VI. Polypodiidae: the Leptosporangiate Ferns

We now take up the leptosporangiate ferns, subclass Polypodiidae, comprising the vast majority of extant fern diversity. As currently circumscribed, Polypodiidae includes seven orders: Osmundales, Hymenophyllales, Gleicheniales, Schizaeales, Salviniales, Cyatheales, and Polypodiales. Polypodiales is the largest of these by far: most of the New England ferns with which you are familiar belong to this order. Polypodiids have megaphylls that bear leptosporangia either abaxially or, less commonly, on the margin of the leaf. They are all spore-dispersed.

A. Polypodiidae: the Leptosporangiate Ferns, Vegetative Features

Now that you have gotten a feeling for the eusporangiate ferns, it's time to focus on the leptosporangiate subclass, Polypodiidae. This, the largest and most diverse of the fern clades has a fossil record going back at least to the Permian period, but its living representatives present ample evidence that present-day speciation is continually yielding new evolutionary entities. To begin with, here is an overview of the Marattiales and the Polypodiidae and their characters.

Features shared by the eusporangiate ferns (Marattiales) and the leptosporangiate ferns (Polypodiidae):

> megaphylls, circinate vernation, sporangia abaxial on megaphyll (or a transformation of this feature), no secondary growth, spores dispersed (not retained to germination), homospory

Features unique to the leptosporangiate ferns:

> leptosporangia (tapetum plus one wall layer, versus two wall layers) with few spores per sporangium, stalk long and slender, antheridia few-celled, protruding (not sunken in the tissues of the gametophyte)

1. Vegetative Features of the Polypodiidae

You are virtually surrounded by a diversity of megaphyllous, non-woody, leptosporangiate plants - that is Polypodiidae. Look at the leaf design of the various types to appreciate their variety. DIAGRAM two different leaves. Label the diagrams indicating the dissection and venation type of each leaf.

a. Phlebodium aureum - Use this plant to focus on basic leaf design and the boundary between stem and leaf. The stem of this plant is covered with papery, tan scales, which are thought to have a role in the retention of water around the stem axis. This creeping stem, called a *rhizome*, gives rise to leaves and adventitious roots as it grows.

The leaves have green stalks called *petioles*. The petioles may at first appear like stems, however their vasculature is bilaterally symmetrical, like leaves, not radially symmetrical, like stems. The leaf blade itself, called a *lamina*, is deeply dissected - we speak of this leaf design in Phlebodium as *once-pinnatifid*. Note that the venation of the lamina is *anastomosing*: the veins diverge and then come together again in a net-like pattern.

b. Now compare Phlebodium with several other ferns in the lab to gain an idea of the diversity of leaf design within the limits apparently imposed on ferns.

i. Cyrtomium: *once-pinnate*, the pinnae broad and their veins anastomosing like Phlebodium, not free like Pteris.

ii. Pteris: *once-pinnatisect* (note the pinnae or leaflets are completely separated from the midrib of the leaf only at the base); basal segments of the pinnae strongly developed, resembling the rest of the pinna

iii. Microsorium: leaves *entire* (undivided).

iv. Doryopteris: leaves *palmately* designed, lobed, not fully dissected into leaflets, giving rise to small plantlets asexually

c. Many ferns have specialized means of producing new plants asexually, that is without meiosis and syngamy: look at three other examples in the lab:

i. Asplenium: plantlets arise on leaf axes
ii. Tectaria: same
iii. Nephrolepis: thin, leafless stems called stolons grow away from parent plant to establish new sporophytes.

d. Stems and Steles

Now is the time to make your own personal sections of a stem in order to get a firsthand understanding of the typical fern stele, in this case a siphonostele with overlapping leaf gaps (otherwise known as a *dictyostele*). Use the living stems of Onoclea sensibilis available in the lab. Make sure you choose a section at least 2 inches long that includes at least two leaf bases.

i. Identify the surface features: remnants of last year's leaves, branching roots, and a cluster of young, coiled leaves (called *croziers*) at the stem apex (you may not get an apex, so look around until you see one.)

ii. Make a clean transverse section through an internode with a new razor blade. You should be able to see a broad pith, a group of vascular bundles in a cylinder near the periphery, a narrow cortex, and a darkened outer region.

iii. Now make a series of similar sections up through the attachment point of a leaf. Lay them out in a series on a microscope slide, so that you know both the order and the orientation of the sections. (It will be easier to keep your sections in the proper orientation if you notch the top of each one with a razor blade.) There will be little difference between adjacent sections, but changes add up. Your teaching fellow will explain the method in more detail.

iv. Once you have your sections, DIAGRAM about six in order, in the same orientation, in your lab book. The goal here is to trace the changes in the vascular bundles, leaf traces, and root traces from section to section, so position is critical for your diagram. You also need to be able to tell vascular bundles from leaf traces from root traces. Here are some hints:

- Make a couple petiole sections in order to learn the appearance of the leaf traces - they usually come in pairs.

- Root traces are single, and they are in longitudinal section in your sections, because they are placed more or less perpendicular to the stem axis.

- The vascular bundles lie in one ring in the stem center.

Now look at prepared slides of Polypodium rhizome transverse sections, in order to see cell detail in one of these stems. Focus on a single vascular bundle (Fig. 15) in the stem at medium or high power.

i. Identify the tracheids: angular cells that have red-staining, lignin-rich cell walls.

ii. Surrounding this xylem is a complete ring of phloem: these bundles are *amphiphloic*.

iii. An endodermis is clearly visible around each vascular bundle.

iv. Locate the *leaf gaps*. (Reread the next to the last paragraph on page 43 to remind yourself what a leaf gap is.)

