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The Gametophyte and Juvenile Leaves of Loxogramme

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Some years ago I described spore germination and prothallus development in two Indian species of *Loxogramme*, based on materials collected in the field along with samples of the sporophyte (Nayar, 1955). The prothalli were collected on the tufted, sponge-like root masses of *L. involuta* and *L. lanceolata*. It was soon evident that reporting mature prothalli as cordate possibly was a mistake. Since then I have made several attempts to raise pure cultures of *Loxogramme* prothalli in the laboratory and to review the nature and development of these prothalli.

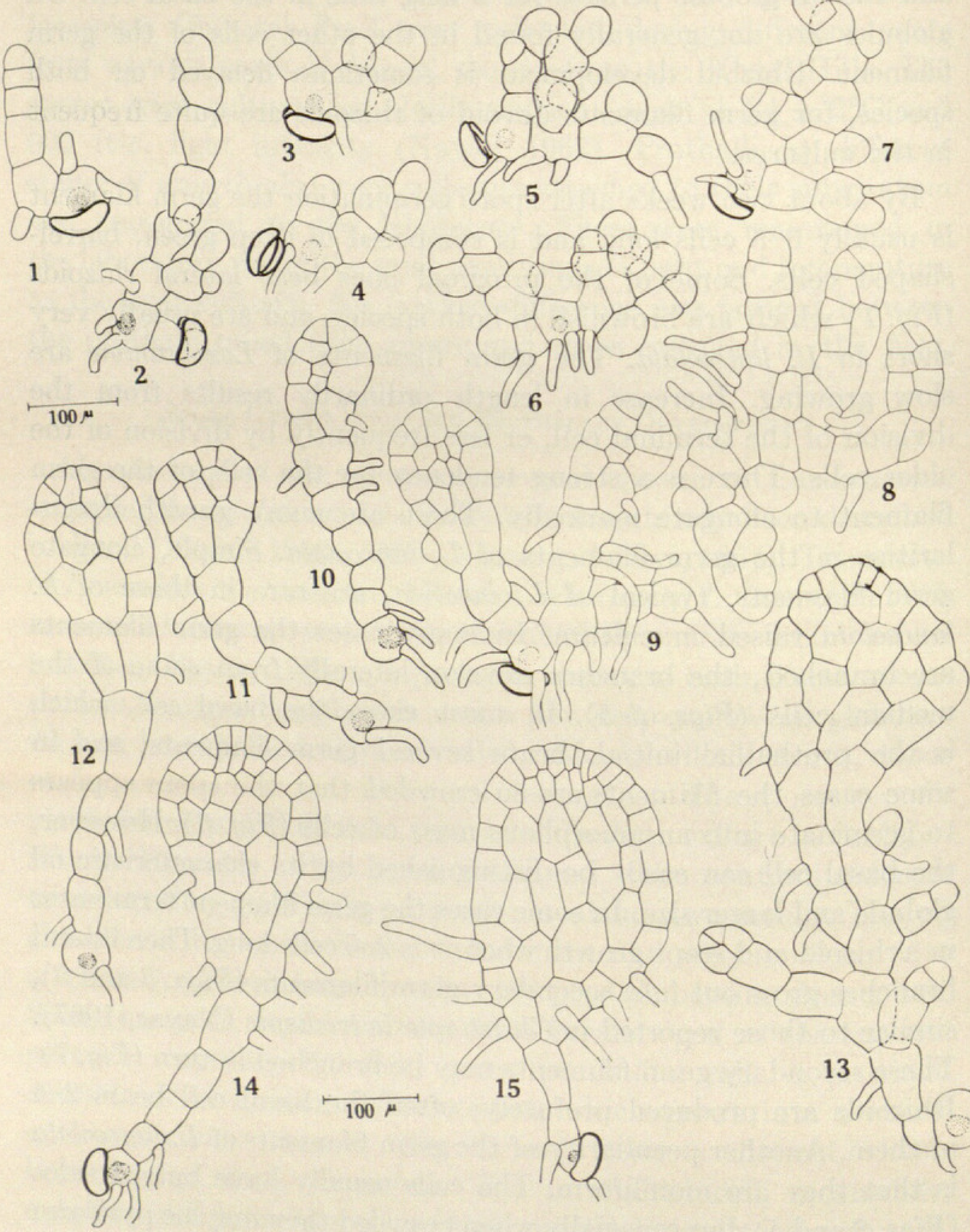
For this study spores of *L. involuta* (Blume) Presl and *L. lanceolata* (Swartz) Presl were collected during different years from various parts of Assam, mostly from the Khasi Hills. The spores were cultured on sterile nutrient agar at 24 ± 2°C and 600 ft-c. light intensity (Nayar, 1962). Prothalli at different stages of growth also were collected attached to their substratum and transferred to the laboratory. These were maintained in the laboratory under the same conditions of light and temperature as the agar cultures. No marked difference was noticed between the prothalli raised from spores and those collected in the field.

