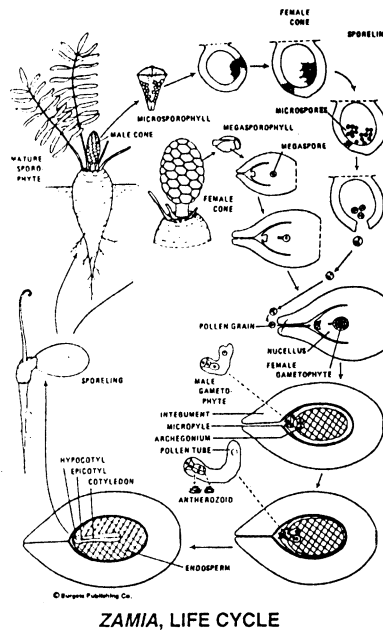


Reproduction in Vascular Plants



Things To Do Before Lab

1. Read this lab text BEFORE coming to lab.
2. As a team, collect a cone from a conifer tree on or near campus. Bring your cone to lab (you'll use it to complete a graded assignment).

Things To Bring To Lab

Make sure you bring your textbook. The photos and figures will help in understanding the material in this lab.

Major Objectives Of This Lab

IT IS MOST IMPORTANT FOR YOU APPRECIATE THAT A *FERN* AND A *PINE TREE* REPRESENT SPOROPHYTES. TODAY, WE WILL LOOK CAREFULLY AT SPORO-PHYTES, BUT WE WILL ALSO EXAMINE GAMETOPHYTES. **YOUR MAJOR GOAL** IS TO LINK THESE TWO GENERATIONS IN YOUR STUDY OF THE REPRODUCTIVE CYCLE OF VASCULAR PLANTS.

1. Focus on details of the life cycles of ferns and gymnosperms.
2. Understand the similarities AND differences in the life cycles of ferns and gymnosperms with reference to Alternations of Generations.
3. Understand the processes and structures that gave rise to the seed plants.
4. Determine the features plants possess that facilitate the movement of plants to a terrestrial environment.

Vascular Plants

In the evolution of plants a major innovation was the development of conducting tissues (a vascular system) to move water and nutrients throughout the plant. This development also resulted in a major explosion of plant size and form since the tissues that made up the vascular system also provided mechanical support. These vascular tissues have lignin incorporated into their cell walls which gave them great strength. The ferns represent the first plants observed in this lab series that have a well-developed vascular system. All plants except the algae and bryophytes make up the vascular plants comprising over 250,000 living species.

Horsetails (*Equisetum* sp.) are a common weed found along the banks of Strawberry Creek. They are easily recognized by their hollow jointed stems, tiny scale-like leaves and terminal cone-like structures (strobili) which bear spores. Their gametophytes are tiny, free-living and bisexual with motile sperm - their life cycle is very similar to that of ferns. They were once considered as fern allies but are now classified under a separate group (Sphenophyta) comprising 15 living species. The morphology of this plant is identical to a 300-million year old fossil plant suggesting that horsetails have an ancient lineage.

Also look at the example of a group of plants which are believed to have evolved before the horsetails. This plant, known as a **whisk fern** (not a true fern) has not yet evolved roots.

During the “Age of Coal” 300 million years ago, seedless vascular plants including the ferns and horsetails were the dominant vegetation type in the swamplands. The gradual accumulation and preservation with time and pressure of this vegetation material as peat eventually became the coal we mine today as a fossil fuel. You will get a chance to view actual fossil remains of plants by making a “peel” from a coal ball during this lab. See the attached “The Peel Technique in Paleobotany” section at the end of this lab.

Ferns

Ferns form the largest group of plants other than the flowering plants numbering about 12,000 species. They are generally small plants growing in woodland habitats under moist temperate or tropical conditions. Some groups, however, occur in mountainous regions of the western states, in habitats that are extremely dry during most of the year. Some ferns can be quite large, up to 24 meters in height and are known as tree ferns. Good examples are found in Golden Gate Park. A few ferns are aquatic. Bracken fern (*Pteridium aquilinum*) is a fern of world-wide occurrence and in many places is considered a major weed. On the underside of most sporophylls, sporangia eventually develop. Sporangia are often aggregated together in patches called sori (singular = sorus). The sori can be arranged in various often beautiful patterns on the underside of the sporophyll. Sori patterns are used by systematists in classifying ferns.

