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MORPHOLOGY OF THE PISTIL IN MALVACEAE-URENEAE

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SUMMARY

The puzzling fact that the pistils of *Malvaceae-Ureneae* have five locules, but ten, instead of five, stylar branches, is made clear by a study of their development.

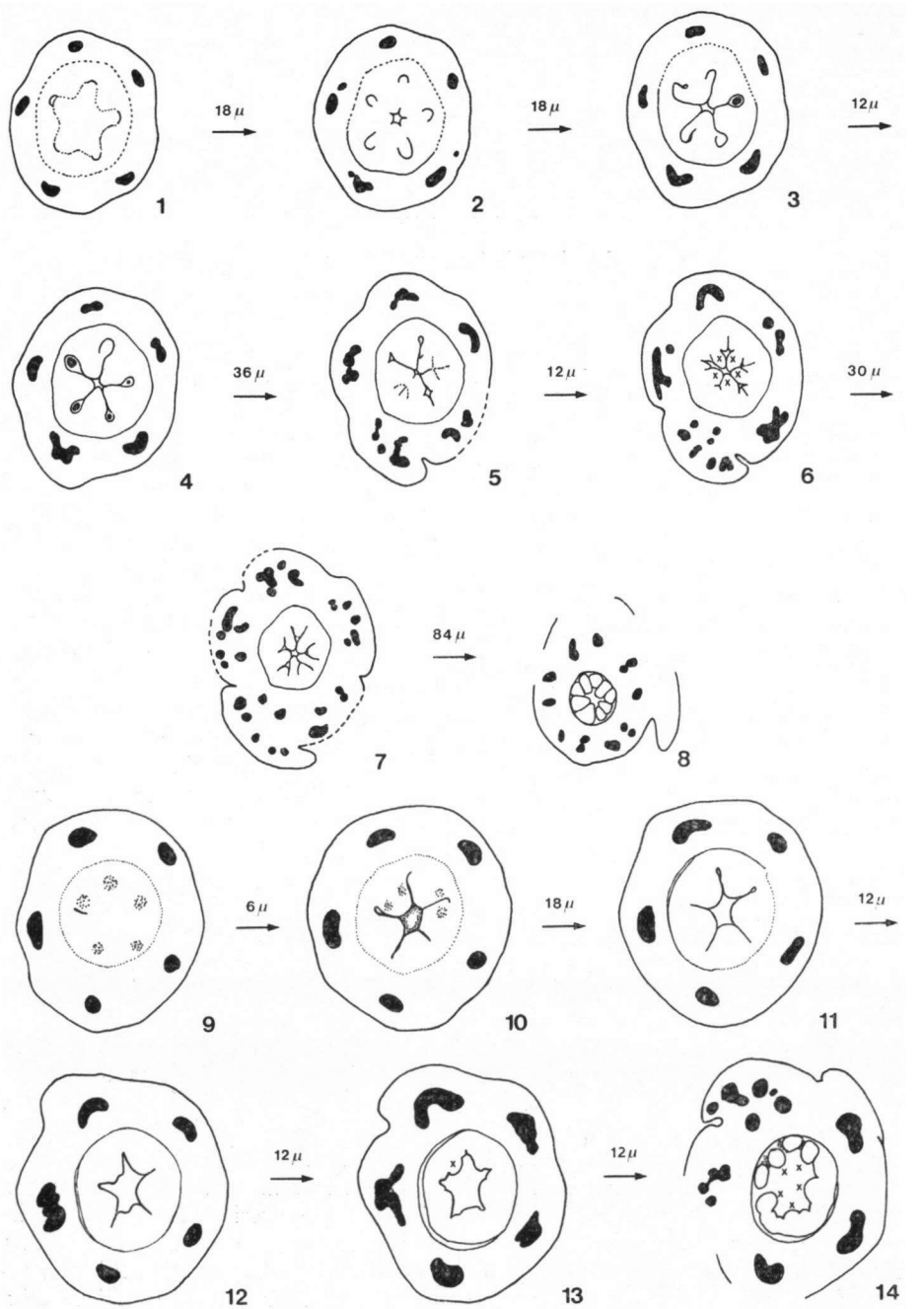
INTRODUCTION

Those *Malvaceae* that have stylar branches twice the number of carpids are grouped together by Schumann (1893) in the *Ureneae*. Normally, there are five locules in the ovary, and there is only one style, which divides into ten, instead of five, stigmatic branches. According to Schumann, in all *Pavonia* species ten carpids develop, of which five are retarded in their development. Moreover, Schumann assumed that the changing position of the carpids (antesepalous in some species, antepetalous in others) depends on whether the antepetalous or the antesepalous carpids are retarded. However, I found (1966) that the location of the carpel primordia in *Malvaceae* depends on the degree of contortion of the petal-androecium system at the time of origin of the carpel primordia.

Probably, Schumann took his knowledge of the development of the carpids from Payer (1857). Payer described and figured the primitive composition of the *Pavonia* pistil. According to him ten protuberances arise and soon become elevated on a common membrane. Whereas at the base of five of them, and on their inside, a locule is formed, the other five remain sterile. Payer convincingly dismissed the assertion of Duchartre (1845) that five carpels have two styles each by '*dédoublement*'.

According to Saunders (1936) the formation of ten separate styles is proof beyond question of her theory of carpel polymorphism. According to this theory the syncarpous pistil consists of five 'fertile carpels' (the septs and placentae of the classical theory) and five 'sterile carpels' (the locules and their walls of the classical theory). Whereas normally only the 'sterile carpels' have styles, in the case of the *Ureneae* also the 'fertile carpels' have. However, the theory of carpel polymorphism is based exclusively on the pattern of the vascular bundles, and not at all on developmental facts (Van Heel, 1969). The fact that locules are formed, but do not further develop, at the bases of the second whorl of carpel primordia in *Urena lobata* and *Pavonia praemorsa*, invalidates her argument.

As ten carpel primordia are initiated, it is evident that ten stylar components and stigmatic branches result in the open flower. However, the question remains how in the open flower the ovary is built up of five carpels, seemingly without any trace of the other five. To answer this question, a study of the development was made, which is here reported, by means of direct photography of early developing stages combined with microtomy. This combination enables a good visualization of the development of flower structure.



1—8: *Pavonia spinifex*, series of c.s. of a young pistil, ×30. 9—14: Ibidem, younger, ×45.

MATERIALS AND METHODS

The species studied are 1. *Pavonia praemorsa* Cav. (cult. Botanic Gardens, Leiden); 2. *Pavonia spinifex* Cav. (cult. Bot. Garden, Groningen); 3. *Pavonia hastata* Cav. (ibid.); 4. *Malvaviscus arboreus* Cav. (ibid.); 5. *Urena lobata* (collected in the field near Bogor, West Java*). Fixation of all material was either in FAPA, or in CRAF.