SPORE GERMINATION AND PROTHALLUS DEVELOPMENT

Morphology of the spores of several species of *Loxogramme*, including *L. involuta* and *L. lanceolata*, has already been described in detail (Nayar, 1963c, 1964; Nayar and Devi, 1964). The spores of *L. involuta* are monolete-bilateral, whereas in *L. lanceolata* both monolete-bilateral and trilete-tetrahedral spores occur mixed together (Nayar, 1963c). A perine is absent in both. Fresh spores contain many large plastids and a few deep yellow oil globules. As can be expected of thin-walled, chlorophyllous spores, those of *Loxogramme* cannot withstand desiccation. The spores remain viable for only a short time, and are difficult to culture in the laboratory. However, when sown fresh and when contamination is avoided, they germinate profusely in about two weeks. The oil globules in the spore become prominent and often coalesce into a centrally placed, large droplet; the plastids become deep green. Soon the spore swells and the exine breaks open at the laesura, partially exposing the deep green prothallial cell. A proximal rhizoid initial is soon differentiated, which grows into a short, achlorophyllous rhizoid. The rhizoids have a distinct violet-brown tinge in *L. lanceolata*. The prothallial cell, meanwhile, enlarges and protrudes as a thick papilla lateral to the rhizoid. The protruded portion is cut off by a transverse wall from the bulbous basal region, and by successive transverse divisions and elongation of the daughter cells develops into a uniseriate germ filament (*Fig. 1*). The basal cell of the germ filament is bulbous, and the oil globule persists for a long time in the basal cell. Oil globules are not generally found in the other cells of the germ filament. Rhizoid development is sometimes delayed on both species, for germ filaments devoid of rhizoids are quite frequent in the cultures.

By about two weeks after spore germination the germ filament is usually 3–5 cells long and is composed of deep green, barrel-shaped cells. Some of the proximal ones bear lateral rhizoids (*Fig. 1*) which are brownish in both species, and are usually very short in *L. lanceolata*. The germ filaments of *Loxogramme* are slow growing. Increase in length ordinarily results from the division of the terminal cell, or less frequently by division of the older cells. There is a strong tendency for the cells of the germ filament to elongate markedly. There are many growth irregularities in the germ filaments of *L. lanceolata*. Simple, elongate germ filaments, typical of *L. involuta*, are rare in those of *L. lanceolata* raised in culture. In many cases the germ filaments are branched; the branches develop laterally from some of the median cells (*Figs. 3–5*). In most cases the basal cell, which is the prothallial initial, bears several germ filaments, and in some cases the filaments are so crowded that the spore appears to germinate into an amorphous mass of cells (*Fig. 6*). However, the basal cell can easily be distinguished by its characteristic oil globule and larger size. In some cases the germ filament terminates in a rhizoid and stops growth when it is 2–5 cells long. Then lateral branches grow out into secondary germ filaments (*Figs. 5* and *9*), similar to those reported in *Christiopteris tricuspis* (Nayar, 1967). These secondary germ filaments may be branched in turn (*Fig. 7*) Rhizoids are produced profusely; often the basal cell bears 2–4 of them. Another peculiarity of the germ filaments of *L. lanceolata* is that they are moniliform. The cells usually have bulged sides (*Figs. 2* and *5*), but especially when crowded they may be pyriform (the anterior end broad and the posterior narrow). Most germ filaments grow erect or oblique to the substratum; branches form in all directions, giving the cultures a characteristic appearance.

