THE GAMETOPHYTE OF *ACROSTICHUM AUREUM* L.

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Introduction

Acrostichum is a pantropic genus of salt-marsh plants, growing in large clusters in coastal areas all over the world. Taxonomically it was one of the most confused genera of ferns: it has long been the practice of pteridologists to associate under it nearly all leptosporangiate ferns with sporangia distributed all over the undersurface of the leaves. Till recently this situation continued, even though the heterogeneous nature of the over-large genus (for example, as conceived by Hooker and Baker, 1868) was long recognised. As now understood, it is a small genus of *ca.* 5 closely similar species (regarded by some pteridologists as only varieties of the type species). The only Indian representative is the type species, *A. aureum* L. (Fig. 33), a salt-marsh fern growing abundantly all over the coastal regions. *Acrostichum* is regarded by most pteridologists (Christensen, 1938; Copeland, 1947; Holttum, 1947; Alston, 1956) as a pteroid fern: among contemporary pteridologists, Ching (1940) treats it as a separate family, Acrostichaceae, which, according to him, belongs to the aspidioid phylum. Based on their observations on the gametophyte of one species (*A. speciosum*), Stokey and Atkinson recently concluded that there is "nothing in the gametophyte structure or development which would indicate a close affinity with the Aspidioid ferns, but there are several characters which would ally it with the Pteroid ferns, and, more particularly with the Cheilanthoid group" (Stokey and Atkinson, 1952, p. 112).

Though morphologically it is one of the better known genera among the Pteridaceae, our knowledge of the gametophyte of *Acrostichum* is restricted mainly to the report on the prothallus of one species, *A. speciosum* Willd. (Stokey and Atkinson, 1952): a passing reference to some of the prothallial characters of *A. aureum* is made by Schumann (1915) in her detailed morphological study of the sporophyte. Spores for the present study were collected from the West Coast of South India and from the

Andaman Islands, and were cultured on Knop's agar medium using techniques reported in earlier studies (Nayar, 1962 *b*). The cultures were maintained throughout at a temperature range of 24 ± 2° C and a light intensity of 600 ft-c. (light supply from Phillips' fluorescent lamps, for 12 hr. duration in every 24 hr. period). Spore morphological observations are based on acetolysed preparations mounted in glycerine jelly (Erdtman, 1952): spore measurements are averages of 20 readings in each plane.

SPORE

The spores of *A. aureum* are trilete (tetrahedral), with a nearly triangular amb having smoothly rounded broad corners and faintly concave sides (Fig. 1). The laesura is tenuimarginate and each arm is *ca.* 21 μ long. The spores measure 53 × 60 μ (P × E). The exine is *ca.* 4·0 μ thick and clearly demarkated into a sexine and nexine: the sexine is conspicuously thicker than the nexine and is densely granulose. Fresh spores contain dense, nearly colourless plastids and one or two pale-yellowish oil globules.

