

Laboratory 15 - Leptosporangiate Ferns: Transitional Groups and Heterosporous Ferns

I. Osmundales

Although the systematic position of the Osmundales remains somewhat unclear, much evidence suggests that the order is an early offshoot of the filicalean line and that modern Osmundales represent a phylogenetic dead-end -- possibly somewhere between the eusporangiate, and truly leptosporangiate ferns. The group is well-represented in the fossil record from the upper Jurassic to present. Observe the organography of the herbarium specimens of *Osmunda* in the laboratory. What is the morphological nature of the structure bearing the sporangia? Does the structure represent a pinnule, pinna, or frond? What does the degree of variability in fertile frond organization suggest about the evolutionary position of members of the Osmundales? Of the group in general? Does the vegetative structure of the plant resemble the typical filicalean fern? Obtain a prepared slide of the rhizome of *Osmunda* and examine the organization of the vasculature. What type of stelar organization does this display? How does the organization of the vasculature differ from that of the typical eustele? What is the position of the phloem? Now examine the structure of the unusual sporangium of *Osmunda*, looking especially at the number of wall layers composing the sporangium and the numbers of spores produced. Is this typical of a leptosporangiate fern? What is the organization of the annulus? How does this sporangium resemble a leptosporangium? How does it resemble a eusporangium?

II. Psilotales

One of the most perplexing questions in plant morphology concerns the systematic position of the Psilotales. Among morphologists, Bierhorst is perhaps the major proponent of the idea that these represent primitive ferns; however, other morphologists have remained quite skeptical. Certainly, a comparison between *Psilotum* and any advanced filicalean fern would lead to the conclusion that they are not closely related. Come to your own conclusions on this matter by examining the living material of *Psilotum nudum* and preserved materials of *Tmesipteris*, the only other genus in the order. How do these species resemble each other? How do they differ? Observe cross sections of the rhizome or aerial stem of *Psilotum*. What type of stelar organization does this represent? Is this specimen exarch, mesarch, or endarch? Is there an endophytic fungus present? Now observe the reproductive organography. Does the mode of insertion of the sporangia differ in *Psilotum* and *Tmesipteris*? Are there significant differences in the organization of their synangia? Observe a cross section of each. What is the organization of the sporangium? Did you notice the unusual tapetal tissues? How does the sporangium of *Psilotum* dehisce? Is an annulus present? Does it function?

III. Marsileales

Observe the living specimen of *Marsilea vestita*. This order of aquatic, heterosporous ferns is characterized by bisporangiate sori located within the sporocarp. The plant is organized into an underground rhizome and floating or emergent leaves. For each of the above, locate the frond, rhizome, and fertile pinnae (sporocarps). Briefly examine a cross section of the rhizome of *Marsilea* to note the conspicuous aerenchyma within the submerged stem. Then turn your attention to the sporocarps in living and preserved material. Consider the morphological nature of the sporocarps. Do they represent an entire simple pinna, a compound pinna, or entire frond? What is considered to be the morphological nature of the two bumps on the sporocarp? Examine closely the positional relationships of the sporocarp and frond. Now obtain a living sporocarp, scarify the outer wall of the mature sporocarp and immerse it in water. What happens? How does the gelatinous tube arise

during dehiscence? What is the arrangement of the sori on the gelatinous tube? Observe a single sorus and examine it closely. Notice both the microsporangia and megasporangia. Are they located on the same sorus? First observe the megasporangium. How many megaspores are produced in each megasporangium? Did you notice the organization of the megaspore into a resistant walled structure with an expanded apical protrusion at the apex? Did you see the sperm lake and gelatinous coat located around the megasporangium and at its apex? Maturation of the megasporangium takes approximately 22-24 hr. Obtain a sorus of a sporocarp which germinated two days ago. Can you identify the archegonium or early stages in embryo development? Are sperm present?

Now obtain a prepared slide of a longitudinal section of a young sporocarp of *Marsilea* and locate the developing microsporangia and megasporangia. Notice the chambering around each sporangial group--what does each of these chambers represent? Notice the arrangement of micro- and megasporangia on the receptacle. Are the megasporangia consistently borne at the apex? It may be necessary to look at a number of slides in order to appreciate the developmental sequence of each of these types of sporangia. Where is the gelatinous tube located within the sporocarp? Now observe the structure of the newly released sperm cells. How much time is required after sporocarp opening is required before the release of the sperms? Are these multiflagellate sperms more complex than others in the homosporous ferns? Next, observe the young embryos. How old are these? Notice the young first leaf, and if possible, the sheath (or calyptra), rhizoids, foot, and root. How does the duration of embryonic development compare with that of the homosporous ferns? With that of the angiosperms?

IV. Salviniales

The Salviniales include the most highly reduced of the heterosporous ferns and possess functionally monosporangiate sporocarps. Obtain living and preserved material of the following:

Azolla sp.
Salvinia sp.

For each describe and draw its organography. What are the most obvious adaptations of these plants to the floating habit? Did you notice the "egg-beater" trichomes on the *Salvinia*? Note especially the phyllotaxis, presence or absence of a rhizome, dorsiventral lobing of the leaf. Is there an evident branching pattern? What is the morphological nature of the structures which appear to be roots in *Salvinia*? In *Azolla*? Do the roots originate from the same place in both genera?

Search the living and preserved material for the presence of sporocarps. Note the position of the sporocarps. How does the insertion and numbers of sporocarps per frond compare with that in the Marsileales? The Salviniales differ from the Marsileales in that each sporocarp itself is indusial in nature and contains only megasporangia or microsporangia at maturity. The smaller mature sporocarps contain a single megasporangium with a single megaspore, whereas the larger sporocarps contain numerous microsporangia. Obtain a prepared slide of a longitudinal section of a sporocarp of *Salvinia* or *Azolla*. A gradation in maturity of the sporocarps will be evident in the section, with a number of developmental stages evident. Find the organization of the mature microsporangium and determine the location of massulae and glochidia, if present. What is the origin of the massulae? Would the separation of male and female sporocarps represent a selective advantage over the bisporangiate reproductive system of the Marsileales?