Formation of a prothallial plate is initiated in the cultures



Spore Germination and Prothallial Plate Development in Loxogramme. Fig. 1. Germ filament of L. involuta. Fig. 2. Same, L. lanceolata. Figs. 3–7. Branched germ filaments of L. lanceolata. Fig. 8. Same, ca. 8 weeks old showing prothallial plate formation. Fig. 9. Same, ca. 10 weeks old. Figs. 10–12. Prothallial plate formation in L.

about 6–8 weeks after spore germination. The germ filaments are usually 5–8 cells long by then, but sometimes, particularly in *L. lanceolata,* there may be an extensive development of the filamentous stage. The distal cells of the germ filament, including the terminal cell, divide longitudinally, and the daughter cells expand laterally to form a dorsiventral prothallial plate (*Figs. 8* and *10*). Individual branches of the germ filament develop into separate prothalli (*Figs. 8* and *9*). About two weeks after the initiation of plate formation an obovate prothallial plate with a smoothly rounded or somewhat flattened distal end is formed (*Figs. 11* and *12*). Cells in the distal region are more actively meristematic and thus progressively smaller. The prothalli of *L. involuta* are comparatively quicker growing, and may develop into a prothallial plate 6–8 cells broad (*Figs. 14* and *15*) about two months after spore germination, whereas those of corresponding age in *L. lanceolata* are often only half as large (*Figs. 8* and *9*). Soon the prothalli become spathulate, and later the distal region expands markedly, making the prothallial plate broader than long (*Figs. 13* and *14*). Rhizoids are produced in profusion from the marginal cells at the posterior end.

The young prothalli of *Loxogramme,* unlike those of most leptosporangiate ferns, never develop a regular meristem or meristematic cell. In some cases, however, an obconical marginal cell is developed which acts for some time as a typical meristematic cell by cutting off daughter cells regularly on the oblique sides (*Figs. 14* and *15*), but its activity is never prolonged. About three months after spore germination the prothalli become nearly circular and about 2 mm in diameter, and are composed of small, densely chlorophyllous cells (*Figs. 16* and *17*). The cells are progressively smaller towards the distal margin. Superficial rhizoids similar to the marginal ones are produced on the posterior half. In *L. lanceolata* the prothalli exhibit a marked tendency to

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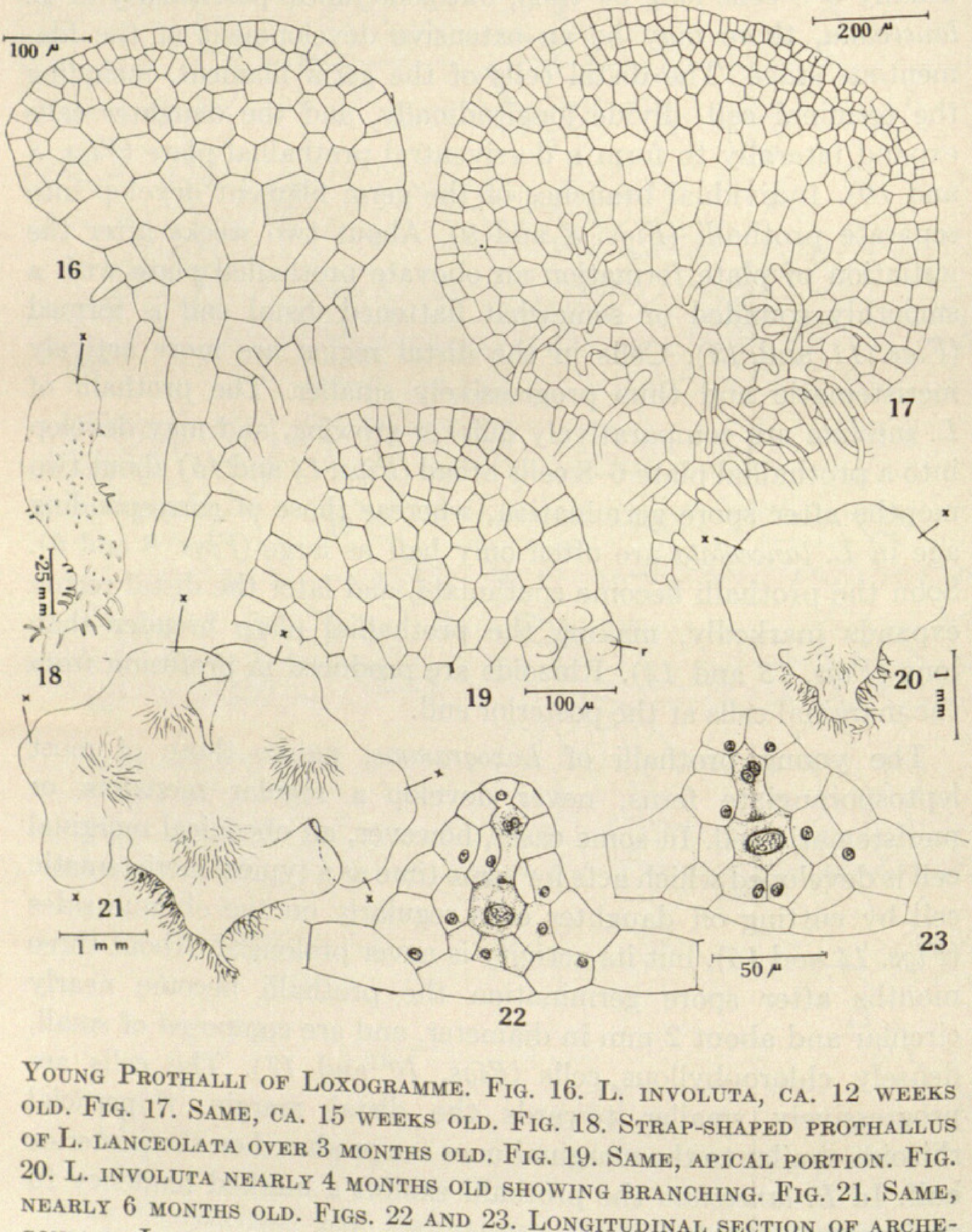
INVOLUTA. FIG. 13. SPATHULATE PROTHALLUS OF L. LANCEOLATA CA. 11 WEEKS OLD. FIGS. 14 AND 15. SPATHULATE PROTHALLI OF L. INVOLUTA CA. 2 MONTHS OLD SHOWING OBCONICAL APICAL CELL. THE SHADED AREAS REPRESENT OIL GLOBULES.

