Structural Botany
Laboratory 2
Embryogenesis, Germination and Seedling Growth

Plant morphology has a long history and a rich tradition of studies that focus on the structure, development and reproductive biology of vascular plants. In the most restrictive sense such studies are limited to external form, but many modern morphologists incorporate data from external structure, organography, internal anatomy (plant anatomy), growth and development, and reproductive biology to address questions of systematics, evolution, and phylogeny.

In this course, developmental morphology is used to emphasize the basic structural similarities and differences among vascular plants, to help build an understanding of plants as growing and reproducing organisms, and to link growth to the underlying regulatory genetics. This laboratory concentrates on **embryogeny** (embryo structure and development), and on the resulting plant structure and function. The basics of plant anatomy, including information from cells, tissues, tissue systems and organs, were the focus of the first laboratory, which is crucial to our analyses. If you are not confident about your background in this area, consult the structural botany chapters of a general botany text such as Raven et al. or a comprehensive Plant Anatomy book such as Esau (1967). The remaining labs in this course focus on comparative and developmental morphology and anatomy of major groups of living vascular plants plus the extinct species that are crucial to our understanding of homologies and plant phylogeny. The groups are presented in an order that roughly approximates the increasing biological complexity, the order that reflects phylogenetic relationships, and also the sequence in which they appear in the fossil record (and in your text book). Background information is also presented in chapters 1 - 6 of Gifford and Foster (1989) and in chapters 1 and 2 of Halle, Oldeman and Tomlinson's "Tropical Trees and Forests" (1978).

1. Embryogeny and the Vegetative Plant Body

Because the sporophyte of vascular plants develops from an embryo, the basic organization and architecture of the plant is derived from this phase of the life cycle. While embryogeny traditionally has played an important role in systematic studies of the free-sporing pteridophytes (e.g., Chapter 6 of Gifford and Foster, 1989), seed plant embryos all have a common basic structure and mode of development. What may these similarities imply about relationships among seed plants? Embryogeny of seed plants can be summarized by examples from the angiosperms, and is included in Raven et al.

Embryogeny of vascular plants begins when the zygote undergoes the first division to produce a two-celled embryo. In pteridophytes and most gymnosperms the embryo is located within an archegonium. The orientation of cytoplasm and organelles in the zygote, the orientation of the first two cells of the embryo within the archegonium, and the organs that arise from each of the two cells determine the "polarity" of the embryo. Polarity often is consistent within major clades, and is used as one of the characters that define many clades. The different types of polarity for the "seed free" plants and their systematic associations are depicted on pages 68 and 69 of Foster and Gifford, (1989). This reference is on display in the laboratory and you should refer to it when learning how to interpret polarity.

In general, if the above-ground organs arise from the cell that faces the neck of the archegonium (viz., toward the outside of the gametophyte), the polarity is said to be **exoscopic**, but if the above-ground

organs arise from the cell facing away from the archegonial neck (viz., toward the inside of the gametophyte), it is **endoscopic**. In embryos where the first division is vertical, the polarity is said to be **prone**.

In the two-celled embryo, the cell toward the neck of the archegonium may become the suspensor, with the cell away from the neck becoming the **proembryo** (i.e., structure from which the embryonic organs arise). All seed plants are endoscopic and most have a suspensor. In contrast, pteridophyte embryos show a wide range of variation in the details of structure and development. However, all lack cotyledons and they have an additional embryonic organ called a **foot**. The foot is the region of the embryo by which nutrients are derived from the megagametophyte. It occurs as a bulge on the surface of the embryo, but is absent from larger **sporelings** and mature sporophytes. The other embryonic organs of pteridophytes include the shoot apex (axis apex in Psilopsida), the first leaf (in all living groups but Psilopsida) and the root apex (in all living groups except Psilopsida). Unlike seed plants that typically develop a primary rooting system from the embryonic root (viz., radicle), the embryonic root of pteridophytes is short-lived, and rooting in more mature plants is performed by adventitious roots (except in Psilopsida and *Isoetes*). Be sure to familiarize yourself with the general embryogeny of pteridophytes in Chapter 6 of Gifford and Foster. We will study pteridophyte embryos in more detail as we cover each major group.

A)   Dicotyledonous Angiosperms

Examine a prepared slide of the genus *Capsella*. Note that the slide bears developing fruits of several flowers, each of which contains several developing seeds. Within these ovules (which are developing into seeds) you will find various stages in embryo and endosperm development. On this and other *Capsella* slides you should find stages in which the embryo is pear-shaped and borne on a single row of cells--a **suspensor**. Free nuclei of the endosperm will also be present. As the embryo develops and the size increases, the cotyledons become much more prominent and a shoot apex develops (the **epicotyl**) between the bases of the two cotyledons. Also, the lower portion of the embryo becomes an elongate **hypocotyl**, at whose lower tip the primary root will develop from a growing point (the **radicle**).