Microtome sections were stained as follows, 1. Safranin (CHROMA), 1% sol. in 50% alcohol, 1.5—2 hrs; 2. Rinse in water, 1 min.; 3. Alcohol 30%, 1 min.; 4. Astra Blau FM (CHROMA), 0.5 g. in 2% tartaric acid, 1 min.; 5. Rinse in water, 1 min.; 6. Aceton, 1 min., 3×; 7. Xylol, 3×; Depex (GURR). This schedule was adapted from Maácz and Vágás (1961) and used for thin (6 μm) sections of the primordial stages**. Its result, especially for the cell walls, is superior to the conventional safranin-fast green staining.

The photography of the primordial stages was as follows: 1. Prepare the objects with the aid of fragments of razor blades mounted on needle holders, leaving enough of the stalks to make handling easy; 2. Stain in a solution of JKJ in 50% alcohol; 3. Prior to photography, mount the objects under water on insect needles. The needles are put in portions of paraffin, fixed along the inside edges of petri-dishes; the whole is painted dull-black to prevent reflections. The stain keeps long enough (1—2 hrs.) to allow photography; 4. For photography the LEITZ Ultropak system with immersion caps was used, mounted on an Ortholux microscope equiped with an automatic camera. A KODAK Panatomic X film was used with a yellow-green filter, valued at 18 Din, and developed in Ultrafin (TETENAL) 1/20 for 8 minutes at 20°C. I think with Sattler (1968 and 1973) that this is a quick, relatively cheap method, which especially brings well forward the cell walls. However, the method is of limited application because of the low depth of focus.

THE DEVELOPMENT OF THE PISTIL

The development is marked by two factors, which are decisive for the adult structure: 1. the ten primordia originate as two successive whorls of five, 2. in the second whorl the development of an ovary is limited according to the species, and ovules are not formed.

In the following the primordia will be called the carpel primordia in accordance with the carpel theory, although possibly the *Ureneae* do not fit the theory, as will be discussed later. By this terminology a whorl of five fertile carpel primordia is followed on the apex by a whorl of five sterile carpel primordia (it is difficult to distinguish an outer and an inner whorl). The origin of the first whorl is shown for *Malvaviscus* in photo 1; the pistil primordia are 1/5—1/4 mm in diameter at this stage. Prior to photography, the young petal-stamen tubes, surrounding and partially covering the pistil primordia, were removed. Along the periphery of the apex five carpel primordia, with a triangular to trapeziform outline, arise, extending laterally. They remain separated by less active radial strips which are about two cell rows wide. It is only after the less active strips of the apex have become higher and broader (3—4 radial cell rows), that they begin to bulge forward, marking the origin of the sterile carpel primordia. The result is that ten less active strips between ten carpel primordia remain along the periphery of the apex (photo 2). Consequently, the fertile carpel primordia have a lead in development. They reach deep into

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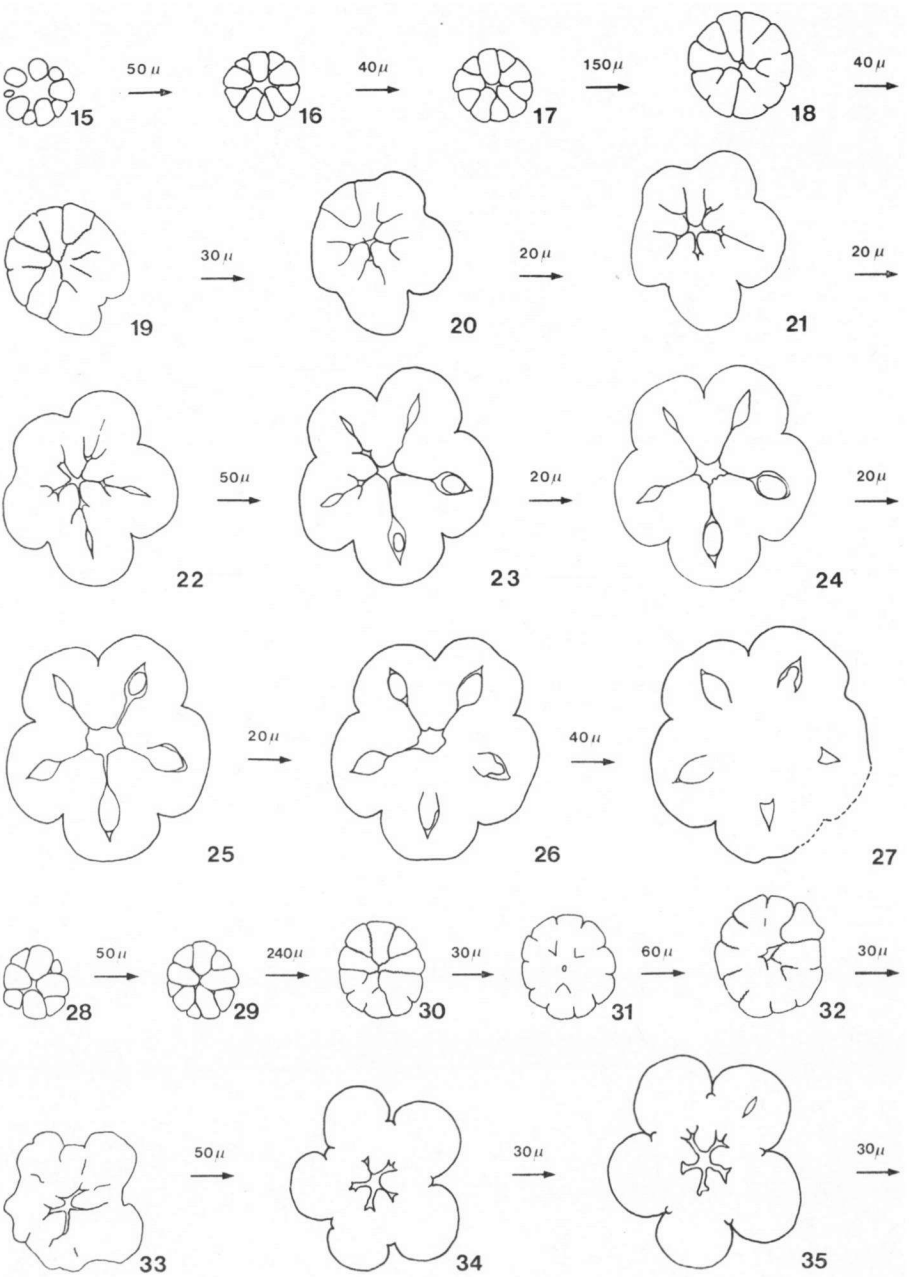
**) My thanks are due to Judith Kramer-Wiltink for taking care of this part of my work.



15—27: *Pavonia praemorsa*, series of c.s. of a young pistil, ×45. 28—35: Ibidem, slightly older, ×45.

