Laboratory 6 - Reproductive Morphology of *Lycopodium* and *Selaginella*

I. *Lycopodium*

A. Morphology of *Lycopodium* strobili and subgenera

1. Subgenus *Huperzia* (old *Urostachya*)

Observe herbarium and preserved specimens of the following species of *Lycopodium*, subgenus *Huperzia* (ref. Gifford and Foster, p. 123), as available:

*Lycopodium lucidulum*
*L. selago*
*L. reflexum*
*L. alopecuroides*

All of these species show strobili which are not compact and merely represent fertile areas of the stem. Arrange these specimens in typological order, if possible, starting with the least specialized strobilous organization and proceeding to the most specialized. What vegetative features do these *urostachyoid* *Lycopodium* types have in common? Obtain pickled material of *L. lucidulum* if fresh strobili are not available. Locate the sporangium, the sporophyll and the shoot apex. Is a ligule present? Obtain and observe a prepared slide of the strobilus of *L. lucidulum* or *L. selago*. Describe the shape of the sporangium. Is the sporangium located on a stalk? Is the line of dehiscence obvious? Is it longitudinal or transverse? Carefully remove a single sporophyll from a plant and observe it under the dissecting microscope. Is the morphology of the sporophyll the same as that of a vegetative leaf? Is the phyllotaxis of the sporophylls similar to that of the vegetative leaf? Is the genus *Lycopodium* heterosporous or homosporous?

2. Subgenera *Lycopodium*, *Diphasiastrum* and *Lycopodiella* (old *Rhopalostachya*)

Observe herbarium and preserved specimens of the following species of *Lycopodium* as available:

*L. annotinum*
*L. cernuum*
*L. clavatum*
*L. complanatum*
*L. inundatum*
*L. obscurum*
*L. pachystachyon*

These species exhibit strobili which are very distinct and not merely fertile, little-differentiated areas of the shoot. The most primitive of these are those in which the condensation of the sporophyll section is not completed and thus a distinct stalk and strobilus are not differentiated. Arrange the above specimens in a typological sequence from the most primitive to most advanced. Now compare the most primitive of this group with the most advanced of the *Huperzia* species. How close is the intergradation? Examine all the true strobilate species and determine how they differ vegetatively from the *Huperzia* species. Obtain a strobilus of one of these "rhopalostachyoids" that has been preserved. Make a longitudinal section along the length of the strobilus. (You might also wish to observe a prepared slide of one of these species!) Are the sporangia dorsal or ventral with respect to
the sporophyll? Is this adaxial or abaxial? Turn your free-hand section over so that the flat side is down and remove a sporophyll to expose a sporangium. Draw the sporangium, noting its shape. Can you observe the possible line of dehiscence in this sporangium? Is the sporangium stalked? Save this strobilus for use later in the laboratory.

The three genera in this group are *Lycopodium*, *Diphasiastrum* and *Lycopodiella*, according to Gifford and Foster, p. 123. Morphologically, how do these subgenera differ? Which is most advanced? Which is most primitive? Are there also gametophytic differences? Which species above belong to which subgenera?

B. Anatomy of the strobilus, sporangia and spores

1. Structure of the strobilus

In section A we learned that the strobilus is a condensed fertile shoot or portion of a shoot. Obtain a prepared slide of a longitudinal section of the strobilus and locate a sporophyll with its associated sporangium. Is the sporophyll vascularized? Describe the shape of the sporophyll. In your species, is there an abaxial extension of the sporophyll above which covers the sporangium? Does this extension occur in all *Lycopodiums*? Does the type of dehiscence change with the presence or absence of an abaxial flap? Is the sporangium stalked? Are the spores all similar (homosporous)? Are spores similar between different sporangia? Is the tapetum plasmodial or cellular? Draw the structure of the sporangium noting wall layer, spores and stalk.

2. Ontogeny of the sporangium

Observe a demonstration slide of *Lycopodium* (m.l.s.) showing the development of the eusporangium. Beside this prepared slide is a photograph indicating sporangial ontogeny, also shown in Fig. 9-9. Can you tell if the sporangium arises from a single cell or patch of surface initials? How many layers of cells are involved in this ontogeny? At what point are the sporogenous cells derived? From what group of cells is the stalk derived? How many wall layers are present at maturity and are all of these present at all stages of ontogeny? In the slide of the *Lycopodium* species you observed, in the bottom row of sporophylls, can you see the entire sequence of early sporangial development? Diagram this sequence as it demonstrates a good, representative example of eusporangiate ontogeny.

3. Spores

Study the ontogeny of spore development in a prepared slide of *Lycopodium*. After you have an idea of spore development, scrape some spores out of a strobilus. Prepare a wet mount slide of these spores and observe them under the compound microscope. Can you locate the triradiate ridge? Why do spores like these have triradiate ridges? (SEM of a spore and its organization in a tetrad is shown in Fig. 9-11, p. 118 in Gifford and Foster. Does this ridge serve any purpose in the life cycle? Is the outer layer of the spore sculptured?

