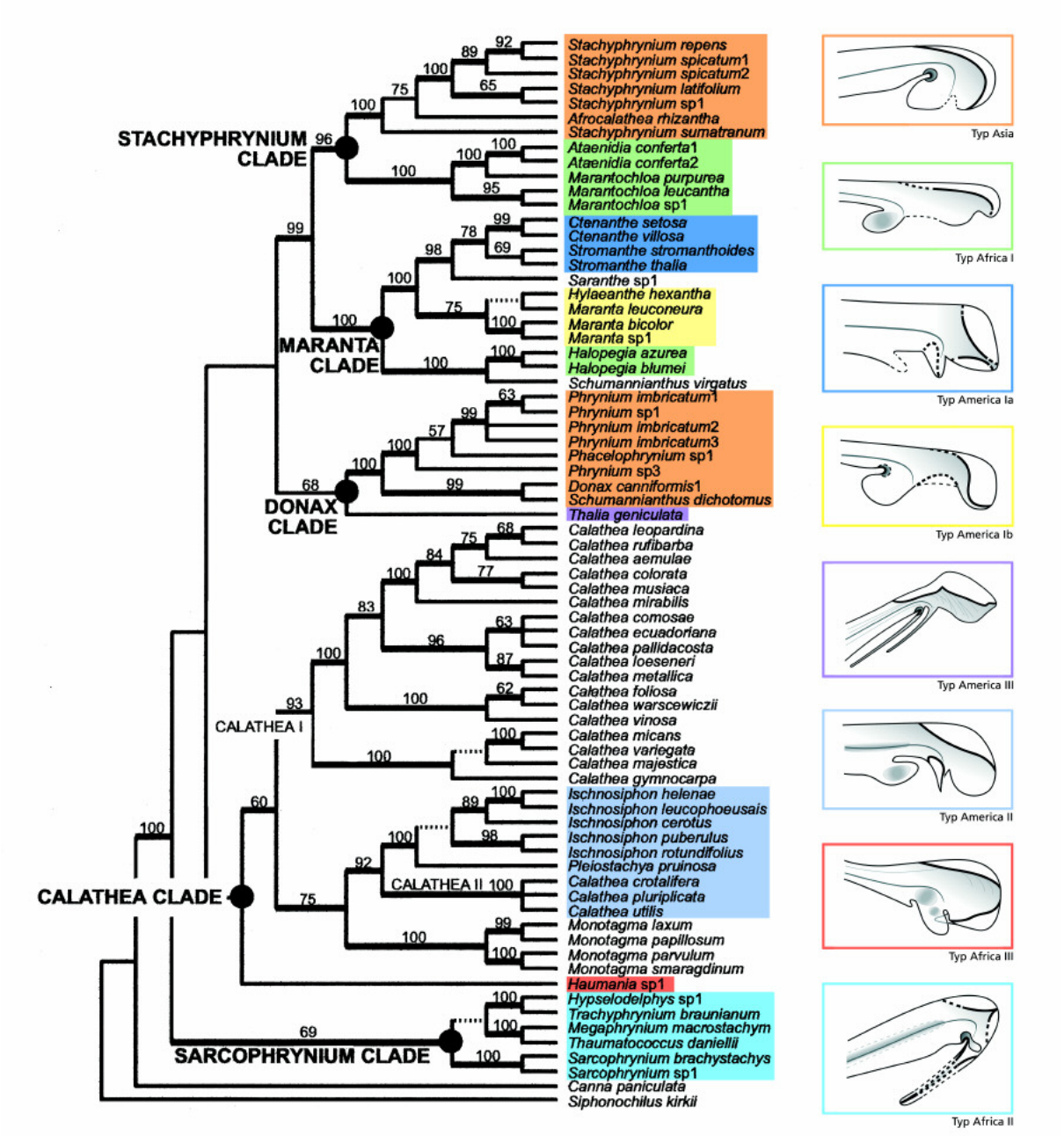

Fig. 4.10: Types of hooded staminodes mapped onto the phylogenetic tree by Prince and Kress (2006). Type Asia (orange) is distributed over the Donax- and Stachyphrynium-clade and Africa I (green) over the Stachyphrynium- and Maranta-clade. America I a (dark blue) and America I b (yellow) are consistent with the tree except for *Halopegia*. America II (lilac) is consistent with the Calathea-II-clade as well as Africa II (light blue) with the Sarcophrynium-clade. *Thalia* (America III, violet) is in a basal position in the Donax-clade and *Haumannia* (Africa III, dark orange) in a basal position of the Calathea-clade.