Make a DIAGRAM of a whole stem section. Be sure to label leaf gaps and leaf traces. Now make a DRAWING showing a close-up of a vascular bundle.

Fig. 15

B. Polypodiidae: Reproductive Features of the Sporophyte Phase

Almost all of the Polypodiidae have sporangia abaxial on the megaphylls and organized into small to large groups called sori (singular *sorus*). The sori vary in shape and position, and the sporangia vary in structure - these sorts of variation have been traditionally used to distinguish the major groups within the Polypodiidae.

We will begin by studying the sorus and sporangia of one species - Cyrtomium falcatum (the Holly Fern) carefully. Then we will go on to compare it with other ferns.

Fig. 16

1. Look closely at a fertile leaf of one of the Cyrtomium plants in the lab (Fig. 16). The sori are abaxial in several rows on the undersurface of the leaf and protected (at least when young) by a tiny outgrowth of the leaf by a tiny umbrella-shaped outgrowth of the leaf called an *indusium*,

2. Now look at prepared sections of the Cyrtomium leaf with sori.

a. Identify the upper and lower leaf epidermis and the spongy and palisade mesophyll (typical of most leaves). The palisade layer - of thin, close-packed cells - is adaxial in the leaf; the spongy layer, of isodiametric, loosely-packed cells, is abaxial in the leaf. Stomates should be visible leading from the spaces among the spongy layer cells out to the leaf exterior.

DIAGRAM a leaf in cross section (indicate the abaxial side); DRAW a sector that includes a sorus. Choose a slide that shows a good medial section through the stalk of the indusium.

b. The sori are on the abaxial side of the leaf, as you can see from their position relative to the spongy layer.

c. Note that the indusium has a stalk (that is a supporting axis like the handle of an umbrella) and a flange (that is the protective part comparable to the umbrella's shelter). Both the indusium and sporangia are attached to a little mound of tissue containing tracheids called the receptacle.

d. The sporangia are of different ages. Some are large and old and contain mature spores. Others are small and hardly developed. The mature sporangium has prominent red-staining cells in a single row that make up what's called an *annulus*, which responds to drying by forcing the sporangium open, then snapping it shut, to disperse the spores. The mature sporangia contain monolete spores in the genus Cyrtomium.

Remember this is a leptosporangiate fern. Even in the younger sporangia, the sporangium wall is one cell thick, not counting the tapetum. This kind of sporangium also typically has a small number of spores, in this case 32, and develops from a single initial, not from a large group of cells. And each sporangium is borne on a long, thin stalk.

3. The submarginal sorus and false indusium of Pteris and Pellaea

Pteris, another greenhouse fern, has an entirely different sorus from Cyrtomium, though the structure of the sporangium is virtually the same.

a. Look at the living plants of Pteris in the lab. Notice that the sorus, though abaxial, is near the margin (submarginal), and that the only protection for the sorus is the incurled margin of the leaf - called a *false indusium*.

b. Look at prepared slides of Pellaea, a genus with sori very much like those you just observed in Pteris. You can see that the indusium is false; it's only an extension of the leaf margin. Notice the trilete spores. DIAGRAM the Pteris leaf with sorus in cross section.

B. Polypodiidae: Gametophytes and Gametangia

Most leptosporangiate ferns have a surface-growing, thin, green gametophyte with a meristem located in an apical notch. According to the classical (but not necessarily common) story of gametophyte development, the antheridia are produced among the rhizoids soon after spore germination, and the archegonia are produced near the notch later in development.

1. First, take a living fern gametophyte from the supply and share it with a lab companion. Mount it in water, with the rhizoids up (ask for help in telling the rhizoids) on a slide and drop a coverslip on top.

Add a little more water to the side of the coverslip from a dropping bottle if there is air under it. Take a look under low power of the compound microscope.

2. First, concentrate on form. One end of the gametophyte is covered by the hair-like rhizoids, which are water-absorbing cells. These are older gametophytes, so they have a well-organized notch meristem at the end way from the rhizoids. Look for the single, wedge-shaped apical initial cell sitting right in the deepest part of the notch.

The gametophyte is flat and very thin. Between notch and rhizoids is a thicker central region called the *cushion*. To either side of the cushion are thinner regions called the *wings*.

3. Now search the cushion region near the notch meristem for archegonia. They look like tiny fingers sticking up from the cushion.

4. Now look around your gametophyte at lower power. You may see little swimming things - which are indeed sperm. See if you can locate the source of these sperm in a small, spherical antheridium near the rhizoid end of the gametophyte. If some of the sperm have not yet begun to swim, look at them under high power to see that they are helical and multiflagellate. If you don't see antheridia and sperm in your slide, look at someone else's.

5. Finally, look at stained slides of Adiantum gametophytes. In these slides, you can see the structure of the antheridia and archegonia much more clearly. Young antheridia, composed of only a few cells, and

mature antheridia, full of spermatozoans, should be visible. Notice that these antheridia are standing out from the surface of the gametophyte, not sunken in the cushion - a characteristic of only the Polypodiidae among the ferns. The archegonia have a neck protruding above the surface of the gametophyte and a dark-staining egg cell hidden beneath in the cells of the cushion. The younger archegonia have intact neck cells; in the older archegonia, the neck cells have disintegrated to open a canal into the egg cell for the spermatozoans.

Make a DRAWING of an antheridium and an archegonium for your notebook.
DIAGRAM a gametophyte to show the location of these organs.