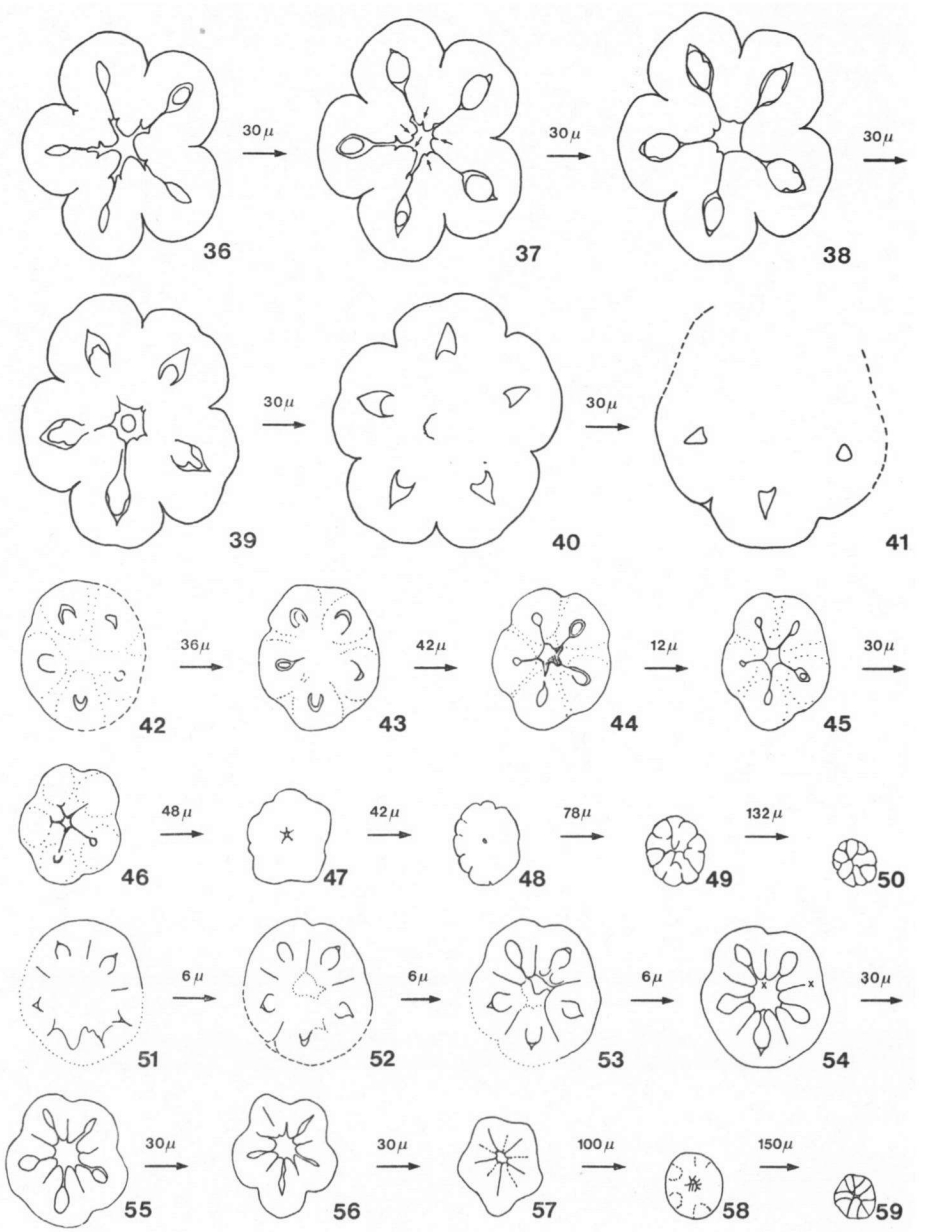
the apex at the onset of the sterile carpel primordia, and may already show the first signs of locule formation. This is the reason why (in adult stages) the proximal pistil part appears to be formed by the fertile carpels only, albeit separated by apex tissue which passes upwards into the tissue of the sterile carpels.

The growth of the fertile and sterile carpel primordia proceeds towards the central parts of the apex, while at the same time the apex itself becomes slightly broader and higher (photo 3, figs. 9—14. Sterile carpels at x). It is clearly shown in photo 4 how the bases of the sterile carpel primordia are situated higher on the apex than those of the fertile carpel primordia. The latter become much wider at the base, occupying the corners of a now slightly pentagonal pistillary primordium. Radial regions of the apex which are situated medianly in front of the fertile carpel primordia, do not take part in the inward development of these primordia. Therefore, the inward growth takes the shape of two radial lateral flanks of a carpel primordium enclosing a locule (photos 4 and 5). The pistil primordia are about 1/3 mm in diameter at this stage. The top part of the primordia overarches the locule and forms its roof. Growth in width forms the back of the locule. The sterile carpel primordia are without prominent locule formation, however. In *Pavonia spinifex*, *P. hastata*, and *Malvaviscus arboreus* locule formation is absent or almost so, in *Urena lobata* it is slight (photo 11), and in *Pavonia praemorsa* small locules are evident (photo 12, fig. 37 at arrows), but they do not develop much further.

The activation of the ten radial strips of the apex, which remain between the ten carpel primordia, is very important for the final structure of the pistil. From that phase onwards the primordia appear as laterally connected, except by their top parts and inward growing parts (photos 3 and 4). In this way the ovary wall originates.

The centripetal growth of the flanks of the fertile carpels needs special attention. Growing inwards, the flanks of adjacent fertile carpels in most cases become connected by the development of a 'bridging' tissue in front of the sterile carpel primordia (photo 6). However, in *Pavonia praemorsa* and in *Urena lobata* adjacent flanks remain separated by 'commissural' slits for a long time (figs. 51—58 at x), until the overall secondary fusion at the end of the development. Upwards these slits pass into the ventral slits of the sterile carpels, which have a limited locule formation in these two species (photo 11, 12, and 14). Also in *Malvaviscus* adjacent flanks were found not to reach one another in all cases (figs. 42—50). As a consequence of the growth of a bridging flank between fertile carpel primordia, the centripetal growth of the sterile carpel primordia is limited at this level of the young pistil, their position remains more to the periphery; they are seemingly carried upwards by the further growth of the fertile carpels. Also as a consequence of the bridging flank, the regions of the upper part of the ovary in most cases are formed by the fertile carpels only. The same is valid for the proximal parts of the style. This also means that the tissue which passes upwards into the sterile carpels (stylar components) can, at anthesis, be recognized only if the development is well known, the more so because of the late extension of the locules. It is situated slightly central to the sinus between two locules, as indicated for *Urena* (photo 14 at x). In the other species, which have a minor sterile locule formation, this zone is still less evident, as shown for *Malvaviscus* (photo 17).