4.4 Discussion

4.4.1 Discussion of the ´bauplan´ of cucullate staminodes

Fig. 4.1 shows a general ´bauplan´ of hooded staminodes that goes most likely – as we have not investigated all of 31 genera - for the entire family. Ontogenetic observations on the development of hooded staminodes of Eichler (1883, 1884 with *Maranta sanguinea*), Kunze (1984 with *Maranta leuconeura*, *Ataenidia conferta*, *Calathea vaginata*) and Ley (unpublished data) are in accord with the appearance of all mature hooded staminodes investigated in this study: the midrib takes a turn by about a right angle so that finally the actual leaf tip is at the abaxial side of the staminode, relating to the main axis of a flower pair. This side eventually forms the later trigger part and the adaxial side forms the hood. Comparative observations on our part have shown that the midrib is discernible already in very small buds (2-4 mm) several days before anthesis and divides the growing staminode in exactly these abovementioned ´halves´, one of which forms the later hood and the other the trigger in all observed genera in the same fashion (A. Ley, with 20 genera, unpublished). The diversity discussed here and taken as a basis for a systematic grouping is an expression of variable proportions of exactly this basic model; the adaxial hood can be more or less supported in its marginal and laminary growth. In addition, the margin of the hood can bear lobes that are able to wrap the style in certain places. As a consequence in some genera (e. g. *Calatheas*) the leaf blade of the hooded staminode can be so enlarged through such lobes that the head of the style and the stigmatic orifice are completely enveloped. In others the surface of the hood is short (e.g. *Marantas*) so that the stigma is left open. The abaxial trigger part can bear 1-2 appendages, the first of which (seen from the leaf apex) is named the ´trigger´ that releases the style movement and can – in most cases – bear at its base the basal plate or a basal swelling. In extreme cases the triggers can be much elongated, some are lengthwise rolled up or even lengthwise splitted in two, and some are simple and flat or cushion-like

swollen. Also the basal plate can be very different in its solidity and shape and extension. The second appendage - if one is formed - is in proximal position to the first and can also be with or without swellings, ledges and differently shaped, but not as ´sophisticated´ as the first appendage. And it is never a ´trigger´ to trigger off the style movement and never does the midrib run into it. However different the actual trigger may be shaped, it is always the ´first appendage´ that the midrib runs into and in this sense all appendages at the tip of the medianus on the abaxial side of the leaf are considered homologous in their position. Any lobe on the hooded part (adaxial side) of the staminode is in this study never called an ´appendage´ and has probably never to do with triggering off the style movement – rather it is supposed to protect the stigmatic orifice and to hold the style in its unreleased position prior to a pollinator´s visit. Another feature that is subject to diversity is the strength of all these elements of hooded staminodes (and also other floral parts) that can vary from very fragile (e.g. *Myrosma*) to extremely solid (e.g. *Megaphrynium*) types, which may possibly be an adaptation between the flowers and their pollinating animals and which remains to be examined. Anyway, in this study characters of robustness are only taken in account for basal plates as explained above.

4.4.2 Discussion of the types

Our simple looking Type Asia of hooded staminodes is in the analysis of Prince and Kress (2006) distributed over two different clades, namely the Stachyphrynium-clade and the Donax-clade. *Schumannianthus dichotomus* and *Donax* are sister-groups (which is consistent with Andersson´s informal grouping of 1998) and together sister to *Phrynium* within the Donax-clade; *Schumannianthus virgatus* which is in a quite different position in the tree (Maranta-clade) we could unfortunately not examine here. *Afrocalathea* and *Stachyphrynium*, on the other hand, are together in the Stachyphrynium-clade. Except for *Afrocalathea* they are all Asian genera (see below).

