American Journal of Botany

Features of Dichotomizing Apices in Flagellaria indica (Monocotyledones)
Author(s): P. B. Tomlinson and Usher Posluszny
Source: *American Journal of Botany*, Vol. 64, No. 9 (Oct., 1977), pp. 1057-1065
Published by: Botanical Society of America
Stable URL: http://www.jstor.org/stable/2442161
Accessed: 23/08/2011 16:11

Amer. J. Bot. 64(9): 1057–1065. 1977.

FEATURES OF DICHOTOMIZING APICES IN FLAGELLARIA INDICA (MONOCOTYLEDONES)[1]

P. B. Tomlinson[2] and Usher Posluszny
Harvard University, Harvard Forest, Petersham, Massachusetts 01366

ABSTRACT

During vegetative growth aerial shoots of *Flagellaria indica* bifurcate regularly at intervals even though axillary meristems are absent. Dichotomy involves no change in the cytohistological organization of the apical meristem; the shoot apex simply enlarges in the plane of distichy, a median furrow appears, and two new equal centers of meristematic activity are constituted in a process of continuous growth. Dichotomy can occur at any stage in the plastochron, and the morphology of the leaf immediately below the fork may be modified under the influence of the two new meristematic centers. The first leaf on each of the new apices is always on the same side, so there is no mirror image symmetry. The suggestion that dichotomy is the result of precocious axillary branching is discussed but rejected.

Recent collections of vegetative aerial apices of *Flagellaria indica* L. (Flagellariaceae) allow successive stages in vegetative dichotomy to be documented, and the anatomy of apices during this process of bifurcation is now reported in detail. In an earlier account (Tomlinson, 1970) essential morphological features of this process of dichotomy had been established, with the prediction that a similar method of branching might occur in other monocotyledons. This prediction has been substantiated by a series of papers which document in greater or lesser degree this distinctive kind of vegetative branching. As summarized by Fisher (1976), examples are now known in the Palmae, e.g., *Chamaedorea* (Fisher, 1974), *Hyphaene* (Hallé and Oldeman, 1970), *Nypa* (Tomlinson, 1971), and in the Strelitziaceae, e.g., *Strelitzia reginae* (Fisher, 1976). Precocious branching, difficult to distinguish from dichotomy, has been recorded by Wilder (1974a, b) in certain members of the Alismatidae where it is characteristic of highly organized, usually reproductive, branch systems which are perhaps not comparable to the simple bifurcation of the vegetative shoot in woody monocotyledons. Furthermore, apical dichotomy has been recorded recently in the dicotyledons in the description of two species of *Mammillaria* (Cactaceae) by Boke (1976). From this it is clear that the angiosperm vegetative shoot apex has a capacity, rarely expressed, for equal dichotomy.

---

[1] Received for publication 11 October 1976; revision accepted 20 January 1977.

[2] Research on woody monocotyledons supported in part by NSF Grant GB-31844-X.

The senior author is grateful for research support from the National Geographic Society, which made the visit to Papua New Guinea possible. The field assistance of Camilla Huxley, Department of Biological Sciences, University of Papua New Guinea is gratefully acknowledged. Anne Faulkner was responsible for all the paraffin sectioning.

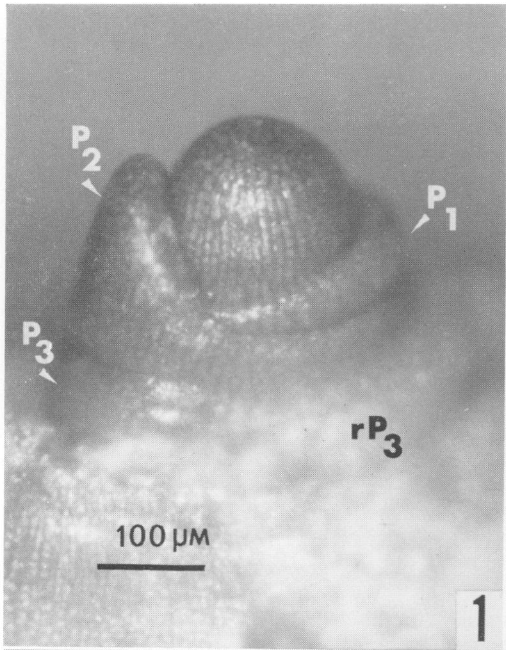
We thank J. G. Torrey and M. H. Zimmermann for critical reading of the manuscript.

→

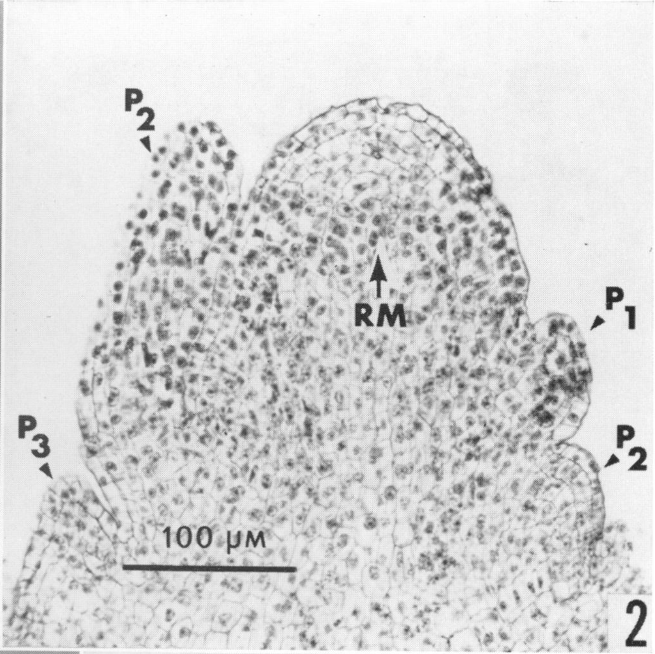
Key to labeling: P_1, youngest leaf primordium visible on the shoot apex; P_2, P_3, P_4, etc., the next oldest successive leaf primordia; P′, P″, leaf primordia formed on apices resulting from dichotomous branching; r, removed, e.g., rP, removed leaf primordium; RM, rib meristem; V, vegetative apical meristem. Note that magnification of all photomicrographs of whole buds is the same, as is the magnification of all corresponding sectioned material.