In later stages the flanks of each carpel touch each other and fuse secondarily. However, long before that stage, the ovule originates exactly on the strip of the apex which was not occupied by the growth of the flanks of the fertile carpel. Photo 8 shows half of a fertile carpel primordium of *Pavonia spinifex*, as photographed after a median longitudinal section was made of a pistil primordium such as shown in photo 7. One of the flanks of the fertile carpel is seen to have grown as far inwards as the region in front of an adjacent terile carpel. The ovule primordium originates more outwards, however, unconnected



36—41: *Pavonia praemorsa*, continuation of figs. 28—35. 42—50: *Malvaviscus arboreus*, series of c.s. of a young pistil, ×30. 51—59: *Urena lobata*, series of c.s. of a young pistil, ×45.

with a '*Querzone*', and most probably unconnected with the carpel flanks. The ovule originates as it were at the bottom of the locule of the fertile carpel, where a locus on the apex remained free. Its development starts by cell division in the second and third layer of cells. In this way the ovule becomes axillary to an enveloping bract. During later stages of ovular growth, microtome sections never reveal any regular 'cellular continuity' with the flanks of the carpel on cross-section; the ovule cells invariably 'come from low down'. Also in later stages the ovules always appear exactly in the median radii of the fertile carpels, if the ovary locules are of equal size and form. It is only when the locules are unequal that some ovules appear contiguous to one of the flanks, suggesting a 'submarginal' placentation on cross-section.

The roof of the ovary and the style are formed as a result of the continuing centripetalous and overarching growth of the sterile and fertile carpel primordia, followed by their adherence and ultimate fusion in the middle. Photo 8 makes clear how the closure and style formation takes place when the inward growing rims of the fertile and sterile carpels will contact one another in the middle. The composition of the roof of the ovary or the proximal parts of the style depends on the measure of centripetal growth of the fertile carpel parts. Usually, this growth is quite considerable, as described above. In that case the proximal parts of the style consist of five fertile carpel parts in the middle and five regions of tissue leading to the sterile carpel parts in the periphery. The distal stylar parts, however, consist of five sterile carpel parts which have grown in centripetal direction, and fertile carpel parts in the periphery (photo 15; figs. 6 and 7. Sterile parts at x). Photo 13 and fig. 23 show the upward transition to the well developed tissue of the sterile carpels in different sectors of a young pistil of *Pavonia praemorsa*. Thus, a style consisting of ten radial components begins to be formed when the pistil primordium is about $\frac{1}{2}$ mm in diameter and height (photo 9). The extreme growth in length of the style, as compared with the ovary, is shown when the primordium is about $\frac{3}{4}$ mm high (in photo 9). Externally, the composition of ten parts is evident, and five shorter stylar parts are (in this case) on top of the fertile carpels. Internally, however, fusion has already started, in a way as described by Baum (1949) and by Boeke (1973). This fusion is marked on cross-section by a zigzag system of cell walls at the sutures (photo 16; figs. 18, 19, and 32). Distally, the constituent parts remain free as stigmatic branches.

In the lower part of the style five strands of pollen tube transmitting tissue are formed by division of the epidermal cells of the flanks of the fertile carpels at those places where they touch and fuse in the median radii of the fertile carpels. The pollen tube transmitting tissue extends upon the funicles of the ovules. Up in the style ten transmitting strands leading into the stigmatic branches are always present (photo 18). All strands fuse into one cylinder of pollen tube transmitting tissue in the middle of the style during the final stages of development. In *Pavonia praemorsa* and *Urena lobata* one to five alternating strands can be formed which terminate blindly downwards, in addition to the usual five strands of pollen tube transmitting tissue. No doubt this is due to the initial formation of locules of the sterile carpels in these species. In *Malvaviscus* this was also observed occassionally.

THE VASCULAR BUNDLE PATTERN IN THE PISTIL

Saunders (1936) presented descriptions of this pattern. Unfortunately, however, she created her own terminology, in connection with the theory of carpel polymorphism, which makes her descriptions rather unintelligible. As intensive anatomical investigation would be needed, a detailed description lies outside the scope of this paper. A first knowledge of, and a comparison with, the more simple cases in the *Malveae*, in which all

carpels are fertile, would be necessary. Therefore a concise description is given here.

Immediately above the level of divergence of vascular bundles for the hypocalyx, the flower base is marked by ten, on cross-section U-shaped or later concentric, large vascular bundles, which are mostly arranged as an inner antesepalous and an outer alternisepalous whorl. The outer parts of the antesepalous main bundles diverge as the sepal median bundles. The outer parts of the antepetalous main bundles diverge as the sepal commissural marginal bundles and the combined petal-stamen bundles. The innermost flank parts of the antepetalous bundles move to the centre as ten pistillary traces, which are located in between sepal and petal radii. It is also possible that, above the level of divergence of the sepal and petal-stamen traces, a closed stele is reconstituted, which on cross-section is five-angled in the petal radii. Presumably, the one or the other possibility depends on the amount of apical growth intervening between the origin of the sepal and petal-stamen primordia on the one hand, and the carpel primordia on the other. If there is a lag between the two, a stele will be reconstituted, from which also ten gynoecial traces originate.

Each pair of gynoecial vascular traces, if taken as antepetalous pairs, divides into two carpellary dorsal bundles to the outside, two placental-stylar bundles to the inside, and, not always, two lateral carpellary bundles in the middle. The dorsal and lateral bundles ramify upwards; the more so as the ovary wall develops. Still more upwards they converge again and contact the stylar bundles. Each ovule receives a single vascular bundle which is connected by two traces with the pair of placental bundles.

From the above descriptions it seems that the stylar bundles are located as two lateral bundles for each stylar component belonging to a fertile carpel. However, it is evident that these bundles are shifted slightly sidewards, and are nearer to the boundaries between the fertile carpels and the tissue which upwards passes into that of the sterile carpels (photos 13 and 17). Upwards, these bundles form commissural bundles between the constituent parts of the style, which belong to the sterile and fertile carpels resp. In the style these bundles develop only after the lateral fusion of the components has eliminated the morphological boundaries between the two sets. It is observed that exactly in those regions where the fusion starts, a cell division process in the dermal and subdermal layers gives rise to commissural stylar bundles (photo 16). Upwards, each of these divides into two lateral bundles for two adjoining stigmatic branches (photo 18). In this way a supply of vascular bundles to the sterile carpel components is effectuated, which the sterile carpels could not receive directly from the base of the flower, by lack of well developed proximal parts.

In *Urena*, ten more gynoecial traces may arise from the antesepalous main bundles in the flower base (also noted by Saunders). They take up a location of placental-stylar traces of the sterile carpels. They occur only if the sterile carpels are developed to some extent.

DISCUSSION

This paper presents a case in floral morphology in which the study of development proves essential. With the discovery that ten carpel primordia originate in two successive whorls, the one fertile, the other sterile, the main cause for the aberrant final structure becomes evident. In *Urena lobata* (Endress, pers. comm.) and in *Pavonia praemorsa* a carpel of the second whorl occasionally can be fertile. This is in accordance with the fact that the carpels of the second whorl clearly have initial locules in these two species. A development of a second whorl of carpels, which together with the first forms one pistilary structure, is known for *Punica*, for *Siphonodon* (Croizat, 1947), and of course for the Navel orange. If,

in the evolution, capsular fruits with many seeds, as in the *Hibisceae* and in the *Malvales* as a whole, are considered primary, and fruits with many monospermic parts advanced, then the case of the *Ureneae* may represent a transitional state.