The distinguishing feature of all ferns is the large leafy structure known as a frond. Fronds can have many shapes and can be variously divided, varying in length from 1-2 cm to 5-6 meters. Technically, the frond is known as a sporophyll (literally, a spore-bearing leaf). Knowing this term is important because it provides you with a beginning for understanding the fern life cycle. A

sporophyll bears sporangia within which spores are formed. Haploid (n) spores are produced by diploid ($2n$) sporophytes as a result of meiosis occurring within the sporangia (Fig. 2.1). Thus, the sporophyll is part of the sporophyte ($2n$) generation.

Staghorn fern (*Platynerium* sp.) is an epiphytic fern. An epiphyte is a plant that grows physically on another plant. How does this plant obtain its nutrients if its roots never touch the ground?

Once produced, the spores are released, and fall to the ground where they germinate and produce the gametophyte (n). So, here we have the two generations, the sporophyte and the gametophyte generations which alternate (Alternation of Generations).

How do we get a new sporophyte which is $2n$ from a gametophyte which is only n ? On a single gametophyte, or on two different gametophytes, sperm (n) and eggs (n) are produced. Following fusion (fertilization) a new embryonic sporophyte ($2n$) is formed. Note that sperm are motile (flagellated) in ferns. Therefore water is required at some portion of the life cycle in order for fertilization to be completed. Thus, ferns have retained certain features which are considered to have been present in the very first land plants.

Marsilea polycarpha is an aquatic fern. Its leaves usually float on the surface of the water and resemble those of a four-leaf clover. Water ferns differ in that they produce two kinds of spores—microspores and megaspores. This condition is called heterospory. Terrestrial ferns produce only one kind of spores (homospory). Seed plants are heterosporous.

Seed Plants

Because the seed habit is believed to have evolved several different times in the seed plants, the condition of bearing seeds is of limited value in determining evolutionary relatedness. Why? Although there are no spectacularly promising candidates in the fossil record for an angiospermous ancestor, it is generally thought that the flowering plants must have evolved from some gymnospermous precursor. The earliest clearly angiospermous fossils date from the late Jurassic (or early Cretaceous), some 125-150 million years ago (mya). By approximately 100 mya, some 35 million years before the dinosaurs became extinct, the angiosperms had become a diverse and successful group. Unfortunately, because of their ephemeral and delicate nature, there are few fossil flowers. As a result, the evolutionary histories of angiosperms and flowers are obscure.

However, the predominance of seed plants in our world today is evidence that seeds were another major innovation in the evolution of land plants. The seed affords a great survival advantage to the plant embryo over their free-sporing ancestors. In seed plants, the megasporangium is now enveloped by one or two additional layers of tissue to form the ovule. The seed is a fertilized ovule and the additional tissue layers form a protective seed coat. In the seed, a young embryo or new sporophyte develops before the seed is dispersed along with stored food. This entire compact package enables the embryo to survive harsh conditions by remaining dormant and then germinating when conditions are favorable.