Fig. 1–6. Side views of vegetative apices just prior to dichotomous branching. **1.** Undivided apex; one leaf (rP_3) removed to allow unobstructed view. **2.** Median longitudinal section through same apex as that of Fig. 1. Note the clearly defined rib meristem (RM). **3.** Broad apex, probably representing initial stages of branching. **4.** Median longitudinal section of apex in Fig. 3. **5, 6.** Two similar stages showing flattened top with enlarged cells (arrows), usually indicative of first stages in dichotomous branching.

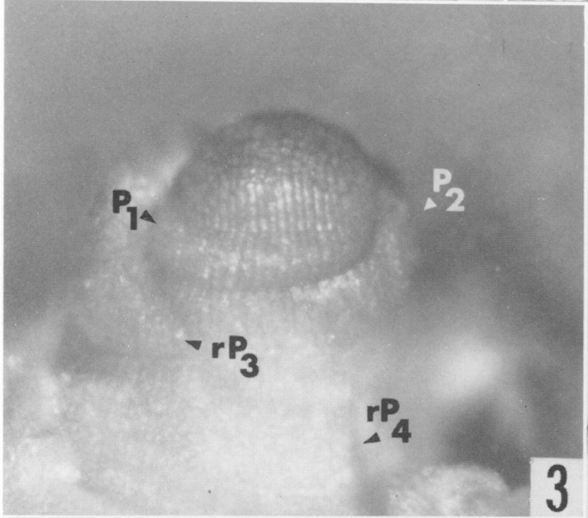
Fig. 7–12. Side views (Fig. 12 oblique top view) of critical stages in dichotomous branching and the establishment of two new growth centers. **7.** Externally this flat-topped apex still appears undivided. **8.** Median longitudinal section through same apex as in Fig. 7. **9.** Two new centers of meristematic activity (V) just becoming visible externally. **10.** Median longitudinal section through same apex as in Fig. 9. Note the periclinal divisions below tunica (arrows). **11, 12.** Two views of the same apex at a critical, early stage in branching. Note enlarged epidermal cells (arrow) between growth centers. See above for Key to Labeling.