During the first phase of development the carpel primordia become trapezoid bodies by apical growth. Subsequent growth in thickness, which is centripetal and lateral, takes place by the overall formation of cell rows. The developing carpels surround a free apex. Regions of the free apex, situated laterally in front of the fertile carpel primordia, in centripetal direction join in with the apical growth, forming the flanks of the carpels. As this occurs over a free apex, which at the same time becomes broader and higher itself, a locule results medianly in front of the fertile carpels. This description is different from such descriptions as 'the margins of the carpels grow (bend) inwards', which is typological language with a developmental tongue and therefore false. The manner of development of the carpel primordia resembles most that of enveloping basal parts of leaves. In fact, the ovule primordia originate on the regions of the free apex that are enveloped by the carpels, not on the flanks of the carpels themselves, as described above. As a consequence the classical theory, holding that the ovules are placed on the margins of carpels in all Angiosperms, cannot stand here; there is no room for heterotopy in the theory. Also the carpels should be named by a neutral term, such as gynoecial appendage, or bract (cf. Sattler, 1974, 1975). It is possible that the ways in which the ovules are placed in a common pistillary structure are more plastic than admitted hitherto. In this connection the development of the pistil in other groups of the *Malvaceae*, such as in the *Hibisceae*, in which the gynoecial appendages have more than a single ovule, should be studied. Because of the variety in gynoecial structure in obviously allied groups, the *Malvaceae* are a promising family for comparative developmental study.

The joint development of the fertile and sterile carpel primordia to form one pistillary wall, is called congenital fusion in the field of non-developmental morphology. The developmental analysis of these processes has started only recently. An account was presented by Sattler on the Fourth Symposium of Plant Morphology at Strasbourg in March, 1977. In no case there is real fusion of organs. It is evident that the meristematic processes are different for various cases of 'congenital fusion'. In our case it may be surmised that a peripheral mantle of the floral apex is potentially active, but that first the activity is limited to two successive whorls of appendages. However, this has not been the subject of our present study. The 'bridging' meristem described above makes the flanks of two adjacent ovule-enclosing gynoecial appendages grow up together because of the meeting in alternate radii of the meristems that give rise to the flanks. Meristematic activity just extends to that region, resulting in an undulating (sinusoid) wall. On the other hand, in the median radii the fusion of the flanks is a real fusion process between two contiguous parts of organs (called 'postgenital' fusion). Also the formation of the style takes place by secondary fusion of the stylar components of both the ovule-enclosing and vacant gynoecial appendages. The problem of 'fusions' is intimately related with the problem of conservatism of the vascular bundle pattern in the changing structure. In our case a new structure is produced in which pollen can reach five ovules by way of ten stigmatic branches of the style. Correspondingly, the vascular pattern changes to give a new feature in the form of ten commissural bundles which, by simple division, supply both the normal five and the extra five stigmatic branches. Thus a new structure induces a new vascular bundle pattern, which is against the idea of conservatism.

It is necessary to find techniques for visualizing the complex patterns of development which take place on the floral apex. The Ultropak photography is one of the ways. The results should be complemented and checked by microtome sections. By combining both techniques a better insight may be reached.

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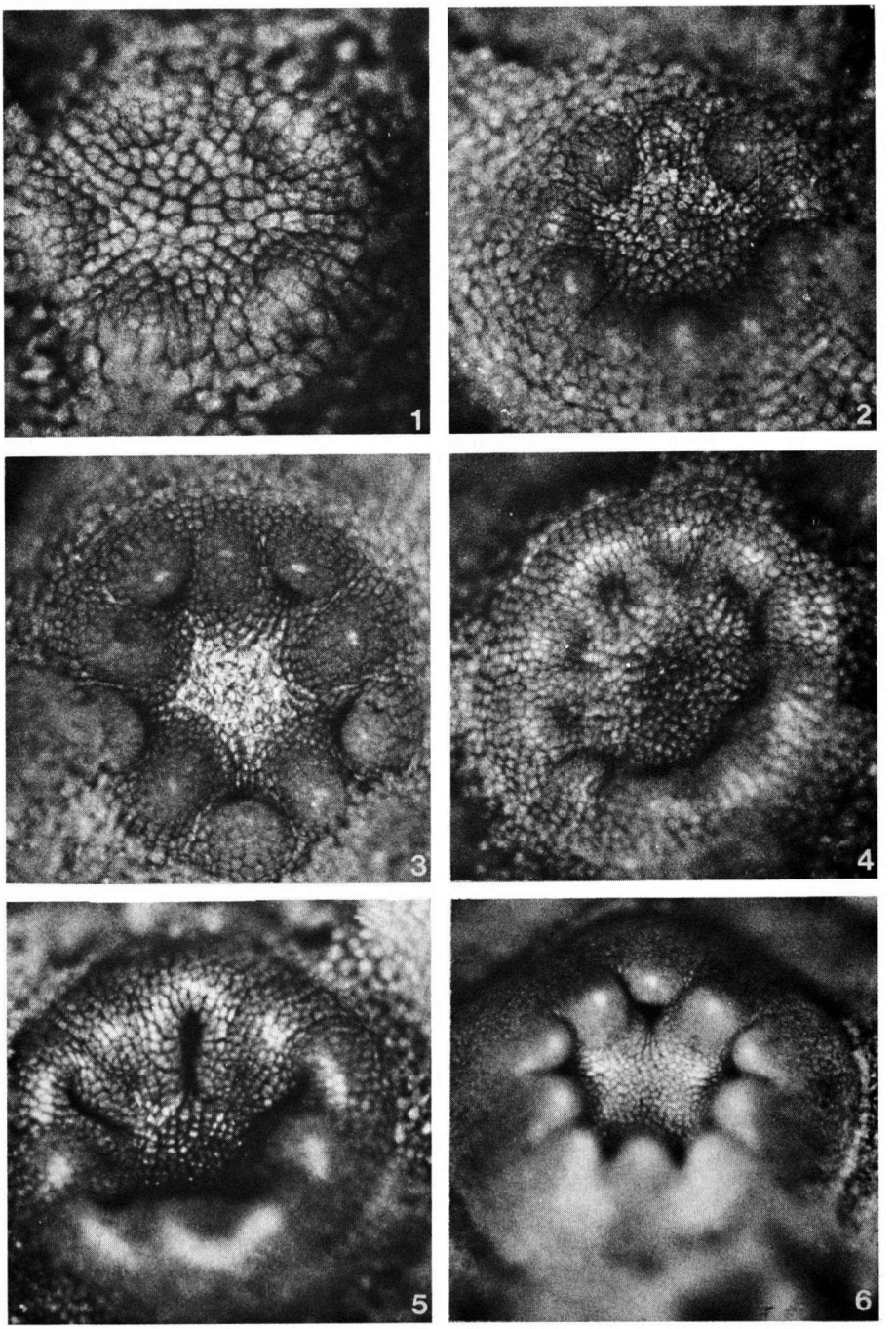
EXPLANATION OF PHOTOGRAPHS