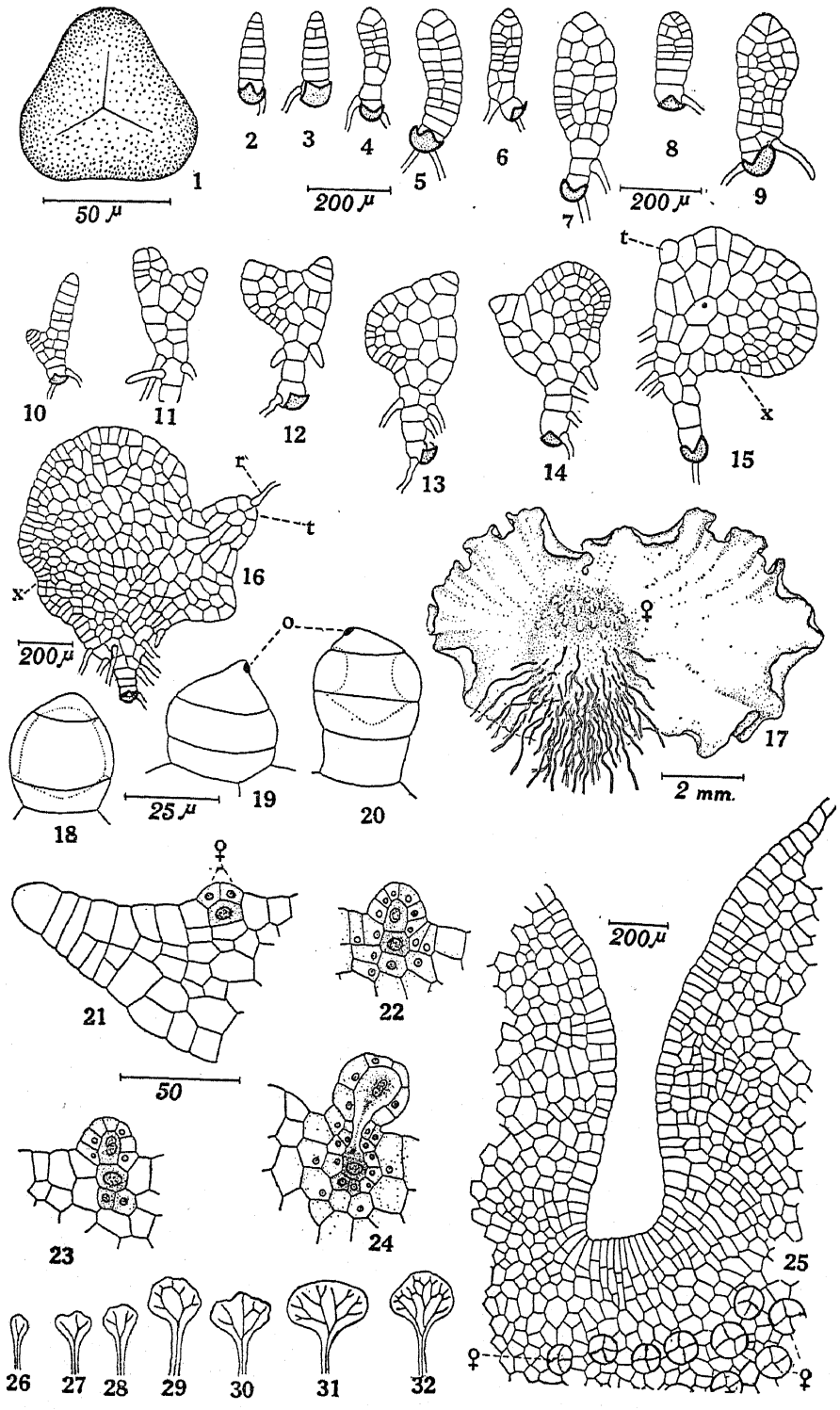
PROTHALLUS

In about 4–6 days of sowing, the spores turn pale-green and germinate by the exine splitting open at the laesura and the first rhizoid emerging as a hyaline protrusion. Chloroplasts are not found included in the rhizoid, but the prothallial cell, which soon elongates as the germ filament, is densely chlorophyllous. The germ filament (Fig. 2) becomes 6–8 cells long in about a week after spore germination. The cells of the germ filament are generally short and broader than long. The basal cell is slightly swollen and enveloped by the spore coat which remains attached to it for long. Generally the germ filaments are more or less tapering towards the apex, the anterior cells being progressively narrow (Figs. 2, 3). Growth of the germ filament is mainly by repeated divisions in the intercalary cells, and, as reported in *A. speciosum* (Stokey and Atkinson, 1952), there is little elongation of cells during the development of the germ filament and the intercalary cells often appear disk-like. Prominent, yellowish-brown oil globules are present in the basal cell, and in most cases these coalesce to form a large, irregularly-shaped mass occupying nearly half the volume of the cell and persisting till a distinct prothallial plate is developed. As the germ filament becomes 6–8 cells long, the anterior cells appear to become more or less quiescent. The terminal cell soon stops growth and may become more or less conical.