The Type Africa I is only partly reflected by the tree of Prince and Kress (2006). The genera *Marantochloa* and *Ataenidia* are unsurprisingly close regarding the shape of their hooded staminodes which appear almost identical. The close relationship of these two was already confirmed by the analysis of Andersson and Chase (2001) in contrast to Andersson´s informal grouping of 1998 with *Ataenidia* in the Phrynium group and *Marantochloa* in the Maranta

group. Prince and Kress (2006) even regarded *Ataenidia* as a ´highly modified member of *Marantochloa´* which was already strongly confirmed by Ley and Claßen-Bockhoff (2005, 2006). On the other hand, comparing *Marantochloa purpurea* with *Halopegia azurea* whose cucullate staminodes are extremely similar the position of *Halopegia* in the Maranta-clade seems surprising at first sight (see below). Likewise surprising is the remark of Prince and Kress about the ´absence of a staminodial appendage´ in *Ataenidia*. From the course of the midrib it is understandable which spot in the leaf margin forms the leaf tip. Proximal to it a distinct appendage or a derivation of it can be determined. It is strongly cushion-like swollen and sometimes a little rolled up. If in *Ataenidia* there were no appendage one would have to regard the homologous positions in many flowers of the genus *Marantochloa* as vestigial, too. This cushion-like swelling in the actual position of a trigger-appendage must be regarded as a rare character. But it is not exclusive to the clade *Marantochloa*/*Ataenidia* as the position of *Halopegia*, which has a cushion-like swelling in the same place, in the Maranta-clade proves. Kunze (1984) equated the swelling of this spot in *Ataenidia* at least in a functional sense with another swelling proximal to the trigger in many *Calatheas*. It is important to realise that in a morphological sense these spots can not be homologous, because in the *Calatheas* the swollen area shown in ontogenetic drawings of *Calathea vaginata* by Kunze is the second appendage or bulge which was already described by Koernicke (1858). This is what we also found in several species of *Calathea* in this study. The position of the first, the actual trigger-appendage is clearly discernible from the medianus that leads to the leaf apex. The sister-genera *Ataenidia/Marantochloa* belong to the Stachyphrynium-clade, together with *Afro-calathea* and *Stachyphrynium*. The main difference between these two types of hooded staminodes lies in the appendages that bear basal plates in the latter and a swelling with no basal plate in the first. They are all African except for *Stachyphrynium* which is Asian.

The type Africa II is a group that is precisely and without exception reflected by the Sarcophrynium-clade of Prince and Kress (2006). They all share a very striking character for which Andersson (1998) created the term of a ´bilobed trigger´ which was taken over by Prince and Kress. The term of course is only usable if one regards the basal plate as a part of the trigger. For the ´flag´ that is extended in distal direction comes from the ´brimmed´ and very stiff basal plate and not from the actual trigger-appendage which is very thin and frail. Both the form and consistency of the basal plate and the membrane that comes out of it are particularly rare characters which are found in no other group. This makes them highly

meaningful and exclusive. The sister-relationship of *Trachyphrynium* / *Hypselodelphys* was already described by Andersson (1998) and supported by the phylogenetic analyses of Andersson and Chase (2001), Ley and Claßen-Bockhoff (2005, 2006) and Prince and Kress (2006). The same is reported for the sister-genera *Megaphrynium* and *Thaumatococcus* that were placed in very different positions by Andersson (1998). But considering the rarity and value of the observed characters this newly found close relationship is not so very surprising any more. Even if one takes solely the trigger appendages and their basal plates as a basis for the grouping one can make out that they are African.

The type Africa III is formed by the genus *Haumannia* alone. In contrast to all other genera whose triggers are always directed in proximal direction, i. e. towards the ovary, the trigger in *Haumannia* is directed towards the flower entrance. Furthermore, it has two folds; one fold is in the place where the appendage rises from the leaf margin, which is exceptional, and the second fold is in the place where the appendage reenters the leaf margin as in general in the family. Instead of a basal plate there is a cushion-like swelling similar to those in Africa I so that a relation between them suggests itself. The consequence of the double fold is that the appendage lies crosswise in the flower tube in a living flower (A. Ley, pers. com.).