P_2
P_1
P_3
rP_3
100 μM

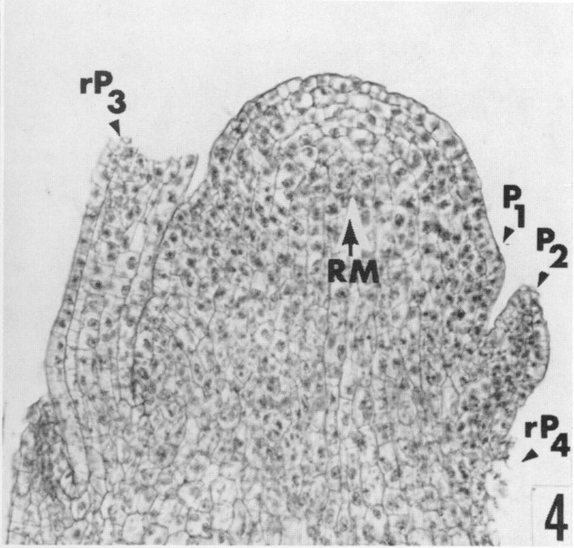
1


P_2
RM
P_1
P_3
P_2
100 μM

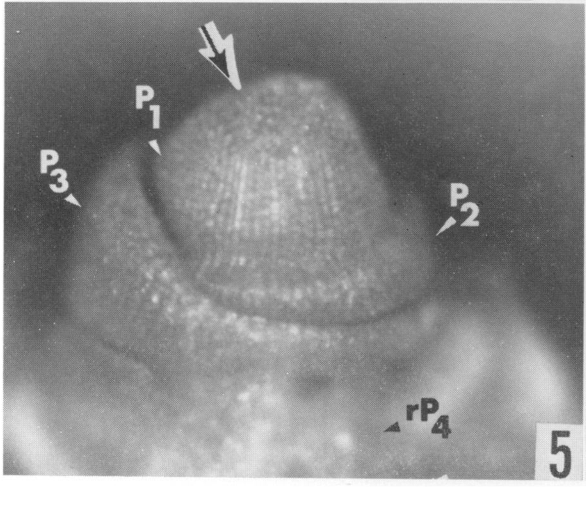
2


P_1
P_2
rP_3
rP_4

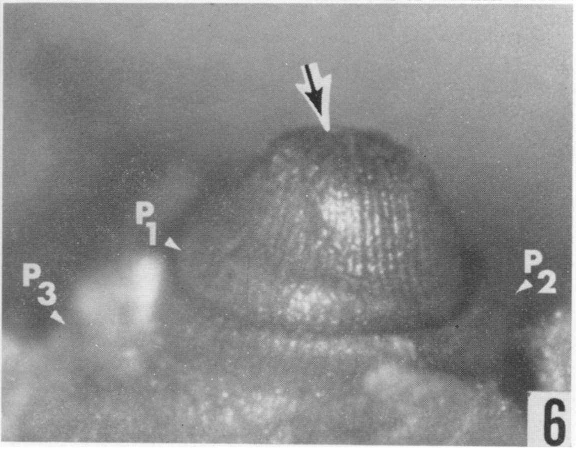
3


rP_3
P_1
P_2
RM
rP_4

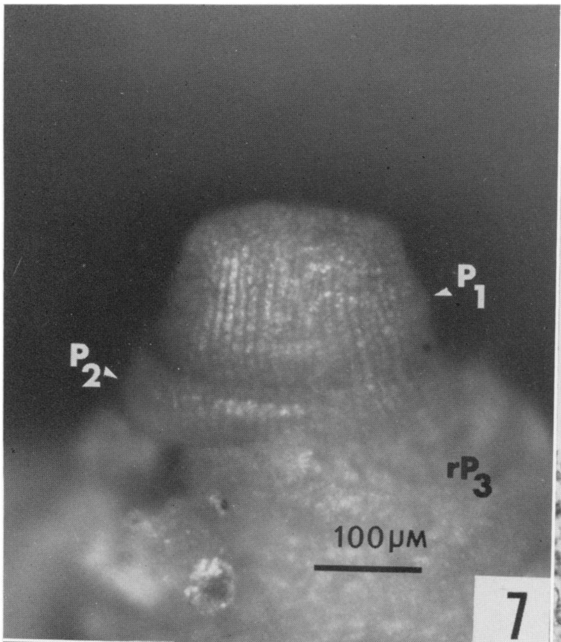
4


P_1
P_3
P_2
rP_4

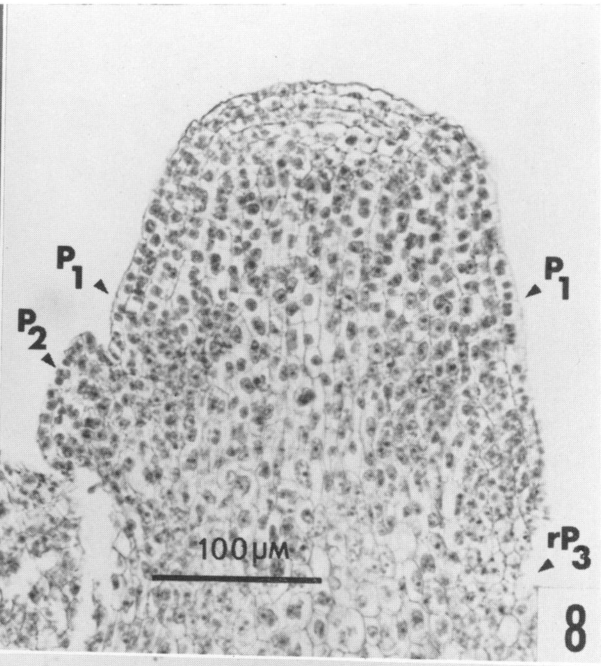
5


P_1
P_3
P_2

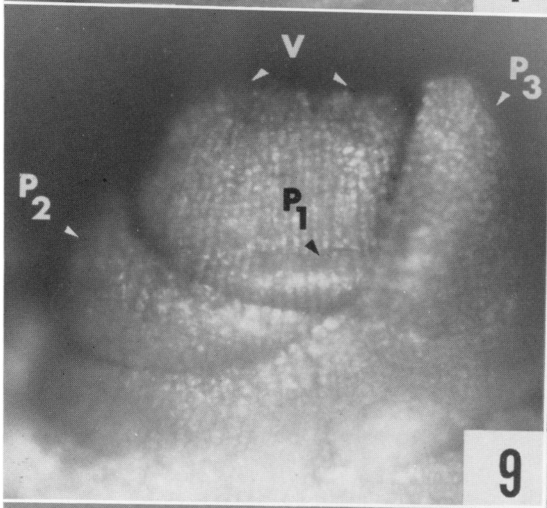
6



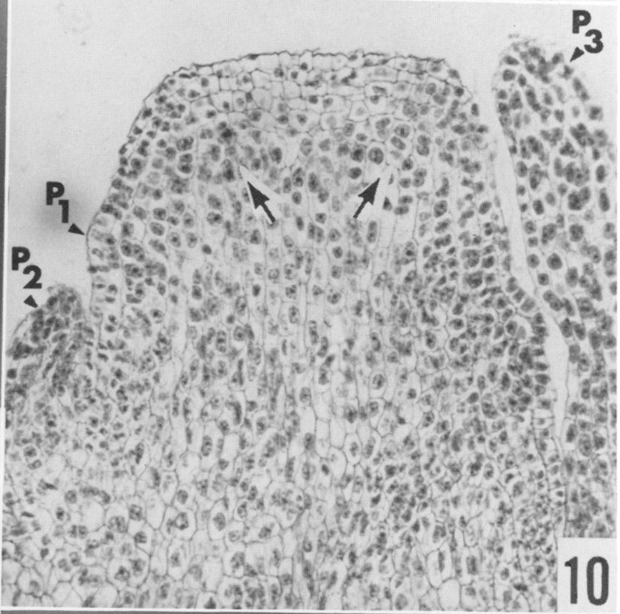
7



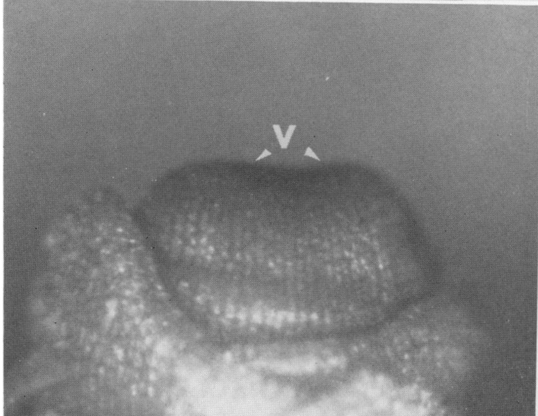
8



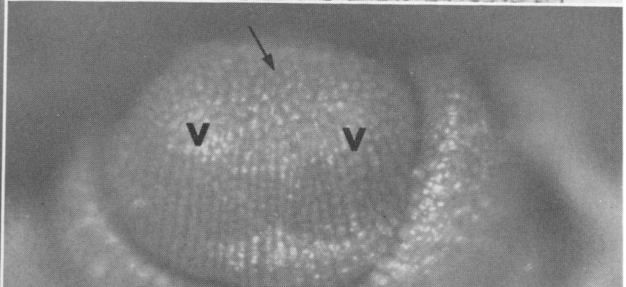
9



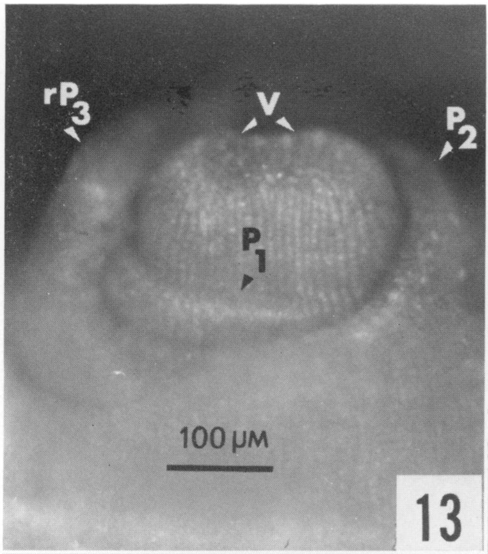
10



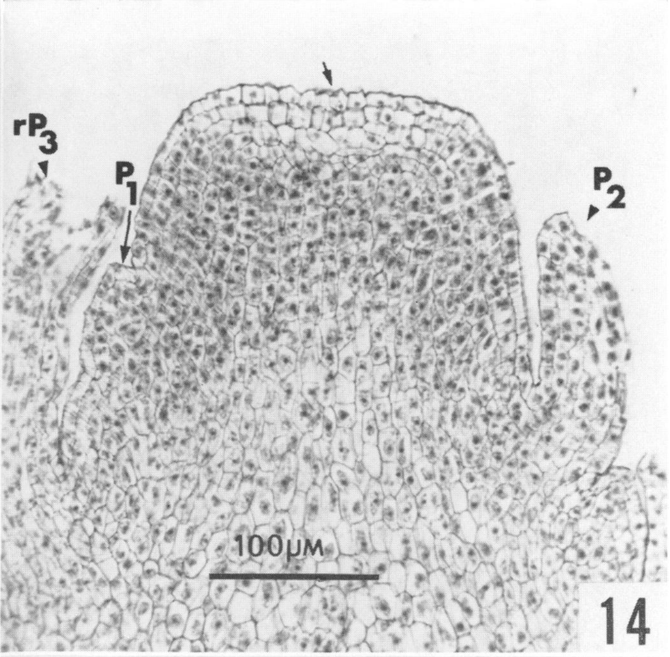
11



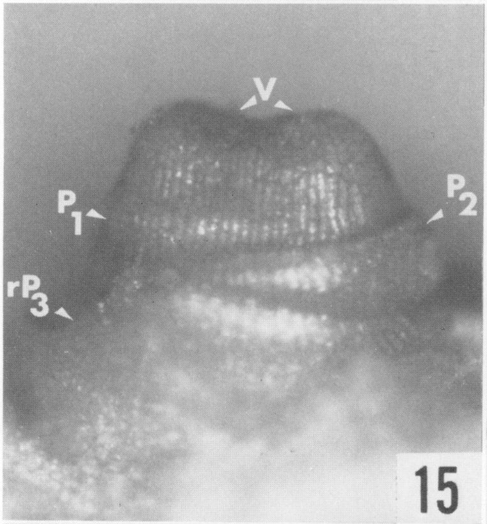
12



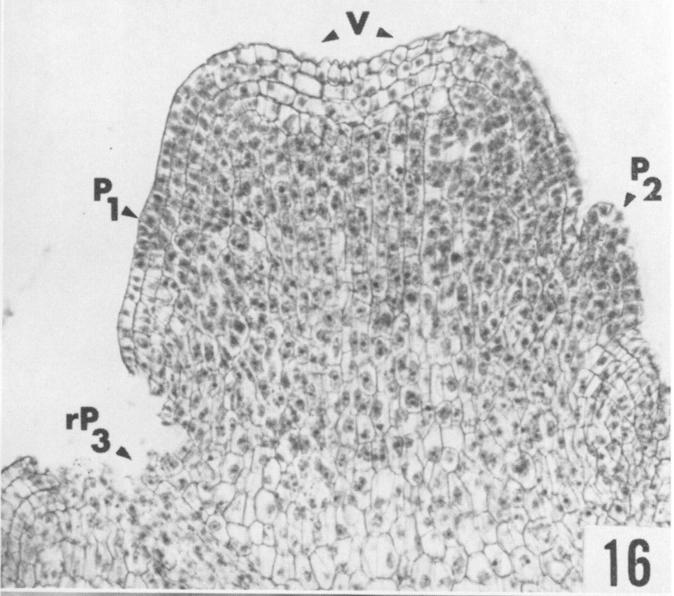
13



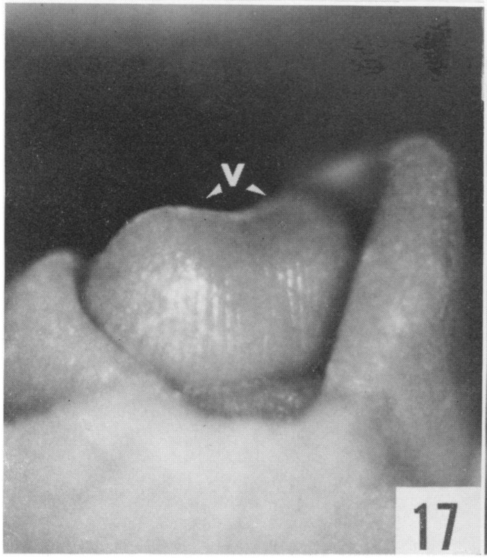
14



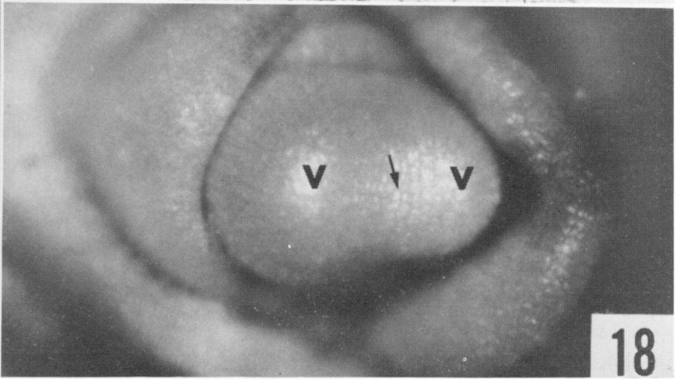
15



16



17



18

We use the term dichotomy in a descriptive sense, without any evolutionary connotation and in preference to Bugnon's (1971) term *ramification terminale.* It is most valuable to be able to look at a shoot apex which divides equally as a normal event from the point of view of the morphogenetic capabilities of the meristem. In particular—what information about histologically distinct zones within the shoot apex is obtainable from a study of the apical meristem as bifurcation proceeds?

Flagellaria is a favorable example for this kind of study since the apex is tall, dome-shaped and symmetrical above the youngest leaf primordium at all stages of the plastochron. Phyllotaxis is distichous so that precise planes of sectioning can be arranged. Shoots are relatively accessible and easy to dissect, compared with *Nypa*, for example. In the early study which established