Slides of somewhat older seeds also are available if your first slide does not have the older stages of embryogeny. Note the disposition of the suspensor and endosperm tissue, and the shape of the mature embryo. Compare what you see in the slides to the figures of developing *Capsella* embryos in Raven, et al.

Now dissect a soaked seed of a bean or pea. Once the seed coat has been removed, only the embryo remains. Carefully separate the two large cotyledons to expose the hypocotyl, radicle and epicotyl. Note that the epicotyl has now grown into two tiny leaves that surround a stem tip. This is termed a plumule. From what parts of the embryo will the primary shoot and root develop?

B) Gymnospermous Forms

The embryos of gymnosperms are basically similar to those of dicotyledonous angiosperms except that they usually have more than two cotyledons. Also, the embryos of gymnosperms are embedded in, and nourished by, tissue of the megagametophyte rather than by the **endosperm** (a fertilization product produced only by angiosperms). Examine a prepared slide of a mature embryo of pine, <u>Ginkgo</u> or a

cycad. Can you identify a suspensor, epicotyl, hypocotyl, radicle and cotyledons? Be sure also to identify the tissue of the megagametophyte.

    B)   <u>Monocotyledonous Angiosperms</u>

    Examine a prepared slide of a transverse section of the ovary of *Lilium*. You will see the three locules, six rows of ovules, and within the nucellar region of these ovules will be early stages in the development of the embryo and endosperm. The embryo will appear as a small cluster of relatively few cells near the micropylar end of the old embryo sac, while the endosperm will consist of scattered, relatively large nuclei lying in a common cytoplasm in the embryo sac. Note that most of the endosperm nuclei are near the margin of the sac. In slides of the later embryo and of the mature seed of *Lilium* you will find the later stages of the Monocot embryo. Among the obvious differences between Monocot and Dicot embryos are that in *Lilium* you will find only one cotyledon (hence the name Monocotyledon), and both the epicotyl (plumule) and radicle are enclosed in sheaths called the **coleoptile** and **coleorhiza** respectively. Figures of *Sagittaria* (pages 608 - 609 in Gifford and Foster, 1989), another Monocot, may be of help in interpreting the embryogeny of *Lilium*. Monocot embryos are also well illustrated in Raven et al.

    Now obtain a soaked seed (actually this is a fruit) of *Zea*, the corn plant. Carefully remove the fused ovary wall and seed coat to expose the large mass of endosperm and the smaller embryo. Identify the **scutellum** (= cotyledon), **coleoptile**, **plumule**, **coleorhiza** and **radicle**. Cut a longitudinal section of the corn fruit and identify the structures in longitudinal section.

2. Unidipolar and Bipolar Growth

    From the embryogeny of pteridophytes and seed plants we now follow growth into more mature plants. These are **sporelings** for pteridophytes and **seedlings** for the gymnosperms and angiosperms. What is the distinction between a sporeling and a seedling?

    The embryos of pteridophytes (except isoetalean lycophytes) have a shoot apex that grows out to form the whole plant (but no primary rooting system). **Adventitious** roots occur along the length of the stems of pteridophytes to root the plants. Seed plants have primary roots that develop from or branch from the radicle of the embryo. All roots that originate in other ways on any organ are termed **adventitious** roots. Typical pteridophyte sporophytes eventually develop a plant that has all of its growth in one direction --**unipolar growth**. In contrast, the seed-plant embryo has an epicotyl that grows upward into the shoot, and a radicle that grows downward to produce the primary rooting system--**bipolar growth**. While some representatives of each group have an architecture that is like that of the other group, the two basic types of embryogeny covered above lead to two basically different modes of growth.

    Examine specimens of the homosporous pteridophyte *Lycopodium* or a similar genus.   See if you can identify the stems, leaves and roots. Does this plant have unidirectional or bipolar growth? Why?

    Examine young seedlings of gymnosperms or dicots. Try to identify the stems, leaves and roots of these. Do they have unidirectional or bipolar growth? From what embryonic structures are each of the organs derived?

Now examine a seedling of *Zea*. Can you identify all of the organs that are present and explain their origins from the embryo? Note that the seedling has all of the organs that occur on the dicot and gymnosperm seedlings, but in addition it has adventitious roots. How can you identify them as adventitious roots rather than branch roots from the primary root? Many monocots have a primary rooting system that only functions until the seedling stage. The primary root then is replaced by a system of adventitious roots.