Formation of a prothallial plate is initiated by longitudinal divisions in the intercalary cells, generally two or three cells behind the terminal cell

(Fig. 3). Most of the cells, except often two or three at either end of the germ filament, now divide transversely again and expand laterally, becoming dorsiventrally flattened (Fig. 4). Soon each intercalary cell divides by a median longitudinal wall and the prothallus is then nearly ribbon-like (Fig. 5). The terminal cell of the germ filament scarcely expands, but, due to the lateral expansion of the penultimate cell, appears nearly conical or even more or less beaked (Fig. 4). The penultimate cell often divides longitudinally soon and take part in the formation of an expanded prothallial plate (Figs. 4, 5), but the terminal cell usually remains quiescent, though rarely it may divide unequally once (Fig. 9). In many cases, two or three of the cells in the terminal region remains uniseriate, forming a small beak on the expanding prothallial plate (Figs. 12–14). In no case an obconic meristematic cell is developed from the terminal cell as reported by Schumann (1915). In one case the terminal cell of a germ filament was observed, in which two oblique divisions cut off an obconical daughter cell (Fig. 8): however, this cell was not meristematic. The basal cell of the germ filament often develops a cluster of rhizoids similar to the first—nearly hyaline, non-chlorophyllous and with a slightly swollen base. Rhizoids are rarely developed by the other cells of the prothallus till the anterior region of the prothallus expands and becomes three or more cells broad. In the posterior half, all, except the basal cell, often divide longitudinally and take part in plate formation (Fig. 9). Branching of the germ filament is rare: but in some cases as the terminal region of the germ filament becomes gradually quiescent, a lateral branch may be developed by one of the daughter cells formed by longitudinal division of an intercalary cell. This branch then continues the growth of the prothallus (Figs. 10, 11). Rarely a group of intercalary cells on one side develops into a broad lateral lobe: this lobe expands and develops into the prothallus while the uniseriate anterior region remains as a beak-like protuberance (Fig. 12). Young prothalli of *A. aureum* are characteristic in being broad and nearly ribbon-like, often with the anterior as well as the posterior ends more or less tapering and the intercalary region composed of many, narrow, transversely elongated, disk-like cells (Figs. 5–9).

The prothallus expands, and, in about 2 weeks after sowing the spores, becomes nearly spatulate. Lateral cells on one side, nearly towards or a little below the middle of the spatulate region (Fig. 7), now divide more frequently than the others: this region later grows out as a broad lobe (Fig. 13). The terminal cell of the prothallus (which remains uniseriate), in some cases produces an apical rhizoid (Fig. 16, *r*) and other rhizoids are developed by some of the basal cells. The lateral lobe expands considerably and consequently the terminal region of the prothallial plate is pushed to one side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FIGS. 1–32

(Figs. 14, 15). Lateral marginal cells of the lobe (generally those facing the basal end of the prothallial plate) are more actively dividing, smaller in size and with denser cytoplasmic contents (Fig. 15, *x*). This region now proceeds to develop into a multicellular meristem (which, in many cases, is facing almost towards the posterior end of the prothallus). Soon the margin at the meristematic region (Fig. 16, *x*) becomes notched by expansion of cells on either side of the meristem. As growth proceeds, the notched region ('apex' of the prothallus) becomes cordate and the prothallus is distinctly lopsided, with a large anterior wing and a small, often inconspicuous, posterior wing. Superficial rhizoids are developed on the lower surface. By unilateral expansion the prothallus soon becomes distinctly cordate, but generally one side is markedly larger than the other. Midrib formation is initiated when the prothallus is nearly 4 or 5 weeks old.

Prothalli reach maturity in 9–10 weeks after spore germination. The mature prothallus (Figs. 17, 34) is naked, cordate, lopsided (one side larger than the other), large, dark-green and often glossy with a deeply cordate apex and a heavy, broad midrib bearing sex-organs and rhizoids. The midrib, in *ca.* 10 weeks old prothallus, is often 10–15 cells thick, but the cells are small and consequently the midrib is not very thick. The wings are ruffled, often crisped, and in many cases with the margin lifted up from the substratum (not funnel-shaped as in *Pityrogramma*, *Onychium*, etc.): the wing cells are uniformly thin-walled. The meristem (Fig. 25) is composed of a large number of slender elongated cells arranged in a row. The midrib bears clusters of nearly hyaline to very pale-brown rhizoids. In some cases the anterior region of the prothallus is also lifted up and rarely may grow almost vertically (Fig. 34).