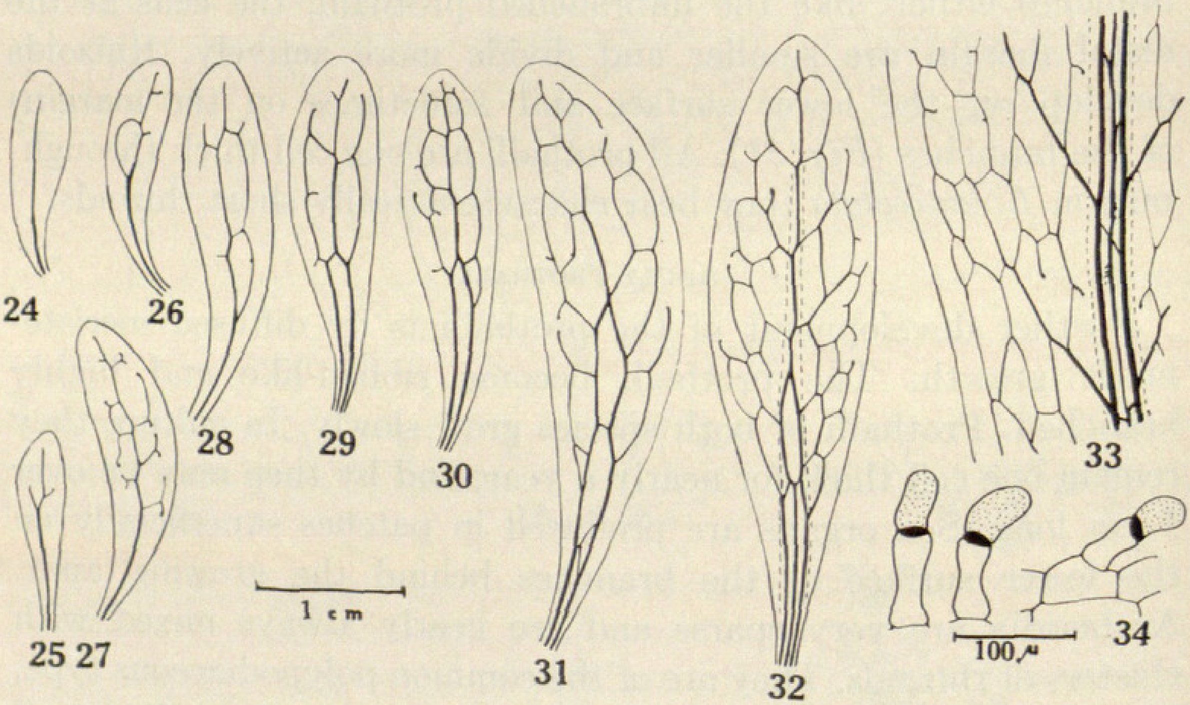


YOUNG PROTHALLI OF LOXOGRAMME. FIG. 16. L. INVOLUTA, CA. 12 WEEKS OLD. FIG. 17. SAME, CA. 15 WEEKS OLD. FIG. 18. STRAP-SHAPED PROTHALLUS OF L. LANCEOLATA OVER 3 MONTHS OLD. FIG. 19. SAME, APICAL PORTION. FIG. 20. L. INVOLUTA NEARLY 4 MONTHS OLD SHOWING BRANCHING. FIG. 21. SAME, NEARLY 6 MONTHS OLD. FIGS. 22 AND 23. LONGITUDINAL SECTION OF ARCHEGONIA OF L. INVOLUTA. THE ABBREVIATION IS: x = MERISTEM.

elongate at this stage and soon become strap-shaped (*Fig. 18*). Rhizoids are produced all over the lower surface and are mostly aggregated in irregular patches; marginal rhizoids are developed profusely on the posterior half. In *L. involuta*, on the other hand, the prothalli expand and become irregular in outline and even irregularly lobed due to unequal marginal growth. The lobes develop into broad branches, often 3–10 at the distal end (*Fig. 21*). Occasionally only two lobes develop and the prothalli appear cordate (*Fig. 20*). There is no well defined meristem in these branches either; like the unbranched prothalli, the cells at the distal margin are smaller and divide more actively. Rhizoids develop on the lower surface and sometimes on the margins of the branches (*Fig. 21*). All prothalli are one cell thick throughout; in *L. lanceolata* they bear characteristically short rhizoids.

ADULT PROTHALLI

Further development of the prothalli is by diffused meristematic growth. The prothalli become ribbon-like and highly branched. Prothalli of both species grow slowly. In culture they remain one cell thick for nearly a year, and by then may be over 1 cm long. Sex organs are produced in patches superficially on the lower surface of the branches behind the growing apex. Antheridia are very sparse and are nearly always mixed with clusters of rhizoids. They are of the common polypodiaceous type, having a central mass of sperms surrounded by a 3-celled wall which is composed of a short, saucer-shaped basal cell, an annular cell, and a cap cell. Archegonia