



Fig. 3. Diagrammatic representation of the various routes by which interstitial cells might be formed from gastrodermal cells.

the mass which had not voided their algae. This process of algal voiding by digestive cells could be observed in the living masses of the tissue by examining them under the compound microscope at a magnification of 160.

Noticeable changes had also occurred in gland and mucous cells by this time. Their cytoplasm was more basophilic than usual, and gland cells located at the periphery of the mass had apparently voided their internal secretions to the surrounding medium. This would account for the highly basophilic nature of the cells, since the area surrounding their nuclei contained a considerable amount of RNA. However the RNA of the dedifferentiated gland cell was not necessarily similar to that of the interstitial cell.

No cnidoblasts were present in any of the preparations at this time. A well formed metachromatic border was observed around the periphery of the mass, probably representing the secretion of mucous cells, and the digestive cells in the periphery were more basophilic than those in normal animals.

After 8 hours, cells morphologically identical to interstitial cells were present in the peripheral border. After 12 hours, the number of small basophilic cells had greatly increased, but no mitoses could be found in the preparations. The gastrodermal digestive cells had become clearer due to the voiding of their algal bodies. No cnidoblasts were present at this stage.

After 24 hours, the number of small basophilic cells had decreased, but large numbers of cnidoblasts had formed. In

each animal, about 24 cnidoblasts contained fully formed nematocysts, many of which discharged upon contact with the fixative. Other cells were in various stages of transition from interstitial cells to cnidoblasts. Polarity may have been already determined at this stage, since half the tissue mass (presumably the distal portion of the animal) was rich in cnidoblasts and interstitial cells, while the other half (the future peduncle and basal disc) was virtually devoid of these cells. The epidermis had formed and was almost free of algae (Fig. 2). In the few animals that were examined after 48 hours, the cells at the periphery of the mass had begun the mucous secretion typical of epidermal cells and were completely free of algae.

We also studied 25 fragments of epidermis, which were treated and incubated in the same way as the gastrodermis. The fragments all rounded up into small balls within several hours of isolation and remained in this form until they disintegrated 48 to 72 hours later. Prior to disintegrating, they showed no signs of differentiating into gastrodermal elements.

Our results thus show that epidermal, epitheliomuscular cells can arise directly from gastrodermal cells which lose their enclosed algae and food droplets and begin mucous secretion. Interstitial cells appearing in the mass do not arise from pre-existing interstitial cells, but from digestive cells, gland cells, or mucous cells which void their internal secretions. Therefore, the interstitial cells do not represent a "modulated" (6) form of a gastrodermal cell, since they are capable of differentiating into cnidoblasts containing mature nematocysts; neither do they represent a persistent embryonic stock which is maintained solely by the division of interstitial cells. Evidently, certain specialized cells in the hydra are not "end points" of development but can acquire new potency if properly stimulated (7).

Since this paper was accepted for publication, we succeeded in obtaining regeneration from gastrodermal fragments which contained only digestive cells and gland cells. Therefore, the mucous cells are not required for the formation of any epidermal cell type.

JULIAN HAYNES

ALLISON L. BURNETT

Department of Biology, Developmental Biology Center, Western Reserve University, Cleveland 6, Ohio