1—3: *Malvaviscus arboreus*, pistil primordium, external view, ×150. 4: *Pavonia spinifex*, ibidem, ×150; 5: ibidem, ×180. 6: *Pavonia hastata*, ibidem, ×180. 7: *Pavonia spinifex*, young pistil, external view, ×150; 8: ibidem, median l.s. of fertile carpel, ×120; 9: ibidem, ×110. 10: *Malvaviscus arboreus*, young pistil, external view, ×90. 11: *Urena lobata*, pistil primordium, external view, ×180. 12: *Pavonia praemorsa*, pistil primordium, external view, ×150. 13: *Pavonia praemorsa*, cf. fig. 23, ×250. 14: *Urena lobata*, c.s. of centre of the pistil at anthesis, ×200. 15: *Pavonia spinifex*, cf. fig. 7, ×200. 16: *Pavonia praemorsa*, cf. fig. 32, ×400. 17: *Malvaviscus arboreus*, c.s. of centre of pistil at anthesis, ×150; 18: ditto, subdistal c.s. of style at anthesis, ×150.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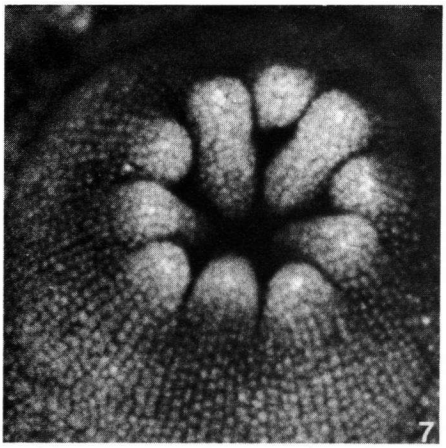