Our Type America Ia corresponds with a part of the Maranta-clade of Prince and Kress (2006) where *Stromanthe* and *Ctenanthe* are confirmed to be sister-groups (as proposed already by Anderssson and Chase 2001). Features that are very elaborate in the Type America II appear here modest and are not always very clearly developed, such as the second appendage which can be present or absent in *Ctenanthe* and *Stromanthe* or the slight tendency of the triggers to roll up. The close relationship of *Myrosma* to the sisters *Stromanthe* and *Ctenanthe* is also supported by the analysis of Andersson and Chase (2001) and by Andersson´s Myrosma group (1998).

Our type America Ib fits with another part of the Maranta-clade, namely the sister-groups *Maranta* and *Hylaeanthe* according to the evaluation of Prince and Kress (2006). Another close relationship between *Maranta* and *Koernickanthe* is reported by Andersson and Chase (2001). Andersson (1998) included *Maranta*, *Koernickanthe* and *Hylaeanthe* in the Maranta-group which is consistent with our type America Ib. The positioning of *Halopegia* in the Maranta-clade after the analysis of Prince and Kress (and together in the Myrosma-group of

Andersson, 1998) seems surprising at first sight in comparison with our morphological findings. But when one imagines the appendage at the hooded staminode in *Halopegia* without its characteristic swelling and instead a small basal plate – or even none, as sometimes in *Hylaeanthe* (also confirmed by our findings and by Heller 2003) – then also the hooded staminode of *Halopegia* can look like a simple Type America Ib. Our decision to place *Halopegia* among the African genera together with *Marantochloa* and *Ataenidia* was primarily based on the presence of a cushion-like swollen appendage without a basal plate and the striking similarity of the entire hooded staminodes between *Halopegia* and *M. purpurea*. In both the appendage is in a very proximal position, i.e. deeper than normally in the flower tube. Obviously is such a swelling not an exclusive character as we thought. Furthermore, also *Hylaeanthe* has an appendage that is relatively deep in the flower tube, more proximal to the ovary than in the sister-genera *Maranta* and *Koernickanthe*. This might also be an adaptive character relating to the function of the staminodes during pollination, as we cannot prove in this study.

The genera *Calathea*, *Ischnosiphon* and *Pleiostachya* form a very uniform type America II that is exactly reflected by the Calathea-clade of Prince and Kress (2006) as well as the Calathea-clade of Andersson and Chase (2001). Even Andersson´s Calathea-group (1998) is consistent with this clade. However, in the recent analysis of Prince and Kress (2006) the Calathea-clade turns out to be non-monophyletic. The species investigated all share the short and stiff and strongly rolled-up trigger-appendage, in comparison with the soft and long triggers in Africa II where only the basal plate is stiff. This is a striking character and a second ´American bulge´ whose function was discussed by Kunze (1984) at the example of *Calathea vaginata*. Similarity between *Calathea lutea*, *Calathea platystachya* and *Pleiostachya pruinosa* was already described by Heller (2003) – not only in a morphological but also functional sense.

Our reasons to associate *Thalia* with the American types (America III) are, first, that most species of *Thalia* are native to America and, secondly, because the American types tend to develop a second bulge or appendage which at first sight appears to be a similar case here, too. Furthermore, *Thalia* resembles no Asian or African type distinguished so far. Koernicke (1858) regarded the appendage in *Thalia* as a single, but deeply lengthwise splitted appendage. As the basal plate is at the base of the second ´ribbon´ this may be plausible. Also