References and Notes

1. D. K. Normandin, *Science* **132**, 678 (1960).
2. P. Brien and M. Reniers-Decoen, *Bull. Biol. France Belg.* **89**, 259 (1955).
3. A. L. Burnett, *J. Exptl. Zool.* **140**, 281 (1959); D. Slautterback and D. Fawcett, *J. Biophys. Biochem. Cytol.* **5**, 441 (1959); A. Hess, *The Biology of Hydra and Some Other Coelenterates*, H. M. Lenhoff and W. F. Loomis, Eds. (University of Miami, Coral Gables, 1961), p. 467; A. L. Burnett, *Ann. Soc. Zool. Belg.* **90**, 295 (1960).
4. Mann Research Laboratories, New York.
5. This whole mount technique is extremely sensitive for the detection of interstitial cells. For example, in *Hydra pirardi*, which loses its interstitial cells during sexuality, we have detected as few as two interstitial cells which had escaped the sexual process and had not differentiated into gametes. Digestive cells are readily recognized by their enclosed algal bodies whose nuclei stain intensely; mucous cells are detected by the presence of high metachromasia in the cytoplasm, and gland cells by their large zymogen granules. The stain might also be considered specific for nematocysts, because in whole animals, these structures stain blue-black and stand out vividly in the tissues. To test the ability of the whole mount method to detect small amounts of epidermis, several fragments of the gastrodermis were purposely contaminated with epidermis. Epidermal cells showed up quite distinctly. In addition, any contaminating epidermis that was present collected and formed a clear cap around a small portion of the border of the fragment. The epidermis did not spread out and form a uniform layer around the mass of gastrodermis.
6. P. Weiss, *Principles of Development* (Holt, Baltimore, 1939), p. 93.
7. Supported by the National Science Foundation and the U.S. Public Health Service.

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A Remarkably Reduced Vascular Plant in the United States

Abstract. *A clone-forming thallus lacking sex organs and propagating by gemmae occurs on rocks and tree trunks in the Appalachian region from Georgia to Virginia, Ohio, and Kentucky. Although bryophytic in appearance, the thallus is identified here for the first time as a greatly reduced variety of the vascular plant known as "shoestring fern," Vittaria lineata (L.) J. E. Smith, or a closely related species. The normally dominant sporophytic phase of the life cycle has been eliminated and the plant exists only as a vegetative prothallus.*

Evolutionary reduction—the abbreviation or loss of organs and organ systems—has long been familiar to students of vascular plant morphology and phylogeny. The example to be reported here, however, is so extreme that the plant concerned, even though widespread and locally abundant in many localities in the Appalachian region, has been neglected in catalogues of the vascular floras of the majority of states where it occurs, with but rare

exceptions (1). Those field observations which have been made come almost exclusively, in fact, from bryologists.

The sterile thallose plants are made up of delicate, pale-green, ribbon-like branches, about 0.5 to 2.0 mm wide, and 1.0 to 10.0 mm long. They form overlapping masses of varying thickness and breadth in quartzitic or sandstone rocks, or on the bark of trees. Originally recognized as a probable fern gametophyte (2), the thallus has been little if at all noticed by tracheophyte botanists. The reproduction of the plant is entirely apomictic by means of narrowly spindle-shaped gemmae or brood bodies borne along the margins of the thallus. No gametangia have ever been found, either in nature or in cultures. The gemmae are presumably carried by the wind or animals or both to the appropriate shaded niches where they germinate and maintain the plant over a geographical range that extends from localities in Georgia, South and North Carolina to as far as Tennessee, Kentucky, Ohio, and Virginia. Thought first to be the possible gametophyte of the filmy-fern genus *Hymenophyllum* (1), it has recently been compared (3) with materials of the prothallus of *Vittaria lineata* (L.) J. Sm. from Dade and Sumter Counties, Florida. There is agreement in all respects, except for the absence of gametangia and young sporophytes in the Appalachian gametophyte.

The determination of the taxonomic relationship of the plant in question rests upon the following comparisons. Its habitat is shaded rocks and tree trunks. The sporophytes of *Vittaria lineata* are well known on trunks of trees in Florida, and they have been reported on quartzitic rock crevices as far north as Lincoln County, Georgia (4). The plant body is a noncordate, branching, ribbon-like thallus, and one cell in thickness. The cell shapes and the thallus outline are apparently indistinguishable from those of the gametophyte of *V. lineata*, as observed in the living materials from Florida, and as described by earlier authors (5). It occurs in populations comprising mats 10 to 100 cm across of intertwined thalli. The extensive sterile mats of prothalli are well known in *V. lineata* in various regions of Florida and the Caribbean (5). The rhizoids are delicate, simple, colorless hairs, arising from superficial cells. The rhizoids of *Hymeno-*