the method of branching (Tomlinson, 1970), only a limited number of apices were available, so all sections were cut transversely to produce a maximum amount of morphological information. A more recent sampling yielded a high proportion of dichotomizing apices. These have allowed a much more thorough documentation of the process of dichotomy, and in particular, the microscopic examination of the apex in longitudinal section at successive stages of its bifurcation. For the first time definitive statements may be made about equal division of highly organized angiosperm shoot apices.

MATERIALS AND METHODS—*Flagellaria indica* as illustrated in Tomlinson (1970)—see also Foster and Gifford (1974, p. 36)—is a scrambling vine, with the aerial shoots, supported by leaf-tip tendrils, arising from a sympodially branched rhizome. Aerial shoots bifurcate at intervals but with a regularity and frequency which varies appreciably from population to population. All aerial shoots eventually produce a panicle of terminal flowers but there may be a slight dimorphism of shoots in that one axis produced by a fork flowers relatively soon while its twin continues vegetative growth for a further period. Sometimes inflorescence axes themselves bifurcate and this is important evidence in interpretating the process of dichotomy.

One hundred aerial shoots of *Flagellaria* were assembled from several clumps growing in back-mangrove communities close to Rigo Road, about 4 miles east of Port Moresby, Papua New Guinea, on March 21, 1976. Axes in which an evident stem dichotomy had occurred recently were rejected so as to increase the chance of locating early bifurcations. Shoots were fixed in F.A.A. at the