C. The gametophyte: antheridia and archegonia.

Since *Lycopodium* is homosporous, the antheridia and archegonia are frequently located on the same gametophyte. Obtain a prepared slide of the gametophyte of *Lycopodium*. Is it endosporic or exosporic? Draw its basic structure. Locate the area containing the endophytic fungus. What is its function? Do all of the *Lycopodium* species have this? How has the presence of the fungus modified the morphology of the gametophyte? Locate the antheridia and examine their distribution on the gametophyte body. Draw their general structure. Try to find archegonia. If you cannot see archegonia on your slide, observe them on a demonstration slide at the front of the room.
archegonia go, it's not too swift. Consult Gifford and Foster for a description of the above-ground type of gametophyte. Which is considered to be advanced, the subterannean or the above-ground gametophyte?

D. The embryo

The embryo of *Lycopodium* is endoscopic—that is, it contains its embryo within gametophytic tissue until, by sheer size, the young sporophyte breaks out of the gametophyte. *Lycopodium* also has a suspensor which acts to push the embryo into the gametophytic tissue. Polarity is established very early in embryo development. It is usually easy to ascertain which areas of the embryo will develop into root, stem, etc. Observe the demonstration slide and photograph. Locate those areas of the embryo which are the presumptive root, foot, leaf, and apex. Is it possible to observe the suspensor? What differences are observable between sporophyte development in the above-ground and below-ground gametophytes.

E. The monotypic genus *Phylloglossum*

If you enjoy rare and esoteric information, undoubtedly the specimen of *Phylloglossum drummondii* will be of interest to you. Although the condition of the specimen is poor, a strobilus, stem and tuber are present. Gifford and Foster briefly mention this reduced genus (pp. 105-107, Fig. 9-1) as a member of the Lycopodiales. Is it considered to be more primitive, intermediate or more advanced than the genus *Lycopodium*? Sections of the tuber are available in the room. How is it organized? Does it have a haplostele, actinostele or plectostele? Where is this genus found as a native? Is it homosporous or heterosporous?

II. *Selaginella*

A. Organography of the strobilus

All members of *Selaginella* have sporophylls condensed into distinct strobili. Observe the following specimens with strobili, as available:

*Selaginella arenicola*
*S. densa*
*S. plumosa*
*S. lepidophylla*
*S. oaxaca*
*S. epirrhizos*
*S. eurynota*
*S. anceps*

For each of the above species, note the position of the strobilus. Is it terminal or lateral? Drawing upon knowledge from previous lab exercises, to what extent is the habitual anisophylly of the vegetative body reflected in the arrangement of sporophylls? Is the arrangement still opposite and decussate? Obtain a pickled strobilus or two of *Selaginella arenicola* or from one of the other living species. Under the dissecting scope, make a careful dissection of the strobilus. Pull off a sporophyll and notice the ligule on the adaxial surface. Remove a sporangium and determine whether its dehiscence is longitudinal or transverse. Is this a microsporangium or megasporangium? Is heterangy present? Can you tell from the outside? What is the segmentation of sporangia in the sporangium—longitudinal or transverse? Are both micro- and megasporangia found in the same strobilus?
B. Anatomy of the strobilus

Obtain a slide of a longitudinal section of a strobilus of *Selaginella*. Locate the sporophyll, ligule and both microsporangia and megasporangia. Is the segmentation of sporangia transverse or longitudinal? Notice the approximate size and shape of micro- and megasporangia. Is the tapetum cellular or plasmodial? Notice the wall of the sporangia. Does it contain thin areas which will become the dehiscence line? To what extent does the abaxial portion overlap the subjacent sporangium?

C. Ontogeny of the sporangium

Observe a prepared slide showing the ontogeny of sporangia of *Selaginella*. How many wall layers are present early in ontogeny? What happens to these layers? Can you identify the tapetal layer of the wall? Is the sporangium stalked early in development? Is it possible to tell at what point the sporangia will differentiate? Does the ligule mature before or after the sporangium? What is the function of the ligule?

D. The gametophyte, gametangia and germination

*Selaginella* is heterosporous. The endosporic gametophytes are not retained within the sporangium but are liberated. We have no material of the microgametophyte or antheridia of *Selaginella*, please refer to Gifford and Foster, pages 139-140 for a fuller explanation of this process. Obtain prepared slides of the megagametophyte showing some stage of archegonial development. Notice prepared slides of the megagametophyte showing some stage of archegonial development. Notice that the megagametophyte breaks out of the spore wall only in response to archegonial development. Can you locate any rhizoids? Observe the three stages on demonstration which show the embryo. Can you discern any embryonic structure such as is shown in Gifford and Foster, Figure 9-13? The embryo, as in *Lycopodium*, is endoscopic with a suspensor. Notice the young sporophyte as it begins to germinate. Now obtain a prepared slide showing the sporophyte at a later stage of development. Locate the shoot apex, the leaves and the root. Can you discern the meristeles at this early stage of development?