the course of the midrib supports this notion. This second ´ribbon´ sensu Koernicke looks less supported than the first when one looks closely at the vascular bundles. Andersson (1998) on the other hand spoke of ´two appendages´ in *Thalia*. But this would say that *Thalia* was the only genus whose hooded staminode bears the basal plate in a different place than all other genera, namely at a second appendage. A second appendage occurs often in American genera (Type America I and II) and never bears a basal plate – just in some cases a swelling whose function in species of *Calathea* was already discussed by Kunze (1984). Up to now all observed genera and species were found to have their basal plates or equivalent derivations, such as swellings or stiffenings, in the same place. It is the place in which the risen trigger-appendage reenters the margin of the hooded staminode. Taking herewith a positional homology for the basal plates in the family as a basis for all, it is impossible to accept Andersson´s notion of two independent appendages, the second of which bears the basal plate. Our findings strongly support the previous interpretation of Koernicke (1859) that in *Thalia* there is one trigger-appendage splitted in two. Furthermore, the positioning of *Thalia* in the Donax-clade ´with moderate support´ (Prince and Kress, 2006), or near the Donax-clade as was already found out by Andersson and Chase (2001), makes any discussion about a second ´American appendage´ obsolete.

Altogether, we never found in this study a hooded staminode without a trigger-appendage, contrary to the opinion of Prince and Kress about *Ataenidia* (see above, Type Africa I). A number of these appendages provided most striking characters that enabled the separation of distinct lineages without doubt or any exception. One of them is the rolled-up trigger with a brim-shaped basal plate (´bilobed trigger´ sensu Andersson, 1998) in our Type Africa II or the Sarcophrynium-clade of Prince and Kress, respectively. Another one is the stiff and rolled-up trigger in our Type America II or the Calathea-clade of both Andersson and Chase (2001) and Prince and Kress (2006). These features may now be regarded as the actual ´shape-characters´ which are obligatory to a group and must be fully expressed. The cushion-shaped appendage in the sister-genera *Ataenidia* and *Marantochloa* (after Andersson and Chase 2001, Ley and Claßen-Bockhoff 2005, and Prince and Kress 2006) is also conspicuous but not absolutely exclusive to the clade and can therefore be misleading as it occurs also in *Halopegia* and more or less also in *Hylaeanthe* (Heller 2003). The thickenings or swellings in the ´American bulge´ in our type America II can also be more or less developed as well as stiffenings along or aside the midrib. Perhaps one must conclude that swellings are no definite ´shape-

characters´ but rather an optional and perhaps continuous feature relating to the function of an organ (e.g. the guidance of a pollinating insect) and that there may be gradual transitions in the expression of such a character. Even for the number of appendages there can be transitions as we have seen in *Myrosma* and in members of *Stromanthe* where the second ´American appendage´ is either very faint or can almost only be suspected. It is always the second appendage that is facultative and that is not the trigger for the pollination mechanism. Also obligatory to the respective group is the basal plate. It is either extremely elaborate, such as the ´brim-shaped´ basal plate in type Africa II, even extending in a ´flag´ that can cover the stigmatic orifice. Or it is simply a small, pointed stiffening in the fold of the leaf margin or in some cases, such as *Hylaeanthe* or the *Marantochloas*, completely missing. A missing basal plate is also a distinct and distinguishing character. The course of the midrib is always the same and does therefore not enable any systematic grouping. But it can be helpful in some difficult cases to find out the position of the leaf tip and herewith the first appendage, inconspicuous though it may be, which is always the trigger-appendage. The degree of support of the growth of the leaf margin of the hooded half of the staminode turns out in this investigation not to provide a very distinctive morphological character. But it may turn out to be an important ecological character as regards the protection of the stigmatic orifice and herewith the regulation of cross pollination versus self pollination. Concluding, among the eight characters selected for the study we found few really distinctive characters whose systematic value was absolutely confirmed by the phylogenetical analyses, namely an extremely elaborate shape of the trigger, an equally elaborate shape of the basal plate and a clear markedness of a second ´American´ appendage, hereby fulfilling the second criterion of homology (Remane, 1956). Where the triggers and basal plates and second appendages are weakly shaped they have no such distinguishing effect, as well as tissue thickenings in some places.

Based on this experience we propose generally for the morphological grouping to select characters according to the following aspects: they must be characters of shape and must be typical of a certain group that is to be investigated, e.g. genus, family, order. They should be rather rare than frequent, rather discrete than continuous, and rather obligatory than facultative. From such characters as few as possible should be taken for the grouping, contrary to previous methods of grouping based on numerous characters (see above: Andersson 1998).