University of Papua New Guinea within 2 h of collection. Subsequently in Petersham they were crudely dissected by removing all but the innermost five or six leaves, transferred to absolute ethanol, and then stained in acid fuchsin (0.5% in 95% ethanol). Final dissection to reveal the shoot apex was accomplished under the highest magnification of a Wild M-5 dissecting microscope. Surface macrophotographs of the stained apices were made by the epi-illumination technique of Sattler (1968) originally used for the study of floral development, but here with equipment adapted to the Wild M-20 microscope.

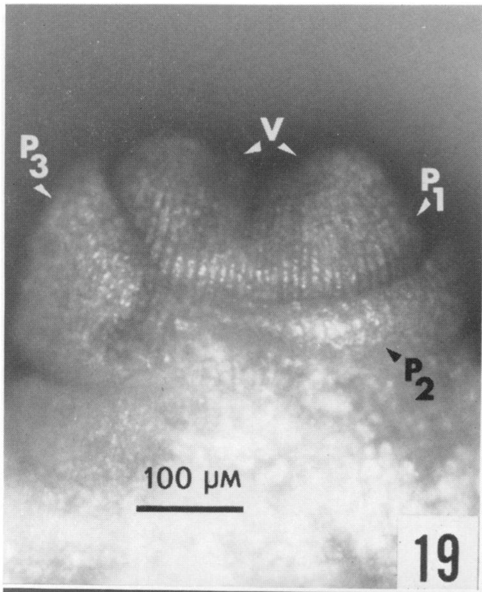
The same apices were then embedded in "Tissuemat" after dehydration with a standard tertiary butanol series. Longitudinal sections in the plane of distichy were cut at 6–10 μm, stained in safranin and fast green, and photographed with the camera attachment of a Wild M-20. These techniques allowed a direct photographic comparison of gross morphology and internal anatomy of the same apex, as is shown in Fig. 1–24.

OBSERVATIONS—*Undivided apex*—Direct visual observation (Fig. 1, 2) confirms statements about the shape and dimensions of the shoot apex made in Tomlinson (1970) on the basis of reconstruction from serial transverse sections. The apex is more or less hemispherical, approximately 180 μm high and 180 μm wide above the insertion of P_1, with a relatively small fluctuation in shape and size throughout a single plastochron. This is because the leaf primordia are initiated laterally well below the summit of the apex, although their encircling growth, characteristic of monocotyledons, is almost completed by the end of the first plastochron.