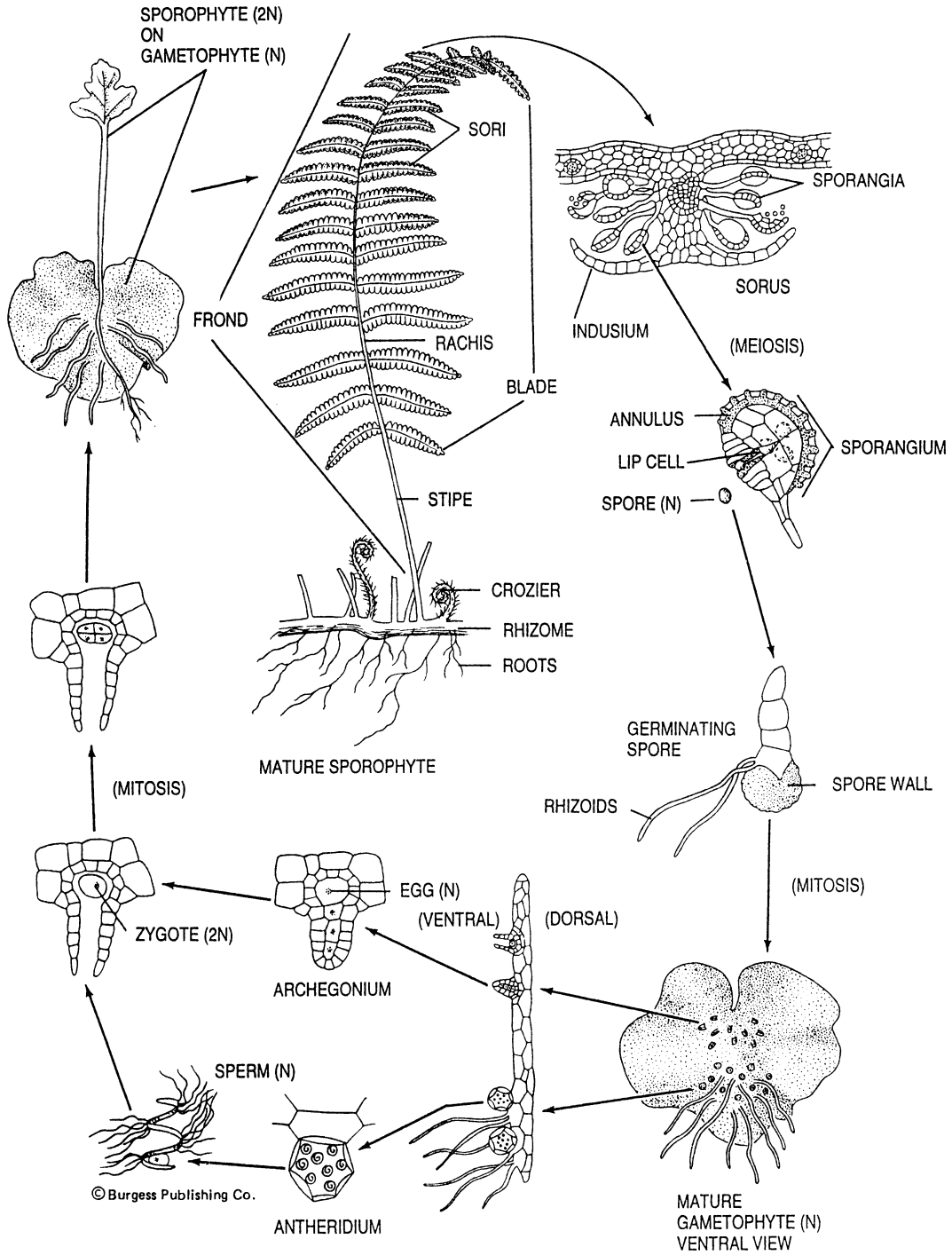


Fig. 2.1
Life cycle diagram of a fern.

Gymnosperms (non-flowering seed plants)

This laboratory and the next considers the reproductive characteristics of two groups of seed plants: gymnosperms and angiosperms (flowering plants). These very different plant groups have two characteristics in common—pollen, and seed—that not only distinguish them from seedless vascular plants (ferns) and bryophytes, but also may have been key factors that enabled gymnosperms and flowering plants to achieve the dominance they have today over so much of the terrestrial world. Relative to seedless vascular plants, conifers and flowering plants are of recent origin, with fossils dating back to the Permian (ca. 225 million years ago) and Jurassic (ca. 150 mya), respectively.

Both pollen and seeds are small, highly dispersible phases of the life cycle. Pollen grains are microgametophytes capable of surviving journeys to other conspecifics (other members of the same species; *con* = same) mediated by such diverse agents as wind, water, insects, birds, and bats, and even mice. Hard seed coats enclose new sporophytes, which are capable of maintaining dormancy through unfavorable seasons or for many years. They can also survive transport through digestive tracts, down streams, or even over oceans. Together, these features allow a plant (sporophyte), which, of course, is rooted in one spot, to mate with a distant conspecific, and to broadcast its offspring over great distances. These are factors of considerable ecological significance.