4.4.3 Discussion of evolutionary pathways

In the maximally parsimonious tree of Prince and Kress (2006) (Fig. 4.10) comprising five big clades the first split divides up the family into the basal Sarcophrynium-clade and four others, namely the Calathea-, Donax-, Maranta-, and Stachyphrynium-clade. The early lineage consisting of *Sarcophrynium* as well as the sister-genera *Hypselodelphys*/*Trachyphrynium* and *Megaphrynium*/*Thaumatococcus* is consistent with our morphological findings (Africa II, Fig. 4.4) showing striking synapomorphies - compared to the *Cannaceae* - in the shape of a staminodium cucullatum with a rolled-up trigger and a basal plate that is brim-shaped and extended in a flag-like fashion (the ´bilobed trigger´ of Andersson, 1998). The next split detaches the Calathea-clade; herein the Calathea-II-clade is in accordance with our America II (Fig. 4.8) with *Calathea*, *Ischnosiphon* and *Pleiostachya* and with the exception of *Haumannia* (Africa III, Fig. 4.5) which is in a basal position here (see below). Unfortunately, the Calathea-I-clade of Prince and Kress (2006) is not covered by our experimental plants while most of our *Calatheas*, such as *Calathea veitchiana*, C. *picturata*, C. *microcephala*, C. *albertii*, C. *lietzei*, and C. *cylindrica* are not included in their study so that we have only *Calathea crotalifera* in common and must refer our results merely to the Calathea-II-clade in the phylogenetical tree. Both together, the Sarcophrynium-clade and the Calathea-clade, share the rolled-up trigger-appendage which appears to be the symplesiomorphic state of it, in contrast to the entirety of the remaining three clades that look more derived.

The next split divides the Donax-clade from the two remaining. In this clade, which is partly consistent with our Asia type (Fig. 4.2), we find as expected the genera *Donax* and *Phrynium* and *Schumannianthus dichotomus* and surprisingly the genus *Thalia* (America III, Fig. 4.9), which is nowadays American as well as African, in a basal position (Fig. 4.10). The last division is continuous right through the Maranta- and Stachyphrynium-clade. In the Maranta-clade the Hylaeanthe/Maranta-group can be comprehended after our morphological findings, namely very simplified and flat trigger-appendages (America I b, Fig. 4.7) and the membership of *Koernickanthe,* which is missing in the tree, is morphologically well-founded (see also Andersson and Chase 2001). To a more basal group in the Maranta-clade belong *Schumannianthus virgatus*, which we have not investigated, and *Halopegia,* that occurs in Africa and some Asian habitats. *Halopegia* is conspicuous for its very apomorphic ´deep´ trigger with a tissue swelling instead of a basal plate that tempted us to include the genus first of all in our Africa I (Fig. 4.3). Sister group to the Hylaeanthe/Maranta-group is the

Ctenanthe/Stromanthe/Saranthe-group which – with the exception of *Saranthe* that we have not investigated – is in accordance with our America I a (Fig. 4.6) including *Myrosma* which is missing in the tree and whose membership is morphologically justified (see also Andersson 1998, and Andersson and Chase 2001). All these genera in the Maranta-clade are in fact American. The remaining Stachyphrynium-clade divides itself in two lineages, namely the African Ataenidia/Marantochloa-group, that shares a synapomorphic ´deep´ trigger and loss of the basal plate and corresponds with our Africa I (Fig. 4.3) - with the exception of *Halopegia* - and the very simplified Stachyphrynium/Afrocalathea-group which is in accordance with the second part of our type Asia (Fig. 4.2), except that *Afrocalathea* is African.