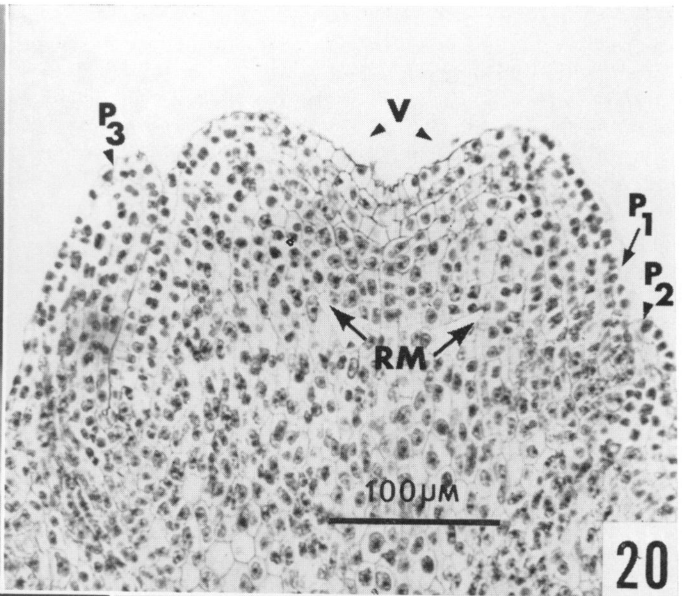
As already recorded, the apex has a 2(-3)-layered tunica, a group of central initials with densely staining walls but relatively lightly staining cytoplasm, and a basal rib meristem (Fig. 2).

←

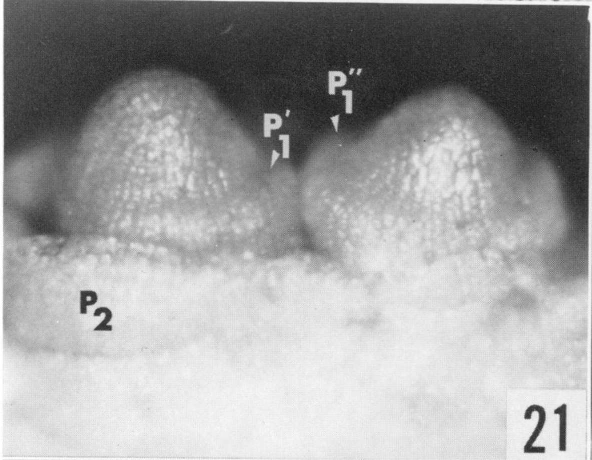
Fig. 13–18. Side views (except top view in Fig. 18) of shoot apices in which the two new centers have already become well established. **13.** The two new vegetative apices (V) are now clearly delimited externally above the last-formed leaf primordium (P_1). **14.** Median longitudinal section through same apex as in Fig. 13. Note the beginning of distortions in the epidermal cells at the tip (arrow). **15.** Even with the clear establishment of two separate apices, a further leaf primordium (P_1) girdling the entire shoot is being initiated. **16.** Median longitudinal section through same shoot as in Fig. 15. Note the further distortions and compression of epidermal cells in central region with indication of anticlinal divisions. The rib meristem is not clearly distinguishable at this stage of dichotomy. **17, 18.** Two different views of the same branched shoot tip. Enlarged epidermal cells can be seen (arrow) between the two new apices in Fig. 18. See page 1057 for Key to Labeling.



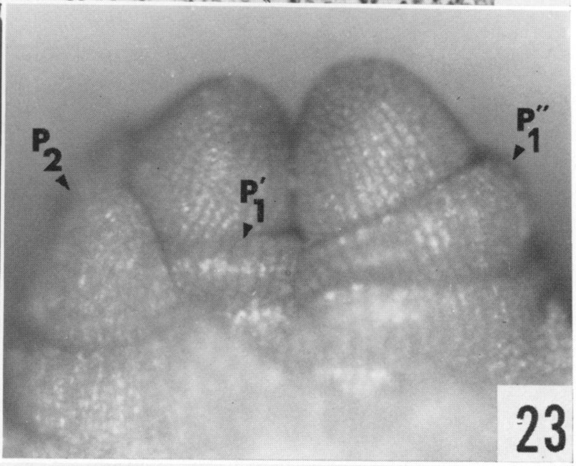
19



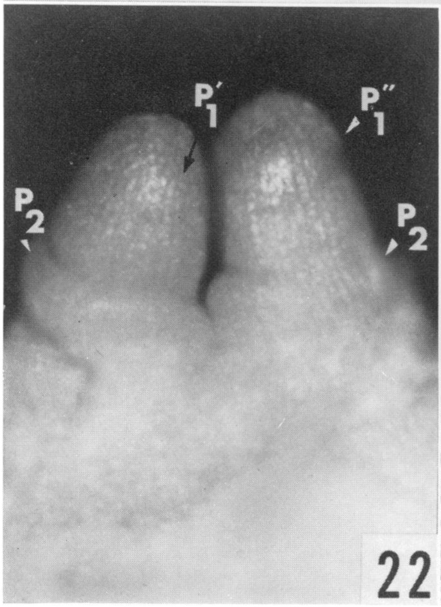
20



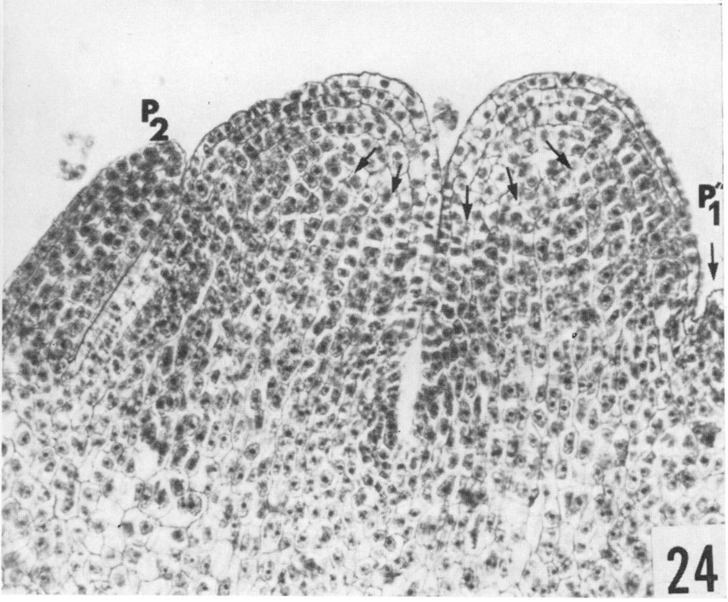
21



23



22



24

At the level of this rib meristem but on the surface of the apex there appear regular files of epidermal cells flanking the apex (Fig. 1). Leaf initiation occurs at the level of or just below the rib meristem and is first indicated by periclinal divisions in the subsurface layers, i.e., both the second tunic layer and within.