are found mixed with antheridia. Where archegonia are borne the thallus is 2–4 cells thick, forming a cushion subtending the sex organs. When archegonia are crowded, the cushions subtending individual archegonia merge to form a single broad patch usually two cells thick. Archegonia are initiated behind the growing apex of the branches where the thallus is still only one cell thick. The archegonial initial becomes slightly larger and its lower wall protrudes markedly from the surface of the prothallus. This cell divides transversely, making the prothallus two cells thick. The daughter cell on the lower surface of the prothallus divides again and develops into the archegonium in the usual way, forming a row of three cells. The outer one protrudes and develops into the neck, the middle one into the egg and neck canal cell, and the lower one into the basal portion of the venter. Mature archegonia (*Figs. 22* and *23*) are small and have a short, nearly straight, papilla-like neck composed of four tiers of three cells each. The neck canal cell is narrow and binucleate at maturity. The basal row of neck cells divides vertically towards maturity, making the neck two cells thick at the base, as in most advanced leptosporangiate ferns.



JUVENILE LEAVES OF LOXOGRAMME INVOLUTA. FIGS. 24–32. PROGRESSION OF VENATION PATTERN. FIG. 33. PORTION OF LAMINA FROM SPOROPHYTE, CA. 6 MONTHS OLD. HATCHED AREA REPRESENTS MIDRIB. FIG. 34. HAIRS OF THE FIRST JUVENILE LEAF.

Mature prothalli of *Loxogramme* are highly branched and ribbon-like. The branches usually have irregular, sometimes lobed sides. They presumably grow for years and form extensive patches. Some prothalli grown in cultures formed masses nearly 2.5 cm broad and had several crowded, lobe-like branches growing nearly erect after about two years. Much bigger masses were collected in the field. The prothalli remain one cell thick, except for irregularly placed areas where sex organs are borne. Midribs are absent, as are trichomes of all sorts.