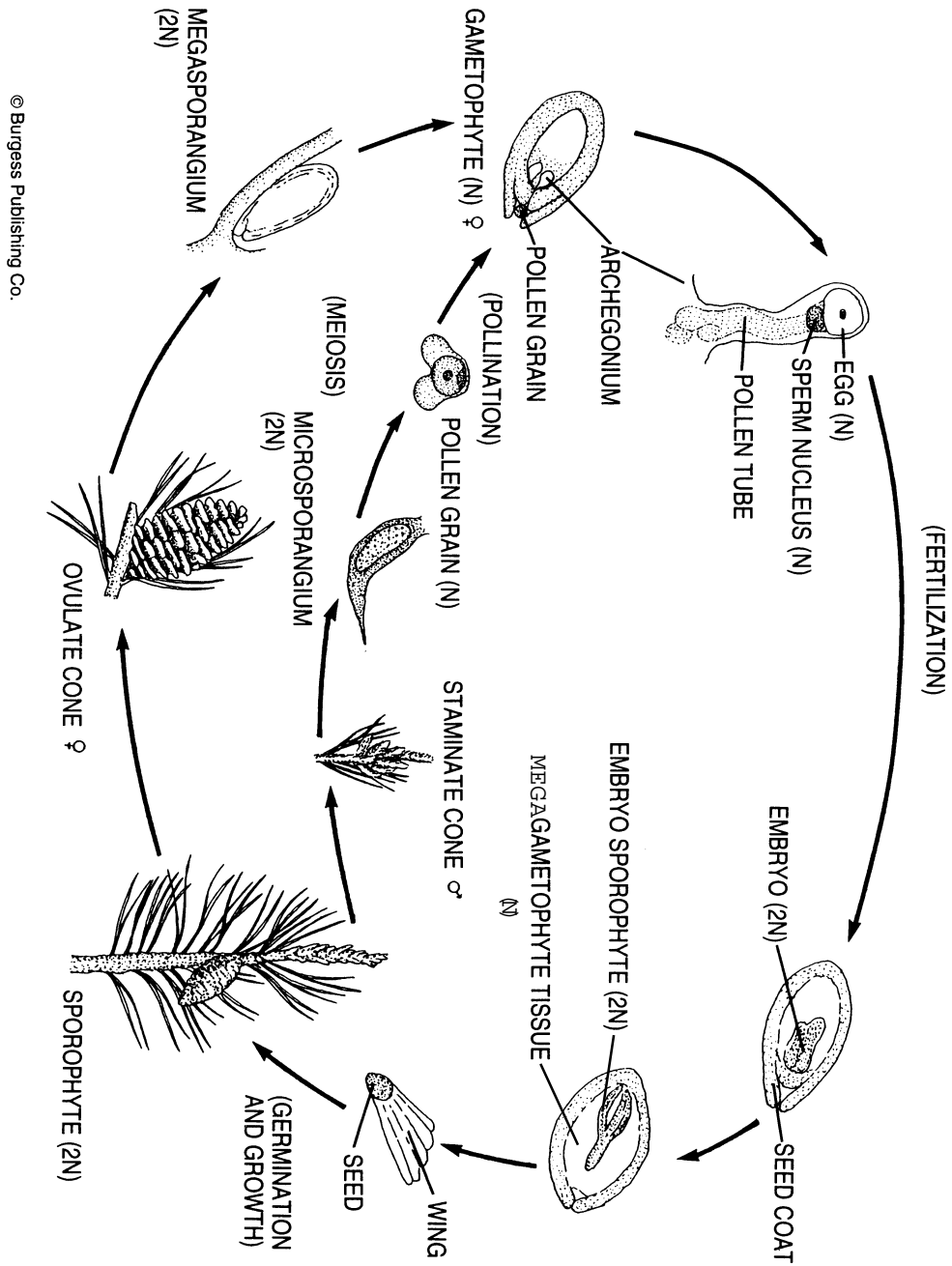
There are four divisions of gymnosperms with living members:

1. **Cycadophyta** (100 species), the cycads or sago palms, of which there are some good specimens on campus near the southwest corner of Hertz Hall;
2. **Ginkgophyta** (1 species), the ginkgo or Maidenhair tree, of which there is a fine specimen just east of Giannini Hall;
3. **Gnetophyta** (70 species), which include such unusual plants as *Gnetum*, *Welwitschia* and *Ephedra* (the so-called Mormon Tea of the Great Basin); and
4. **Coniferophyta** (550 species), the conifers, which are by far the largest, most species-rich, and abundant of the gymnosperms.

The gymnosperm with which this lab will be primarily concerned is a pine, a member of the Coniferophyta. Since the other groups may differ radically, do not generalize pine reproductive characteristics to all gymnosperms.

Sexual Reproduction in Pines

As noted in the previous lab on ferns, sometimes plants can have two different types of sporangia (megaspore and microspore). Both types give rise to spores (megaspores or microspores). In gymnosperms, microspore and megaspore are produced in separate cones, even though both are commonly found on the same sporophyte, or tree (Fig. 2.2).



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Fig. 2.2
Life cycle diagram of *Pinus*.