Dividing apex—Gross morphology—The first evidence of an incipient dichotomy is the enlargement of the whole apex in a manner which precludes this being the result of leaf initiation. Early recognition of this phase is somewhat subjective but becomes easier as the apex manifestly exceeds its normal dimensions (Fig. 3, 5, 6 and 7). At first, enlargement is always most pronounced in the plane of distichy, to which bifurcation is always restricted. The earliest superficial indication of dichotomy is the development of an apical group of enlarged densely stained cells (Fig. 5, 6, arrows) followed soon by an apparent slight median furrow at right angles to the long axis of the swollen apex (Fig. 7, 9, 11, 12). This is evidently the original summit left behind by the upward growth of the two new shoot apices. This is followed by the early establishment of two new centers of meristematic activity (Fig. 12, 13, 15, 17, 18). These centers become progressively more distinct (Fig. 19, 21) until a stage is reached where each daughter meristem is capable of functioning as a discrete unit, as indicated by the development of separate primordia on them (Fig. 22, 23).

Dividing apex—Anatomy—Corresponding external and sectional views of apices are provided as pairs in Fig. 1 & 2, 3 & 4, 7 & 8, 9 & 10, 13 & 14, 15 & 16, 19 & 20, 23 & 24. Notable early features are the continuity and discreteness of the tunica and central cells during the division process, and the initial enlargement and increase in number of the group of central cells (Fig. 4, 8, 9). These cells reach a maximum number at about the time two new centers of meristematic activity are first recognizable (Fig. 10–14) and the reconstitution of two new apices is established by differentiation of the median apical cells as a densely staining group (Fig. 16, 20). Up to this point there has been no major change in the cytohistological organization of the meristem, but early dichotomy is marked by periclinal divisions below the tunica layer corresponding to each new center of activity (Fig. 8, 10, arrows). The rib meristem layer is least discrete in the middle of this bifurcation process, when the apex reaches its maximum size, but again becomes evident in later stages, especially when the daughter axes are quite discrete, widely separated, and producing leaves independently (Fig. 24).

Dividing apex—Leaf arrangement—It is clear from the range of examples observed and the few illustrated that dichotomy can occur at any period within the plastochron (Fig. 5, 6, 11). Apices at comparable stages of development can have the youngest leaf primordium (P_1) of varying sizes. One consequence of this observation, shown in Fig. 12 and markedly so in Fig. 19, is that encircling growth of a young leaf primordium may be sufficient to enclose the whole axis even after two centers of meristematic activity are distinguishable. Furthermore, examination of mature axes shows that this enclosing leaf is often modified by the distinctive circumstances of its development and accounts for the existence of leaves with two blades attached to a single sheath (as illustrated in Tomlinson, 1970) and other abnormalities. Sometimes, again as illustrated in Tomlinson (1970), there is a lateral, sclerotic protuberance in the sinus of the lateral furrow and the initiation of this structure as a part of the leaf sheath is suggested in Fig. 19 and 12.

The first leaves on the twin axes always occupy the same position on corresponding sides, as reported earlier and illustrated in Fig. 22 and 23, so that the two shoots have the same symmetry and are not mirror images of each other. The first leaves on the daughter shoots can appear relatively soon after dichotomy (Fig. 21) or relatively late (Fig. 22) as far as morphology can suggest. This variability accounts, in part, for variation in the height of individual apices after bifurcation. No measurements were made of the length of leaf primordia immediately below and above a dichotomy before they were dissected away, although this procedure could have provided comparative data which might have

←

Fig. 19–24. Side views, showing late stages in dichotomous branching, as the new apices begin to initiate their own leaf primordia. **19.** The youngest leaf primordium (P_1) is being initiated very close to the already divided shoot tip. **20.** Median longitudinal section through same shoot tip as in Fig. 19 with the incipient rib meristems (RM). Reestablished rib meristem (RM) can be seen below growth centers; anticlinal division of epidermal cells in the furrow may have occurred. **21.** Two newly formed apices just initiating their first leaves (P_1', P_1''). **22.** Apices which have elongated for some time after branching before beginning leaf primordia (P_1', P_1''). Note that the last formed leaf primordium of original unbranched shoot (P_2) has been "broken up" during the development of the two new apices. **23.** A late stage in dichotomous branching with leaves on daughter shoots (P_1', P_1''). **24.** Median longitudinal section through the same shoot tip as in Fig. 23. Note that although two separate apices have now formed, the rib meristems seem continuous in both apices (arrows).

thrown light on plastochron rates in different shoots.

Axillary branching—Axillary branching which occurs in the sympodial rhizome of the plant has not been studied. No axillary branching occurs in the vegetative aerial axis; in the large number of shoots either dissected, or sectioned longitudinally and transversely, no evidence for any dormant or vestigial lateral meristems has been seen. This confirms what seems evident on mature shoots. Axillary branches, of course, occur in the terminal panicle, and during dissection a few shoots were encountered with early stages of inflorescence development, showing lateral branches in the axils of bracts. This condition is always readily distinguishable from the bifurcation of vegetative axes; there is no possibility of confusing the two processes. We have already commented on the frequent occurrence of inflorescences with forked axes, showing that dichotomy and panicle development are two independent but sometimes superposed processes, which distinction we regard as highly significant.