Antheridia are produced from the cordate stage onwards and are superficial, developing behind the meristem. On the mature prothallus they are restricted to the midrib region on the lower surface. The antheridium (Figs. 18, 19) is large, elongate-globose when mature, and with a bluntly tapering apex tilted slightly to one side, as reported in *A. speciosum* (Stokey and Atkinson, 1952). Antheridia are more or less profuse on young thalli, but as archegonia begin to develop, antheridial development generally ceases on the main thallus. Occasional stalked antheridia (Fig. 20), with short disk-like stalk cells, are observed, especially on old prothalli. The opercular cell protrudes as a beak, the apex of which opens as a pore to release the sperms (Figs. 19, 20, *o*). Archegonia are formed only after midrib formation. They are more or less similar to those reported in

A. speciosum (Stokey and Atkinson, 1952), but the neck is comparatively shorter, being only 4 or 5 cells long (Fig. 24). The neck canal cell is usually binucleate, but, as in *A. speciosum*, was sometimes found to contain four nuclei: three-nucleate neck canal cells, however, were not observed in *A. aureum*. The development of the archegonium is of the common type (Figs. 21–23). Archegonial initials are differentiated generally 5–7 cells behind the meristem of the prothallus (Fig. 21). During development, the neck canal nucleus divides prior to the differentiation of a ventral canal nucleus (Fig. 23). As the archegonia attain maturity the prothallial cells surrounding the egg divide and form a well-differentiated venter composed of a row of regularly arranged small cells (Fig. 24).

JUVENILE SPOROPHYTE

The early juvenile leaves of the sporophyte are generally spatulate and nearly entire (Figs. 26–29): the simplest ones may be broadly obcuneate with a rounded apex. The single vein supplying the lamina is forked once or twice. Generally the very first juvenile leaf is spatulate with the vein dichotomising two or three times (Fig. 31). Two or three of the succeeding leaves may be similar but larger. Often a large central areole, bearing excurrent veinlets, may be formed by fusion of some of the ultimate veinlets (Fig. 29). A midrib is developed by the third or the fourth leaf, and soon areoles are developed on either side of it (Fig. 30). The apex of the lamina becomes pronounced: a distinctly oblong lamina with a central midrib and profusely reticulate venation resembling the adult pattern is developed by the eighth to the tenth leaf (Fig. 32). All the juvenile leaves possess a simple lamina with a nearly entire margin: though herbaceous, the lamina is thick, in marked contrast to the thin membraneous juvenile lamina of most of the ferns (*see* Fig. 34). The early juvenile leaves are naked: multicellular hairs and paleae are developed at the stipe base. Multicellular hairs with slightly swollen terminal cells occur sparsely over the stipe of the third juvenile leaf onwards: similar but smaller hairs gradually spread over the main veins (but are sparse) in later-formed leaves.

DISCUSSION

The prothallus of *A. aureum* is closely similar to that of *A. speciosum* (Stokey and Atkinson, 1952), in its morphology and development, even-though there are minor differences especially with regard to the development of a prothallial plate. In *A. aureum* the prothallial plate expands laterally by the increased activity of some of the lateral intercalary cells of the ribbon-like thallus and develops into a broad spatulate plate in which a