Microsporangiate strobili, microsporophylls, and microsporangia. Microsporangiate cones (in the vernacular, male cones; more technically, strobili) are smaller, structurally less complex, and more ephemeral than their megasporangiate counterparts. A pair of microsporangia are borne on the abaxial (lower) surface of each microsporophyll. Microsporophylls are borne spirally along a specialized axis, which, as a unit, constitute the microsporangiate strobilus. Within each microsporangium there are many microsporocytes, or pollen mother cells, which are the cells that will divide meiotically to produce the haploid meiospores (microspores, in this case). A microspore, in turn, will divide mitotically to produce an immature 4-celled microgametophyte that is housed within a tough protective case, the pollen wall. That is to say, pollen grains are microgametophytes. It is at this stage of development, which is before the gametes themselves are formed, that large numbers of pollen grains are released to the vagaries of the wind. Some very small proportion of these reach the female cones, where the microgametophyte will commence its development and form the gametes (non-motile sperm).

Megasporangiate strobili, ovuliferous scales, bracts, and ovules. In common parlance, megasporangiate strobili (female cones) are known simply as pine cones. They are much larger, structurally more complex, and persist longer on their parental sporophytes than do microsporangiate strobili (male cones). Female cones consist of a central axis that bears a number of spirally arranged ovuliferous scales, each subtended by a sterile bract. Each ovuliferous scale has two reduced megasporophylls (= a leaf bearing a megasporangium). A pair of megasporangia are thus borne on the upper surface of each ovuliferous scale. The megasporangium, or nucellus as it is called in gymnosperms and angiosperms, is contained within the ovuliferous scale of the parental sporophyte. Except for a small pore that faces the central axis of the cone (the micropyle), the megasporangium itself is completely covered by a thick layer of parental sporophytic tissue called the integument. This integument will eventually harden into the strong seed coat that will house the embryonic new sporophytic generation when it is dispersed from the parent tree. The integument, and its contents (megasporangium, megagametophyte, and/or egg) comprise the ovule.

Pollination. In the spring when pollination occurs, young female cones will elongate slightly, such that the ovuliferous scales separate to a small extent. Pollen borne on the wind comes to rest between these open scales, and some may sift down toward the central axis of the cone. At this time there is a droplet of sticky fluid near the micropyle, and as it dries out, any pollen in it will be drawn through the micropyle and onto the surface of the megasporangium. After pollination, the ovuliferous scales again become tightly appressed, where they will remain until the seeds are dispersed.

Pollen tubes and fertilization. It is at this stage, with pollen adjacent to the megasporangium, that the microgametophytes will again commence growth. Pollen grains germinate to form a tube that will grow through nucellar tissue and deliver the sperm to the egg. However, at this point in time, there isn't an egg; the megasporocytes have not even undergone meiosis! About a month after pollination, the megaspore mother cells (megasporocytes) will undergo meiosis. Only one of the four haploid cells produced meiotically by each megaspore mother cell (the functional megaspore) will divide mitotically to form the megagametophyte. The other three will die. When fully developed, the megagametophyte may consist of some 2,000 cells, and is retained within the megasporangium (nucellus). It is almost a year later when the two or three archegonia will form toward the micropylar end of the megagametophyte. The pollen tube eventually reaches the archegonium containing the egg cell, and delivers the two recently formed sperm cells. One sperm fertilizes the egg, and the other dies.

Seeds. Although there may be several zygotes formed within each ovule, generally only one matures. Seeds then, generally have only one embryo. The single embryo is nourished by the remains of the megagametophyte, and will develop within the confines of the seed coat. At the time of seed dispersal, the embryo consists of a single axis with root and shoot apical meristems and several cotyledons.

Plant Biology Comes Alive!

This interactive portion of the lab consists of three activities (Mystery Identification and your pine cone). Your GSI will base your grade for this part of the lab on the participation of all team members and demonstrated biological understanding. Each team member will receive the same grade.

Mystery Identification. Examine the Mystery Organism located on the front bench. As a team, decide whether this organism is a fern or a gymnosperm. Assuming you could analyze this material in any way, how could you obtain support for your identification? Write your identification and your justification on the chalkboard. When discussing the Mystery Organism at the end of the lab period, your GSI may ask you to explain your response to the class.

Your pine cone. When instructed by your GSI, show your cone to the class. As a team, tell whether the is male or female. Also, indicate whether pollen or ovules are present and where they are located on the structure. Be sure to state the ploidy level of the structures that are present and relate this to the life cycle of gymnosperms.