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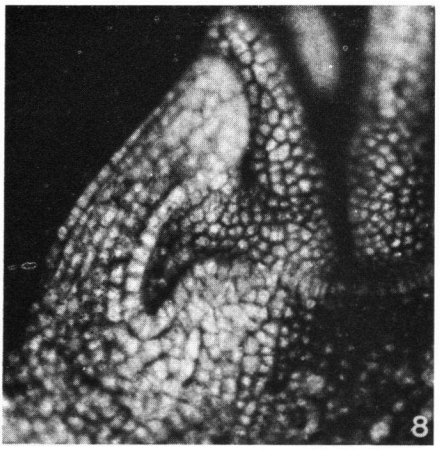
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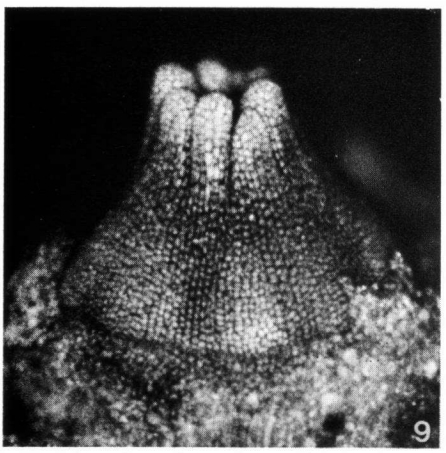
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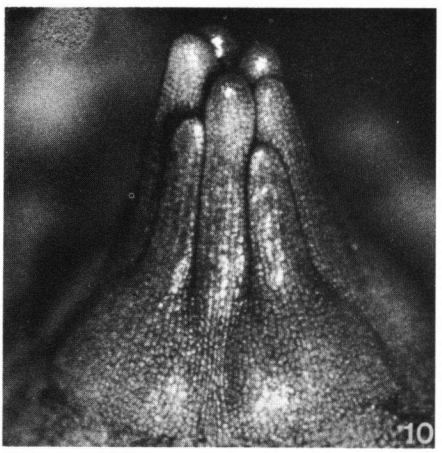
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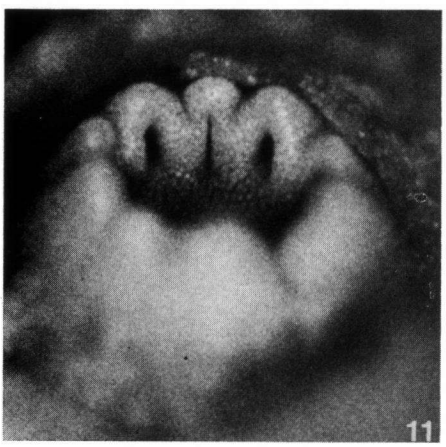
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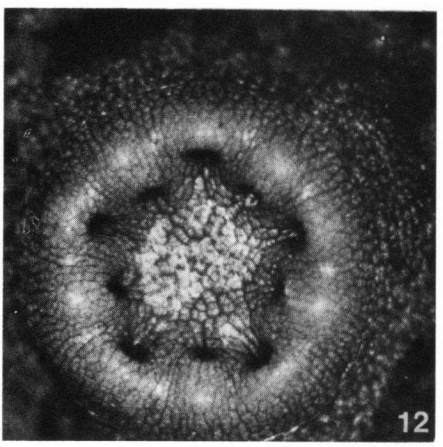
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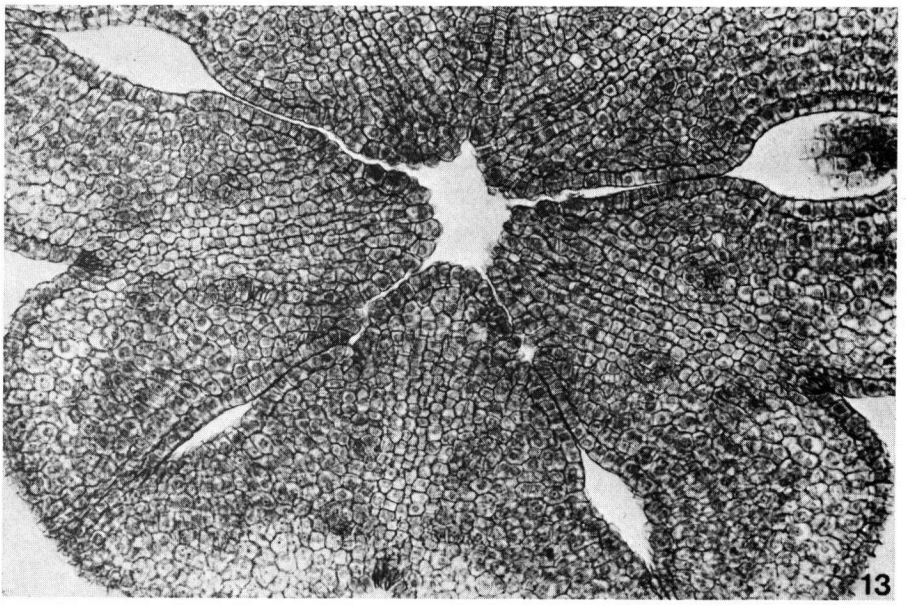
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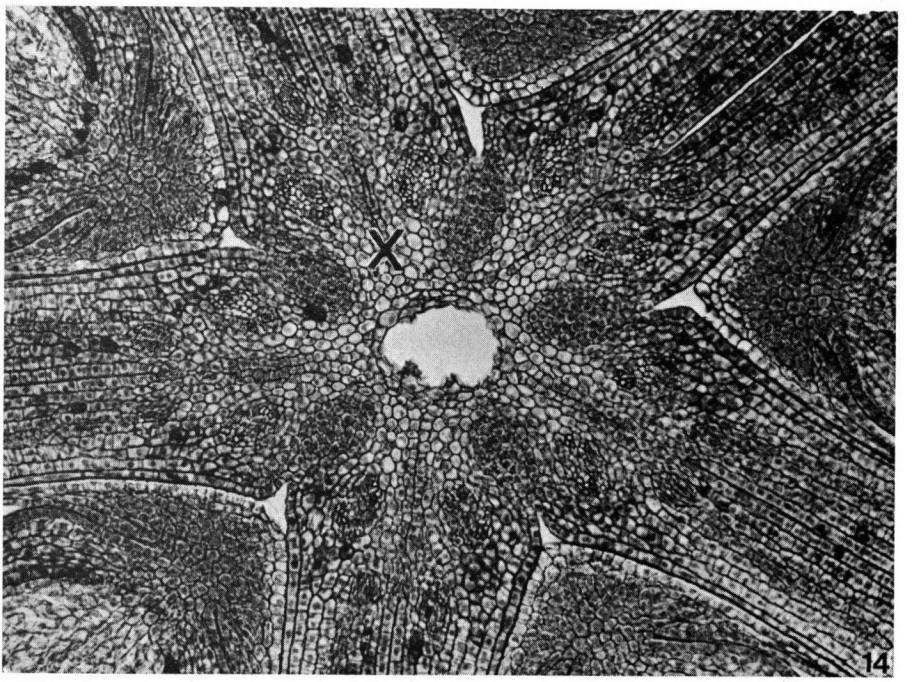
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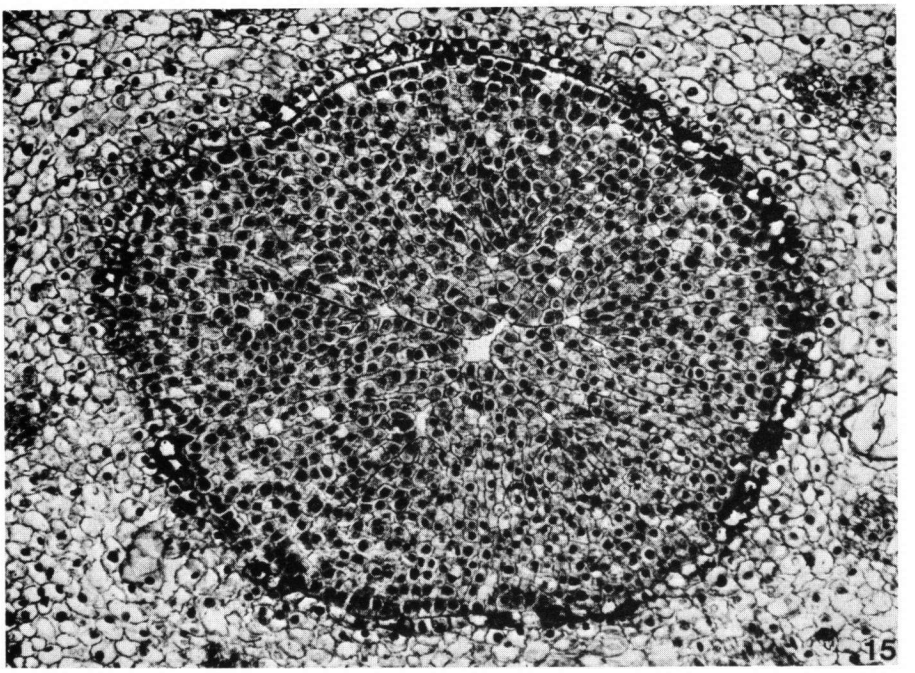
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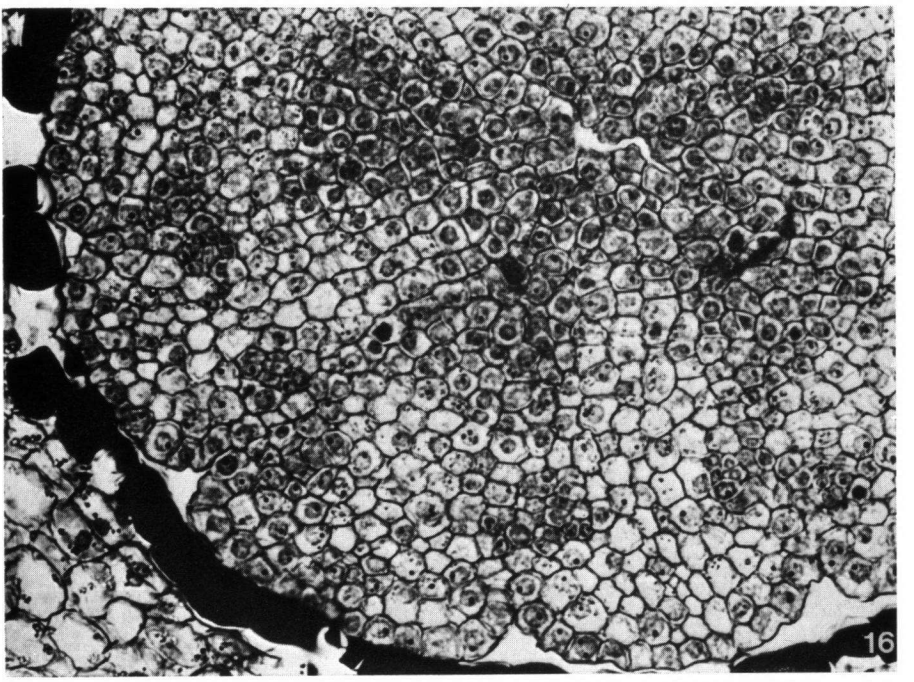
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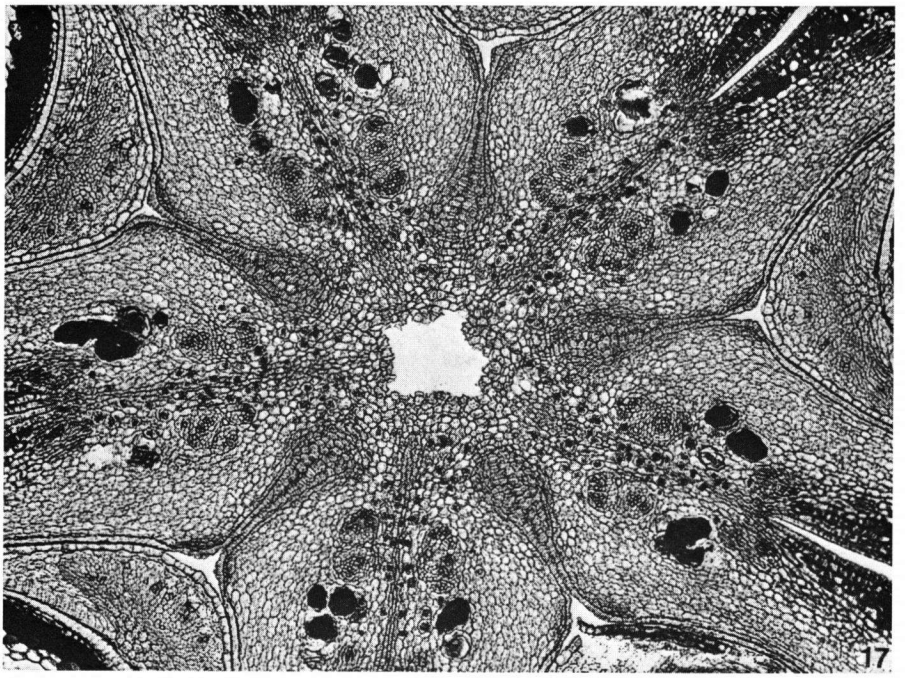