3. Branching

Most plants produce branched shoot systems. The mode of branching, frequency of branching, angle(s) of branching, and differential rate of growth of branches all contribute to the mature architecture of the plant. In the past few years the **Architectural Analysis** of plants has become an important tool for understanding plants (particularly trees) as biological organisms, and has led to the establishment of a new subdiscipline within plant morphology. For those of you who would like to know more about this subject, there is a review by P.B. Tomlinson (Tomlinson, P.B. 1983. Tree Architecture. American Scientist 71: 141-149) available in the lab.

Branching can be classified either as **apical** or **lateral** (or **axillary**). Apical branching occurs when the stem apex **dichotomizes** to produce two (or more) apical meristems. The products of the **dichotomy** may be nearly equivalent (**equal dichotomy**) or one may grow out larger than the other (**unequal dichotomy**). Sometimes the products of apical branching are so unequal that one appears to be stem and the other a lateral branch (**pseudomonopodial branching**). Apical branching is most common in pteridophytes. Examine the pteridophytes in the laboratory to determine what type(s) of branching characterizes each.

Lateral branching occurs most commonly as the result of a small mound of meristematic tissue being left behind (in the axil of a leaf) by the growing stem apex. The axillary meristem may grow out into a lateral branch immediately (**syllepsis** or sylleptic branching), or it may occur as either a dormant bud or a tiny meristem that is not readily observable. When laterals have this type of discontinuous development, the branching is referred to as **prolepsis** (proleptic branching). Evergreen plants and annual plants that have continuous growth typically display syllepsis. Deciduous plants and those with periodic growth have proleptic branching. Among the examples of living and dried plants in the lab., which display syllepsis and which display prolepsis? What other features associated with each type of branching are found on the plants?

Another type of lateral branching occurs in *Equisetum* and is unlike the branching in any other living vascular plant. In this genus the branches occur on alternate radii from the leaves at each node. Examine branching specimens of *E. arvense* and *E. hyemale*, and compare the relationships of the branches and leaves in these species to those in the other plants with lateral branching. What are the similarities and differences?

In plants with lateral branching, either the stem or the lateral branch can grow out as the main axis of the plant (i.e., the trunk of the tree). When the stem is dominant and the lateral branches are subordinate, the branching is **monopodial**, but when the lateral branch grows out to continue the trunk the branching is **sympodial**. Take a few minutes of lab time to go out in the parking lot and down onto the old river bed across Oxbow Drive. Examine the different trees, shrubs and "weeds" around you. Which are

monopodial and which are sympodial? Can you identify proleptic and sylleptic branching in any? What is the difference between monopodial and pseudomonopodial branching?

The last type of branching that we will consider is adventitious branching. Any bud or branch that occurs as the result of something other than apical or lateral branching is termed adventitious. Many adventitious branches occur as the result of the redifferentiation (or dedifferentiation) of parenchymatous tissue to form an active meristem. Sometimes an axillary bud can be dormant in the cortex of the stem for years before growing out. In such cases it often is difficult to tell if the branch is lateral or adventitious in origin. Buds and branches of equivocal origin are referred to as **epicormic**. Look over the plants in the laboratory to see if any have adventitious branches. Did you find the **plantlets** on *Lycopodium*? What do you think was their origin?

4. Primary and Secondary Growth

Any growth that occurs as the result of continuous cell divisions from the apical meristem of either the stem or the root is considered to be primary growth. Why does this include most of the growth in leaves? Primary growth is responsible for the production of new organs and for increase in length of the stems and roots. Once cell divisions behind the apex have ceased for even a short period of time, primary growth has ended. All additional growth to that part of the plant is secondary. Secondary growth results from the activity of **lateral meristems** or **cambia**. The most common of these are the **vascular cambium** that produces secondary xylem (wood) and secondary phloem, and the **cork cambia** that produce the bark on trees.

A plant like a pine tree or a sycamore tree has secondary growth that increases the girth of the trunk and branches as the plant grows. What would happen to such plants as they grew if there were no secondary tissues? Could they ever become trees? Why or why not? Do you know of any trees without secondary growth?

Most living pteridophytes and most monocots have only primary growth. How do tree ferns manage to stand up without secondary growth? Can you determine from the outside of a plant which parts are the result of primary growth and which are the result of secondary growth?

The above exercise should serve as an introductory guide to the factors of growth that produce the external form of vascular plants. Also, it should prepare you to understand better the similarities and differences among the major groups of vascular plants that we will soon be covering.

References

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