meristem is differentiated on the side facing the posterior end of the prothallus, contrary to the condition in *A. speciosum*. The major features which characterise the prothallus of *Acrostichum* are: the intercalary growth of the germ filament by the addition of more cells rather than by cell elongation, the completely lateral development of the meristem, the omission of an apical-cell-stage in development, the nature and behaviour of the terminal cell of the germ filament, the asymmetric nature of the mature prothallus, lack of hairs on the prothallus, and the beaked nature of the opercular cell of the antheridium. A lateral development of the meristem is found in several genera of the Pteridaceae, including *Pteris*, but is not a universal feature in the family: it is more conspicuous in *Pityrogramma* and *Onychium*, but in these genera the terminal region of the germ filament is not quiescent as in *Acrostichum*. A condition similar to that in *Onychium* etc., is reported in some schizaeoid ferns (Atkinson, 1960; Kaur, 1961). Among the aspidioid ferns (from which group Ching derives the Acrostichaeceae) the meristem usually is lateral in the early stages of development, but this initial asymmetric development, as pointed out by Stokey and Atkinson (1952), does not appear to be comparable to the distinctly lopsided development of *Acrostichum*. In many of the aspidioid ferns, the terminal cell of the germ filament stops growth and ends in a hair: a meristematic cell is then developed by an oblique division in one of the penultimate cells (Nayar, 1960 *a*; Nayar and Kaur, 1963; Nayar and Chandra, 1963). The beaked nature of the quiescent terminal cell of *Acrostichum* does not appear to be comparable to this terminal hair of the Aspidiod ferns. Moreover, the prothalli of all aspidioid ferns, as far as are known, are profusely hairy in contrast to the naked thalli of *Acrostichum*. Also, the spores of the Aspidiaceae are of the monolete type, enveloped by a distinct perine (Nayar and Devi, 1963, 1964). The trilete spores of *Acrostichum* ally it to the Pteridaceae, a family characterised by trilete spores. However, the spores of the pteroid ferns, *Onychium*, *Pityrogramma*, *Actiniopteris* (Nayar, 1962 *c*), etc., possess a characteristic equatorial ridge.

The juvenile leaves of *Acrostichum* also differ from the usual type in the Pteridaceae in possessing a simple, entire lamina: a lobed or dissected lamina characterise the Pteridaceae (Nayar, 1962 *c*, 1962 *d*, 1962 *e*; Nayar and Kaur, 1963). The early juvenile leaves of some of the cheilanthoid ferns (Nayar, 1956, 1960 *b*, 1962 *a*, 1963; Nayar and Bajpai, 1964) tend to be less dissected to nearly entire, but they soon become lobed as a midrib is formed, and are profusely hairy.

SUMMARY

The spores of *A. aureum* are trilete and granulose. On germination, a 6–10 cells long germ filament is produced, in which growth is mainly by intercalary formation of new cells rather than by cell elongation. One or two of the terminal cells become quiescent soon, and the intercalary cells form a prothallial plate. Cells on one side of the plate are more active than those on the other, and a broad ameristic lateral lobe is developed by their activity. As the lobe becomes spatulate, a multicellular meristem is differentiated from marginal cells on the side facing the posterior end of the germ filament. By the activity of the meristem the prothallus becomes cordate, with the meristem at the bottom of the notch. A midrib is formed behind the notch and the prothallus grows to become asymmetrically cordate. The mature prothallus is naked and with ruffled wings. Juvenile leaves possess entire, naked lamina.

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Explanation of Figures

Figs. 1–32. Fig. 1. Proximal view of spore. Fig. 2. A 5-day-old germ filament. Fig. 3. Germ filament, showing initiation of plate formation by longitudinal division in an intercalary cell. Figs. 4–6. Formation of a prothallial plate. Fig. 7. A 2-week-old prothallus, showing increased meristematic activity of lateral cells on one side. Figs. 8, 9. Young prothalli, showing divided terminal cell. Figs. 10, 11. Development of a lateral lobe in young prothalli. Fig. 12. Young prothallus, showing an enlarged lateral lobe and quiescent terminal region. Figs. 13–15. Stages in the development of a lopsided prothallial plate (*x*, meristematic region, *t*, terminal region). Fig. 16. A 3-week-old prothallus, showing development of a notched 'apex' (*x*), and the terminal region of the prothallus (*t*) ending in a rhizoid (*r*). Fig. 17.

Mature prothallus (♀, archegonium). Fig. 18. Mature antheridium. Fig. 19. Antheridium, showing dehiscense (*o*, pore-like opening). Fig. 20. Stalked antheridium. Fig. 21. L.s. of apical region of mature prothallus, showing initiation of archegonium (♀, archegonium). Figs. 22, 23. Stages in development of young archegonium. Fig. 24. Mature archegonium. Fig. 25. Apical region of mature prothallus (♀, archegonium). Figs. 26–32. Juvenile leaves.

EXPLANATION OF PLATE VIII

FIG. 33. *Acrostichum aureum* growing in a marshy area on the Kerala coast.

FIG. 34. Cultured prothalli of *A. aureum*.

(*j*, juvenile plants. The arrow on the right points to the uplifted terminal region of a prothallus.)