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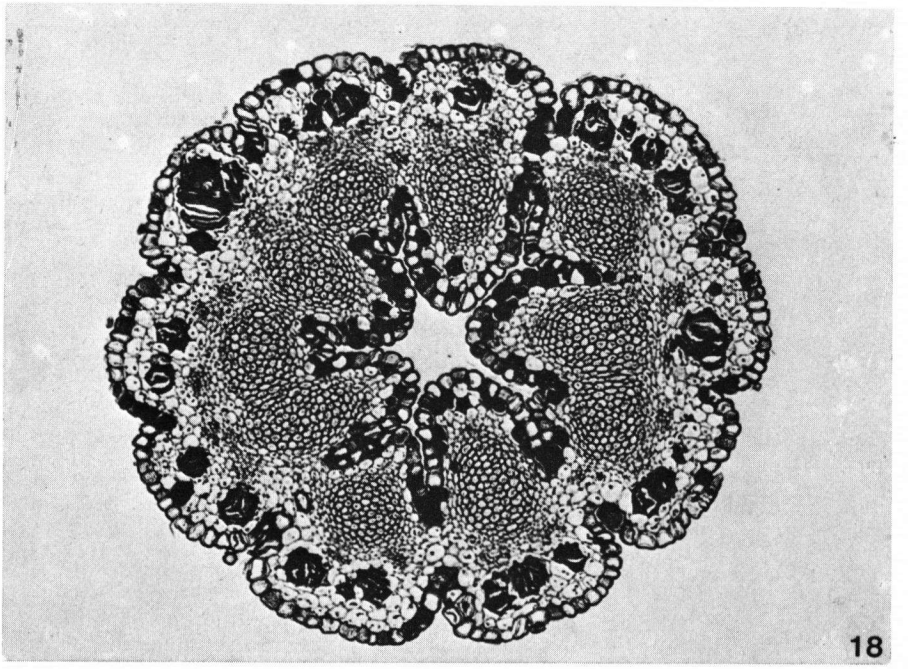
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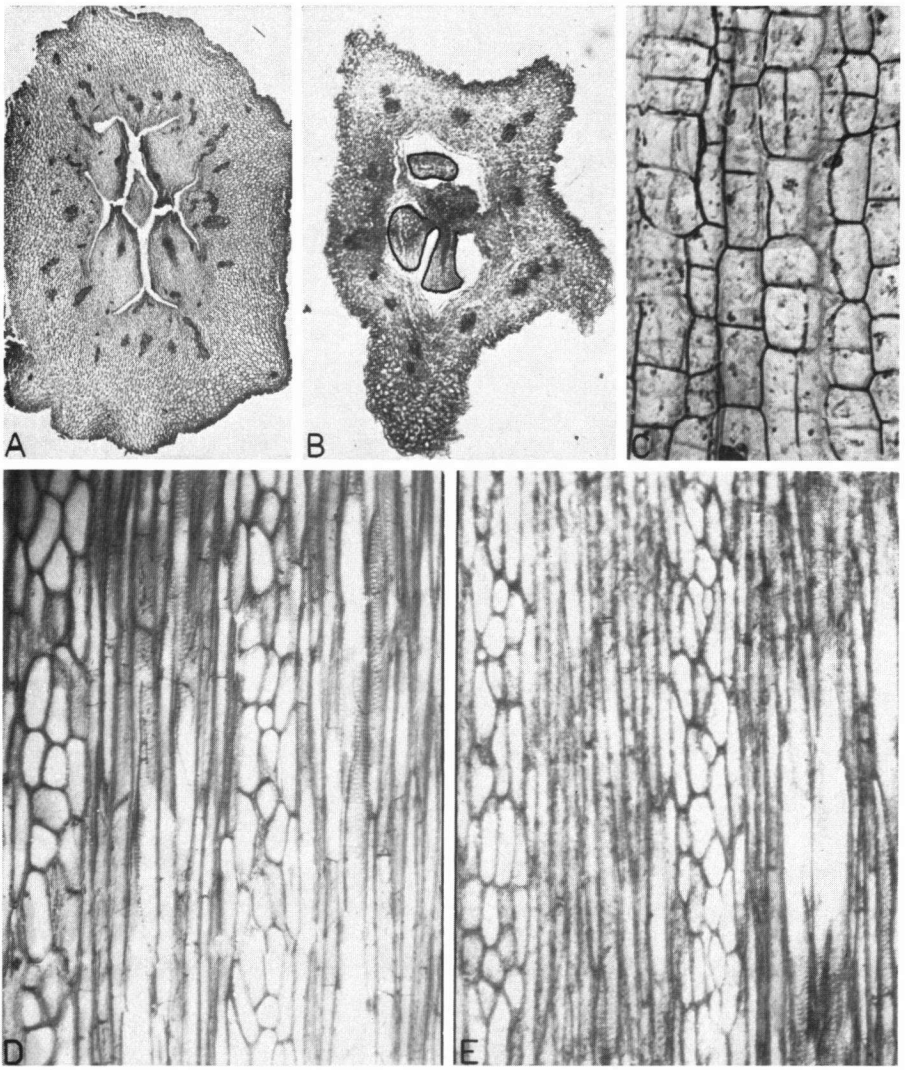

Plate 1 A. *Memecylanthus neo-caledonicus (Deplanche 413*, K), transverse section of flower, showing fleshy hypanthium (base of calyx, corolla tube, and stamens), ×20. — B. *Periomphale pancheri* (*Schlechter 15426*, L), transverse section of ovary, showing axile placentation (ovules outlined), ×75. — C. *Periomphale balansae* (*Balansa 2776*, K), tangential longitudinal section of stem, ×260. Casparian strips (darkly stained with safranin) of the endodermis form a net-like pattern. — D. *Memecylanthus neo-caledonicus*, tangential longitudinal section of secondary wood, showing tall rays, ×120. — E. *Periomphale balansae*, tangential longitudinal section of secondary wood, showing tall rays, ×120.