phyllum differ from those of *Vittaria* in having heavy brown walls, in their tendency to branch, and in originating from marginal cells. The gemmae are narrow, spindle-shaped filaments composed of four to ten cells, with greatly reduced terminal cells, the latter with fewer or no chloroplasts. In the filmy-fern *Hymenophyllum*, prothallial gemmae are recorded for only a minority of the known gametophytes, and these are sharply different in morphology, being oval, two to four or more cells wide, and composed, when mature, of 20 to 40 cells (6). The sterigmata are made up of marginal and submarginal cells, spherical or ovoid in form, lacking chloroplasts usually, and they show one or more darkly colored abscission layers. These cells, which produce the gemmae, differ in several respects from the gemmiferous structures known in other fern families (for example, Hymenophyllaceae). Stem scales of the sporophytes have secondary, dark, wall thickenings; the leaves are spatulate to linear. Although no naturally occurring sporophytes have ever been found attached to the Appalachian gametophyte, abnormal apogamous buds arising after 2 years in artificial cultures of this plant (7) revealed scales with heavy brown walls and, rarely, narrow simple leaves. "Clathrate" scales or paleae (scales with dark secondary wall thickenings) are characteristic of Vittariaceae, but are unknown in the Hymenophyllaceae, in which the trichomes are only uniseriate hairs.

That the widespread Appalachian gametophyte is probably a prothallus of *Vittaria lineata* is not necessarily surprising. The gametophyte in either the sexual or asexual condition has apparently been observed by botanists in the Caribbean region since as early as 1741 when Dillenius first described it (in *Historia muscorum*) as "Lichenoides gelatinosus tenue reticulatum"; the plant later designated as "*Riccia reticulata* Swartz 1798" is evidently the same (5). It seems to be an established fact that the gametophytes of *Vittaria* can maintain themselves independently even in the tropical and subtropical regions where the sporophytes abound.

The species relationships of the Appalachian gametophyte are most likely with *V. lineata*, of which, indeed, it is probably an exceedingly reduced taxonomic variety. This species, the shoe-string fern of the West Indies and the

warm regions of North and South America, is by far the most common and widely distributed of all the New World members of the genus and family. Except for an isolated record of *Vittaria filifolia* from Florida, *V. lineata* is the only species which occurs in the United States and in close proximity at Graves Mountain, Georgia, to the geographical area of the sterile prothallus in question. The latter extends its range hundreds of miles beyond the area of the sporophyte, and is evidently much more tolerant of cold winters.

Thus we are dealing with a taxon (probably a derivative of a still-living species, or possibly a related plant, now extinct) which represents a remarkably reduced vascular plant. So far as has been determined, the Appalachian gametophyte has lost its ability to form gametangia; and the entire sporophyte generation has been excluded from the natural biology of the plant. Whether it proves to be a genetically distinct geographical variety of *Vittaria lineata*, as is most likely, or a separate species of the same or a closely allied genus, it seems to hold the current record for maximal over all reduction among the vascular plants of the United States. It should be interesting to discover whether forms of pteridophytic plants in other lands have similarly eliminated the sporophytic phase of the organism, and exist now only as simple, gemmiferous thalli, distributed long distances beyond the sporophytic members of the same genus and family (8).

WARREN H. WAGNER, JR.

Department of Botany,
University of Michigan, Ann Arbor

A. J. SHARP

Department of Botany,
University of Tennessee, Knoxville

References and Notes

1. E. T. Wherry, *Guide to Eastern Ferns* (Science Press, ed. 2, Lancaster, Pa., 1942), pp. 70-71; H. H. Vannorsdall, *Ferns of Ohio* (Wilmington, Ohio, 1956).
2. By A. J. Sharp, in 1930, and subsequently by bryologists L. E. Anderson, M. Fulford, and R. M. Schuster.
3. By W. H. Wagner, Jr.
4. J. H. Pyron and W. H. Duncan, *Am. Fern J.* 29, 142 (1939).
5. E. G. Britton and A. Taylor, *Bull. Torrey Bot. Club* 8, 185 (1902).
6. K. Goebel, *Ann. Jard. Bot. Buitenzorg* 7, 74 (1887), Figs. 77, 78, 99, 107.
7. A. G. Stokey, personal communication.
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