Considering the assumed course of the development given in the parsimonious tree of Prince and Kress (2006) (see Fig. 4.10) it turns out that the two first clades, namely the Sarcophrynium-clade and the Calathea-clade, are those that are conspicuous for their lengthwise rolled-up trigger-appendages which we have named particularly ´elaborate´ triggers. The rolled-up shape of triggers appears to be a primal character shared by an African and an American group of the family, a symplesiomorphy. When we start from the assumption that the ancestral area of the family is Africa (see also Andersson 1981 and Andersson and Chase 2001) then the American Calathea-clade must be the next relations. While Africa is obviously a centre of diversity of genera, some of which are even monotypic or very poor in species (*Thaumatococcus*, *Trachyphrynium*, *Ataenidia*, *Afrocalathea, Halopegia*) the size of the Calathea-clade reflects an enormous radiation resulting in some 300 species of *Calathea* alone (Andersson and Chase 2001). So it suggests itself that a ´primal Calathea´ came from Africa to America. The other American genera may have secondarily ´simplified´ their cucullate staminodes while the lineage *Ctenanthe*/*Stromanthe*/*Myrosma*/ (*Saranthe*), our America I a, still retains partly the old characters in the shape of small ´second appendages´ und more or less rolled-up triggers (Fig. 4.6). The group *Maranta*/*Hylaeanthe*/*Koernickanthe*, our America I b, is most strongly derived and simplified with flat triggers and without second appendages (Fig. 4.7) where according to the tree also *Halopegia* belongs. Also the group *Ataenidia*/*Marantochloa* (Africa I, Fig. 4.3) appears secondarily simplified in comparison with Afrika II (Fig. 4.4). This may make our erroneous assignment of *Halopegia* to Africa I, in particular considering a swelling at the trigger, understandable. Both groups, America I b and Africa I, appear to be the most strongly

advanced each in their continent and are therefore – at least as regards the morphology of the cucullate staminodes - very similar.

The last section is the Asian (Fig. 4.2) which is also twice secondarily strongly simplified, namely in the Donax-clade and the Stachyphrynium-clade in which likewise the cucullate staminodes are very similar to each other in their simplicity. Here the basal position of *Thalia* in an Asian clade is interesting – if with little support (Prince and Kress, 2006) – it seems to connect all three continents (see below). Despite the doubtful situation of *Thalia* it seems to be cogent to acknowledge the lengthwise splitted trigger-appendage as a strong apomorphy to the genus alone.

Very interesting is also the basal position of *Haumannia* (Africa III, Fig. 4.5) in the Calathea-clade (Fig. 4.7). Like that in *Thalia* the hooded staminode in *Haumannia* is morphologically very peculiar in displaying even two apomorphies, such as a completely missing basal plate and a doubly folded trigger-appendage. *Haumannia* that is actually endemic to Africa appears morphologically African and genetically American and does not look actually like our virtual ´primal Calathea´ with a rolled-up trigger. But we do not know how strongly derived the contemporary types Africa II and III and America II look nowadays in comparison to their ancestral form. Also the modern *Haumannia*, basal though its position in the Calathea-clade may be, appears very derived. It may be secondarily simplified in a way similar to the *Marantochloas* and *Ataenidia* as regards the ´swollen´ trigger after a possible re-migration from America back to Africa in a secondary dispersal event. This ´modern African simplification´ might be an adaptation to certain African pollinators, involving perhaps a simpler pollen transfer mechanism, which we cannot prove here. The position of *Afrocalathea*, which is endemic to Africa, in the Asian Stachyphrynium-clade might be interpreted either as an example of a convergent development in both continents due to the aforementioned mutual dependence of plants and their pollinators or as a result of a secondary dispersal event, too. Secondary migration must also be suspected for *Halopegia,* which is in the Maranta-clade, but occurs in African and Asian habitats and for *Thalia* that occurs in America and Africa (see also Andersson 1981).

Altogether, we are in agreement with previous authors (Andersson, 1981 and Andersson and Chase, 2001) on the conclusion that most probably the ancestral area of the family is Africa. Thence a ´primal Calathea´ may have settled in the New World – whether prior to the continental drift or after (see also Andersson and Chase, 2001) cannot be proved here – giving rise to an enormous radiation of species. The last migration is most likely the one to Asia

where the cucullate staminodes developed to simple modern forms without any exception found in this study. When looking at these assumed derivations one must always allow for the interaction between flowers and pollinators which we know very little about at present. Therefore, ecological investigations and experiments on the trigger-mechanism are doubtless the most important in the next time.

