

Laboratory 12 - The Eusporangiate Ferns

The ferns are typically segregated into two general groups: eusporangiate and leptosporangiate ferns. The extant eusporangiate ferns are placed in two orders differing widely in their morphology: the Ophioglossales and Marattiales.

I. Ophioglossales - *Botrychium*

A. Organography

Observe herbarium specimens of the following species of *Botrychium* as available:

Botrychium biternatum
B. dissectum
B. lunaria
B. virginianum

Identify the sterile and fertile portions of each specimen. Make a careful observation of how the sporangia are borne on the shoot. The most advanced members of the genus are generally considered to be those that show a reduced level of branching within the aerial portions. Place each of the above specimens into a typological series leading from the seemingly most primitive species to those which appear most advanced. Observe a number of specimens of *Botrychium virginianum* to familiarize yourself with the amount of variability that occurs within this single species.

What two morphological theories have attempted to account for the unique aerial organography of *Botrychium* and other Ophioglossales? Which seems the most probable based on the available evidence?

B. *Botrychium* stem and rhizome

Observe a slide showing a bud of *Botrychium* and observe the special demonstration slide of a median longitudinal section of the apex. The apical bud of *Botrychium* is unusual because it contains the numerous primordial buds for leaves that will not mature for several years. Locate the leaf primordia, and if possible, identify the shoot apex and apical cell. (If you really see the apical cell, please share it!)

Next, observe a prepared slide showing a transverse section of the rhizome. Notice that the primary vascular tissues are arranged into an ectophloic siphonostele--that is, a continuous cylinder of phloem outside the xylem. A pith is located in the center. You will probably not see a ring of vascular tissue because of the presence of leaf gaps. To familiarize yourself with this, attempt to diagram what your slide shows in a three-dimensional drawing. In many cases, the rhizome of *Botrychium* possesses a reasonable amount of secondary growth. Locate the secondary xylem, secondary phloem and, if possible, periderm. If you have any additional time, you might like to examine a cross section of the rhizome of *Helminostachys*. How different is this from *Botrychium*?

C. *Botrychium* sporangia

Observe a pickled specimen of the fertile shoot of *Botrychium* and place it under the microscope for observation (don't chop it up as there isn't a lot of material). Notice that the eusporangia are separate and appear stalked and are not associated with strobili. Notice the large number of sporangia on the fertile shoot (pinna). Obtain a prepared slide showing sections of the eusporangia. From how many cells does this sporangium arise? How many wall layers are present in the mature sporangium? Is *Botrychium* homosporous or heterosporous? About how many spores are produced per sporangia, but please don't count them (about 2,000)?

D. *Botrychium* gametophytes and embryogenesis

Within the Ophioglossales, gametophyte development is strictly exosporic. Observe the organization of the archegonium and young embryo in the prepared slide on demonstration. Is it possible to locate archegonia or antheridia? Do they occur on the same plant at the same time? How big is the archegonium? How about the embryo? You might like to refer to photographs of the subterranean tuberous gametophyte in Bierhorst to orient yourself. How does embryo development occur within the genus *Botrychium*? Is it endoscopic or exoscopic? For a precise answer to this question, examine Bierhorst, p. 148. Nowhere else within a single order is there so much variability of embryo development as is found in the Ophioglossales.

II. Ophioglossales - <i>Ophioglossum</i>
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A. Organography

Observe herbarium specimens of the following species of *Ophioglossum* as available:

Ophioglossum crotalophoroides
O. engelmannii
O. vulgatum

Notice that the general organography of *Ophioglossum* is structurally similar to that of *Botrychium* but outwardly displays a greater simplicity in both its sterile and fertile areas. In what ways does the external organography of *Ophioglossum* seem to be more advanced than that found in *Botrychium*? A spike? Venation patterns? Gifford and Foster contend that the larger epiphytic species of *Ophioglossum* are more primitive than the smaller, inconspicuous types. What do you think?

B. *Ophioglossum* stem, root and stipe

Obtain a prepared slide showing the transectional anatomy of the above organs (there are several, but please share). Observe the rhizome first and notice the ectophloic siphonostele, as is present in *Botrychium*. Is secondary growth present? Next, examine the anatomy of the stipe. Notice the discrete vascular bundles. These will later divide, some branching out into the fertile segment of the shoot system and some into the sterile segment.

C. *Ophioglossum* fertile spike and sporangia

Obtain preserved material showing the fertile spike of *Ophioglossum* and observe it under the dissecting microscope. Notice that, in contrast to *Botrychium*, the eusporangia are embedded within the fertile spike tissue. Obtain a longitudinal section of the fertile spike (a synangium?) and draw the

details of sporangial structure, especially noting, if possible, how each sporangium receives its own vascular bundle. What might this indicate about the phylogeny of *Ophioglossum*?

III. Marattiales

The Marattiales include all of the fern-like plants that produce eusporangia on the abaxial face of the leaf. The Marattiales have a number of features in common with the Ophioglossales--including eusporangia, similar ontogeny of archegonia and antheridia and the late differentiation of embryonic organs. Because the vascular system is so complicated--a polycyclic dictyostele with commissural strands--it would be best to forego the vascular system at this time and concentrate on the unique reproductive morphology.

A. Organography

Observe the following herbarium specimens of these members of the Marattiales as available:

Angiopteris evecta
Danaea crispera
Marattia salicina

For each of the above, note the fern-like foliage and also the amount of dissection of each individual frond. For each of the above, sketch the nature of the sporangia, noting whether they are separate, slightly fused, or totally synangiate. What is the method of dehiscence for each type (see Gifford and Foster, Fig. 12-15). On which surface of the leaf are the reproductive structures found. Observe the herbarium specimens and photographs available in the lab. Notice the hard, thick stipules which are present at the base of the leaves. Certain genera of Marattiales can reach more than a meter in height.

B. Synangial organization

Obtain longitudinal prepared slides showing the structure of the synangia in *Angiopteris*, *Christensenia*, *Danaea* and *Marattia*. How many wall layers are present? What is the morphological origin of the wall of the synangia in *Marattia*? Are the spores homosporous or heterosporous? What type of gametophyte will eventually be formed? What will the initial polarity of the embryo be? What characteristics link the Marattiales with Ophioglossales? Which with the Filicales?