DISCUSSION—Bugnon (1971) defined types of branching in the higher plants and his requirements for dichotomy (*ramification terminale*) are fully met by *Flagellaria*, since it is clear that there is continuity of growth during the process of bifurcation. We have provided the following evidence to substantiate our interpretation. First, there is no morphological indication of rhythmic growth (shortened leaves or internodes) at any stage in the extension and forking of aerial shoots of *Flagellaria*. Second, dichotomy occurs at any stage of the plastochron. Third, the apex expands prior to bifurcation, largely because of an increase in cell number (cf. cell size in Fig. 2 with that in Fig. 4; Fig. 8 & 10; Fig. 14 & 16). Fourth, there is no cytohistological evidence for maturation of cells and the subsequent reconstitution of new meristematic centers by dedifferentiation (cf. Prévost, 1972); tunica, central initials and, to a greater or lesser degree, rib meristem remain evident throughout the process of dichotomy. Fifth, the two apical meristems resulting from division are equal in size from the first; this equality is more clearly revealed by direct observation of longitudinal sections than in the reconstructions from serial transverse sections offered by Tomlinson (1970).

We therefore conclude that dichotomy is not the result of invagination of a single meristem; instead, a single apical meristem becomes two during continuous growth. The fact that an inflorescence can bifurcate is important evidence for the method of development because its axillary branches are produced while the inflorescence apical meristem goes through the process of dichotomy.

We have considered alternative interpretations of our observations. Dichotomy simply as the development of a precocious axillary branch would be supported by the leaf arrangement consistently shown at bifurcation because one axis would always occupy the position of an axillary branch subtended by the enclosing leaf, while one leaf above the fork would be in an adaxial (prophyllar) position although it would always develop as a foliage and not a reduced leaf. In our interpretation of this branching process, there is no main and no lateral axis; consequently, a terminology based on axillary branching (e.g., prophyll) should strictly not be admitted. However, with distichous phyllotaxis there are only two possible types of shoot symmetry which can result from dichotomy—the daughter axes either are or are not mirror images of each other. Fisher (1976) has shown that in *Strelitzia reginae* both alternatives can occur, so that no constant arrangement exists. In *Nypa* (and possibly *Hyphaene*), both with spiral phyllotaxis, mirror-image symmetry of daughter axes seems constant (Tomlinson, 1971). In *Flagellaria* morphological evidence certainly supports the interpretation of precocious axillary branching, but it is the only evidence for this interpretation. We consider this interpretation untenable in face of the developmental evidence which shows synchrony in the appearance of daughter shoots, coupled with the absence of axillary vegetative meristems from the aerial shoots.

The organizational interrelationship between the two axes of a dichotomized shoot is clearly different from that which exists between a parent and lateral axis in axillary branching, since leaf primordia initiated during bifurcation may partly or wholly enclose the two new centers of meristematic activity leading to abnormal leaf types. This interrelationship is not to be expected where one axis (main) imposes some control over another axis (lateral). Modified leaves of this type are not a feature of any kind of axillary branch.

Is dichotomy pathological? We have observed occasional buds infected by insect larvae, but too infrequently in relation to the numerous dichotomies observed, and without regular association with forks where they do occur, to suggest a causal connection. One cannot rule out the possibility of dichotomy being induced by a viral pathogen (cf. fasciation) in which case many, if not all, populations would be infected, as is suggested by the existence of herbarium specimens showing forked axes obtained throughout the range of the species. Such a hypothesis is as equally difficult to prove as disprove. However, the regularity of dichotomy and the minimal disorganization of the dividing meristem argue against the process being pathological.

Accepting dichotomy in aerial shoots of *Flagellaria indica* as a normal developmental process allows one to move on to a study of other aspects. The development of the vascular system at the moment of bifurcation is of obvious interest in relation to current concepts of vascular development in monocotyledons (Zimmermann and Tomlinson, 1972). Experimental control over the process of dichotomy should be sought, and *Flagellaria* offers advantages in the accessibility of its shoot apex, which is relatively large. We hope to make further reports in the future.

LITERATURE CITED

BOKE, N. 1976. Dichotomous branching in *Mammillaria* (Cactaceae). Amer. J. Bot. 63: 1380–1384.

BUGNON, F. 1971. Essai pour une caractérisation des types fondamentaux de ramification chez les végétaux. Mem. Soc. Bot. Fr. 1971: 79–85.

FISHER, J. B. 1974. Axillary and dichotomous branching in the palm *Chamaedorea*. Amer. J. Bot. 61: 1046–1056.

———. 1976. Development of dichotomous branching and axillary buds in *Strelitzia* (Monocotyledonae). Can. J. Bot. 54: 578–592.

FOSTER, A. S., AND E. M. GIFFORD, JR. 1974. Comparative morphology of vascular plants. ed. 2. W. H. Freeman Co., San Francisco.

HALLÉ, F., AND R. A. A. OLDEMAN. 1970. Essai sur l'architecture et la dynamique de croissance des arbres tropicaux. Masson et Cie, Paris.

PRÉVOST, M. F. 1972. Ramification en phase végétative chez *Tabernaemontana crassa* Benth. (Apocynaceae). Ann. Sci. Nat. (Bot.) Ser. 12. 13: 119–128.

SATTLER, R. 1968. A technique for the study of floral development. Can. J. Bot. 46: 720–722.

TOMLINSON, P. B. 1970. Dichotomous branching in *Flagellaria indica* (Monocotyledones), p. 1–14. *In* N. K. Robson, D. F. Cutler, and M. Gregory [eds.], New research in plant anatomy. Bot. J. Linn. Soc. London. Vol. 63. Suppl. 1.

———. 1971. The shoot apex and its dichotomous branching in the *Nypa* palm. Ann. Bot. 35: 865–879.

WILDER, G. J. 1974a. Symmetry and development of *Butomus umbellatus* (Butomaceae) and *Limnocharis flava* (Limnocharitaceae). Amer. J. Bot. 61: 379–394.

———. 1974b. Symmetry and development of *Limnobium spongia* (Hydrocharitaceae). Amer. J. Bot. 61: 624–642.

ZIMMERMANN, M. H., AND P. B. TOMLINSON. 1972. The vascular system of monocotyledonous stems. Bot. Gaz. 133: 141–155.