Juvenile Sporophytes

As in other polypodiaceous ferns, the juvenile laminae of *Loxogramme* are simple and entire. The following description is that of *L. involuta* alone because juvenile plants of *L. lanceolata* were not available for study. The first few leaves have spathulate or strap-shaped laminae which taper downward to a narrow base (*Fig. 24*). A single, unbranched vein traverses the lamina and ends well behind the rounded apex. In succeeding leaves the vein is unequally 1- or 2-forked at the apex, and a midrib is differentiated by successive unequal forking and overtopping (*Fig. 25*). Such pinnately branched veins sometimes are found as early as the second or third juvenile leaf. Soon the basal lateral veins become forked (*Fig. 27*), and the branches (which face each other) of successive veins fuse to form areoles (*Figs. 26–29*). Sometimes the laminae are inequilateral with areoles only on one side of the midrib (*Fig. 28*). In some small leaves the single vein entering the lamina forks at the tip and the two branches form a terminal loop as in *Vittaria* (Wagner, 1952). A series of elongate, costal areoles develops on either side of the midrib of the laminae of larger, successive leaves (*Figs. 29* and *30*). Leaves from the fifth to seventh onward are supplied by a pair of parallel vascular strands, each of which bears the lateral veins of its side, and which fuse distally. Regular vascular commissures between the two main vascular strands form a row of narrow areoles which constitute the midrib of the leaf (*Fig. 31*). Meanwhile, the lateral veins become prominent, a second row of smaller areoles forms beyond the costal row (*Fig. 31*), and short, free-ending veinlets which point toward the midrib develop within the costal areoles from the outer vein of the areole.

Larger juvenile laminae develop several rows of areoles beyond the costal ones, and main lateral veins become more conspicuous. The midrib in these leaves has three main vascular strands (*Fig. 32*). A venation pattern quite similar to that of adult leaves is formed by plants nearly six months old (*Fig. 33*). In some cases early juvenile leaves are forked, with each lobe receiving a branch of the forked vascular strand entering the leaf base. Early juvenile leaves, including the first leaf, bear a few clavate hairs (*Fig. 34*) on the lower surface and on the margins. These hairs are bicellular, with a slender, elongate stalk cell and a rather swollen, probably glandular, densely protoplasmic terminal cell. They are similar to those reported on adult leaves (Nayar, 1955).

DISCUSSION

This study corrects an earlier report on prothallus morphology and growth in *Loxogramme* (Nayar, 1955). The prothalli are of the ribbon-like, branched type and develop not by the activity of an obconical meristematic cell, but from a diffuse meristematic area. Although the prothalli of *L. involuta* may approach a subcordate shape (*Fig. 20*), this is due to diffuse development of lateral branches by the cessation of the meristematic activity of the medianly placed cells at the apex, and not by the development of a median apical meristem as reported earlier; the branches ultimately become elongate and ribbon-like. The elongate, ribbon-like, mature prothalli are much like those reported in some microsorioid genera of the Polypodiaceae, e.g., *Kaulinia* (Nayar, 1963a), *Leptochilus* and *Paraleptochilus* (Nayar, 1963b), *Colysis* (Nayar, 1962), and *Christiopteris* (Nayar, 1967). So also are the juvenile leaves, with their simple, entire laminae traversed by a solitary vein, but which later develop a midrib by overtopping. These facts appear to support Holttum's (1947, 1949) belief that *Loxogramme* is polypodiaceous. In addition, the germ filaments of *L. lanceolata* occasionally branch profusely and stop terminal growth, while the lateral branches develop into individual prothalli, similar to those of *Christiopteris tricuspis* (Nayar, 1967). In many cases *L. lanceolata* produces several aggregated, branched germ filaments from a single spore.

Loxogramme is sometimes considered a grammitid fern. But the prothalli of Grammitidaceae are basically cordate albeit narrow, elongate, and often strap-like (Stokey and Atkinson, 1958). They also bear characteristic trichomes, which are highly branched and glandular or elongate and acicular. A midrib, which is sometimes interrupted, is found in all. Branching of the prothalli is uncommon. An apical meristematic cell is regularly formed during early stages of prothallus development. This is replaced by a multicellular meristem. The prothalli multiply vegetatively by fragmentation of the germ filament. The antheridia possess slender, elongate, barrel-like basal cells. The early juvenile leaves of the grammitid ferns, insofar as known, have lobed laminae. All of these features differ from *Loxogramme*. However, the very slow prothallial growth rate, the protracted filamentous stage of *L. lanceolata*, the characteristic branching of the germ filaments, the tendency for one spore to produce multiple germ filaments, and the tendency toward moniliform germ filaments recall the prothalli of grammitid ferns. Probably these features are adaptations to similar environments.

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