5. General conclusions

All results of our investigations taken together meet in the conviction that the hypothesised key innovation in *Marantaceae* can be confirmed and that it lies in the very close cooperation of the style and the cucullate staminode, ranging from their mutual development in bud to the hydraulic tissue of the style to the peculiar shapes of the cucullate staminodes.

In *Marantaceae* the style is thick and fleshy and consists of so many cell layers that under ´straitjacket-conditions´ it can be tensioned for the movement and during ageing a tissue of hydraulic function can be established. Contrary to this there is in the sister group *Cannaceae* a thin and flat petaloid style (Petersen 1981). Although a tendency to an increased porosity of the style parenchyma is already laid out in *Cannaceae* the style tissue cannot make use of that feature in the service of pollination.

The cucullate staminodes in *Marantaceae* with their unique ´bauplan´ and strikingly divers appendages are able to keep a firm hold on the style prior to a pollinator´s visit until the opportune moment for pollen transfer; the maturity of the style must be on one hand sufficient to perform the full movement and on the other hand not over-mature because a wilting style could not be released any more. And regarding the diversity of the trigger-appendages it appears very likely that only particular pollinators can trigger off the movement in a certain genus or species of the family – other insects may only visit the flowers without pollinating - so that through the cucullate staminodes the styles are probably ´detained´ to wait for the best-adapted pollinator, so as to avoid hybridisation. In summary, the cucullate staminodes have the task to prepare the style for the movement, to control the time of pollination, possibly to select pollinators, and they are supposed to have a certain share in the regulation of self-pollination versus cross-pollination (Hildebrand 1870, Eichler 1884, Schumann 1902) And we feel certain that they also control the release of the movement (see below). In comparison to this in the sister *Cannaceae* there is in the homologous position of the cucullate staminode a simple and flat staminode that does not enclose the style and can have none of the aforementioned functions during pollen transfer. The great precision of the pollen transfer in *Marantaceae* is probably also supported by the callose staminodes of the inner androeceal whorl which we have not investigated in this study. As for the evolutionary ´success´ of this precision, it appears likely that it lies less in the multiplying of individuals because despite the elaborate pollen transfer seed set through cross-pollination is generally low (A. Ley, pers.

com.) and at least 44 species (8% of the family) help themselves with autogamy (Kennedy 2000) and vegetative propagation through rhizomes. The main aspect of success may rather lie in the avoidance of hybridisation and, in consequence, in the ecological niche-finding. If this could be confirmed, we would eventually be convinced that the great radiation of the family was in fact an adaptive radiation as a result of a key innovation.

As for the release, in view of the size of the family we must allow for deviations from the general scheme anyway. But at least, we tried to approach the physiological contributions to the trigger-mechanism in the style-staminode-complex. For this, several electrophysiological experiments were carried out in styles of *Maranta leuconeura* and in styles and cucullate staminodes of *Calathea veitchiana* (unpublished data). These experiments turned out to be extremely difficult. What we expected was at least a measurable alteration of a membrane potential in one or both organs as a result of style release by hand manipulation. Unfortunately, the release of the movement always resulted either in a break of the electrode itself or – through the mechanical inertia of the electrode - in a tear of the style tissue so that the expected local potential changes or even conducted action potentials could not be detected. We do not know at present how this problem might be solved by a better technical method. We succeeded after all in detecting a resting potential of about –160 mV in both organs and species (unpublished data). This, however, is not very meaningful because any plant or animal cell membrane shows a resting potential. Therefore, the physiological part in the release must remain open for the time being and subject to conjectures.

What remains to be done in future are observations of plant-pollinator-interactions at the native habitats so to shed more light on the ecological aspects of the adaptive processes in the family. The hypothesis concerning the avoidance of hybridisation should be tested by crossing experiments and the examination of chromosome numbers in the family. Furthermore, the share that other floral organs apart from the cucullate staminodes have in the precision of the pollen-transfer, such as the callose staminodes and maybe even the styles themselves, should be looked into.

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