Chapter 9

Plant Fossils in the Laboratory

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Introduction

Most students, when they think of fossils, think of dinosaur skeletons, ancient shells, and the occasional senior faculty member. If they think of plant fossils at all, they usually visualize the "petrified forest" or reconstructions (paintings or dioramas) of primeval swamp forests. Few students have any real experience with plant fossils; nor do they have any idea of the scientific information that can be gleaned from them.

This exercise, which includes coal-ball peeling and microexcavation, is designed at the most basic level to simply acquaint the students with the reality of plant fossils and to give them "hands-on" experience in working with them (which should stimulate their interest). Depending on the level of knowledge of the students, the aims of the course, and the desires of the instructor, this exercise can be easily modified.

Even at the most basic level it does require at least some familiarity with plant tissues and structures. However, the exercise itself can be used to sharpen powers of observation and to excite interest in those observations of tissues and structures, since most students do enjoy making coal-ball peels and are amazed at the quality of thin sections they personally turn out. Interdisciplinary courses can use this exercise as a springboard or corollary activity to considerations of topics relating past events to present concerns, *e.g.* past plant history and present energy concerns, or paleoecology, evolution, paleoclimatology, paleogeography, *etc.* Other aspects which could be emphasized include the types of fossils and how each is formed, the types of information available in each, the nature of the surrounding matrix and its effects on the form and usefulness of the fossils, evidence of evolutionary changes between past and present plant structures and plant groups, and the changes in the composition of plant assemblages.

The actual laboratory activities include two common and simple techniques of fossil preparation which are used on two different types of fossils, plus demonstrations of other fossil types and techniques as desired. The time required to prepare the laboratory is about 1–2 hours once all the materials have been acquired. Student activities plus brief introductory remarks and instructions should fit within a single 2 or 3 hour laboratory period. Depending on the availability of materials, students can work singly or in groups, but it is recommended that each student have a chance to make a coal-ball peel.

Student Materials

The study of plant fossils (remains or evidence of the existence of prehistoric plants) can provide answers to questions that range from such esoteric considerations as the nature of the first land plants and their evolutionary changes, to more practical ones such as the likelihood of the presence of oil or coal deposits in a certain area. Frequently the study of plant fossils can also contribute to our understanding of past climate and geological changes, which in turn can shape our views of present conditions and trends. The purpose of this exercise is to acquaint you with some of the more common types of fossils, the kinds of information which can be derived from each, and some of the techniques used to study fossils.

Types of Fossils and Information Available

Examine the fossil types on demonstration, noting how they differ. Consider the potential information in each and try to think of ways to get that information. Write down your ideas.

Petrifactions

In these types of fossils the plant parts were only slightly altered before and during infiltration with water high in mineral content. The mineral solutions frequently include dissolved calcium carbonate, silicon dioxide, or iron sulfide. The solutions would permeate the intracellular spaces and lumens of cells, thus preventing further decay. The solutions would later evaporate leaving the minerals in place as rock, thus preventing further shape distortion. When recovered, such petrified (or PERMINERALIZED) fossils often show beautiful preservation of cellular composition with little distortion of shape. They can provide information on both internal anatomy (stelar patterns, cell types, xylem maturation patterns) and external morphology (stem size, leaf presence, branching) of the specimen. Chert beds now exposed in South Africa and Australia, which were formed over 1 to 3 billion years ago, contain remains of some of the earliest known forms of life, while the Rhynie chert beds of Scotland have provided us with valuable fossils of what are thought to be some of the earliest land plants (alive over 350 million years ago). The vast coal fields of the world which were formed over 280 million years ago often contain plant-filled calcium carbonate concretions known as "coal-balls". Such coal-balls provide us with much valuable information concerning the types of plants and plant assemblages in existence then, as well as information about the ancestors of some of our present-day plants.

Compressions

In fossils of this type the plant parts were trapped between layers of sediment. As the layers of sediment built up, the weight compressed lower layers into rock. The plant parts were obviously squashed, and in addition much of the organic material was altered—"volatilized" or "coalified"—so that all that remained was a flat sheet of carbon where the plant was. The nature of the sediment (coarse or fine sand, clay, or silt), its deposition, and the post-depositional changes that followed greatly affect the quality of plant remains and thus the information available. The general outline and habit of the plant is available, as are various surface features such as enations, leaves, leaf venation patterns, glands, and hairs. The finer the texture of the rock matrix the finer the details available. Portions of cuticle may also be present which can contribute information on cell arrangement, surfaces, stomate presence and type, *etc.* If reproductive organs are present it is usually possible to recover relatively unaltered spores or pollen which can be useful in determining the relationships of the plant(s). Seeds may also be recovered. Occasionally specimens will be found with portions which still have some internal structures preserved (petrified), thus providing anatomical as well as morphological information.

Impressions

Here plant parts are similarly trapped in sediments, but all traces of organic matter have been destroyed, leaving only the impression. Such fossils can provide information on the basic shape and size of the plant and, depending on the texture of the matrix, possibly some evidence of the surface features such as glands, hairs, and stomates. Occasionally spores or pollen grains may be isolated intact from the matrix.

Casts and Molds

Casts are formed when hollow areas of the plant, such as a hollow pith or rotted trunk, are filled with rock material which then retains that inner shape once the plant parts are gone.

Molds are formed when rock material surrounds the plant material and solidifies to retain an impression of the plant's external features after the plant decays. If the hollow center of a mold (where the plant decayed) is then filled with another type of rock material, this rock material will then form another type of cast.

Questions

1. What conditions would appear to be necessary for fossilization to occur?
2. What types of areas today would be likely sites for the preservation of tomorrow's fossils?
3. How can you explain that compression fossils are much more common in occurrence than petrifactions?
4. Compare the types of information available in each type of fossil. In which fossil types would you expect to find internal structure? Cuticle? Spores?
5. Compare the types of information (from what parts of the plant) which can be derived from the two types of casts described.
6. Are the most recent fossils always in a higher quality state of preservation than those formed earlier in time? Why or why not?

7. Why is the composition of the matrix important? (What effects does it have on preservation? What effects does it have on preparative technique selection?)
8. How could you use plant fossils in the study of paleoclimatology or paleoecology?

Techniques of Fossil Preparation

As you noticed in the above section, different types of fossils present different types of problems with respect to getting at their information. For the remainder of the laboratory period we will be taking a closer look at two solutions to the problem of information liberation. The instructor will provide detailed instructions on both methods to the entire class, and then you will split into groups to apply the techniques. Plan to spend approximately half your time with each technique. Make sure that you try both.

Peel method for preparing thin sections of coal-balls or other calcium carbonate matrix petrifactions

1. Obtain a split and roughly ground portion of a coal-ball.
2. Polish the cut surface with #400 (or #600) carborundum in a wet slurry on plate glass. Be sure to move the specimen in large circles all over the glass surface so as to not wear the glass unevenly. (Why not?) The polishing step provides a flat uniform surface.
3. Rinse the polished surface thoroughly with tap water to remove all traces of polishing powder and rock particles.
4. Immerse (about 3mm) the polished surface in a dilute (2%–5%) solution of HC1 (CAUTION: DO NOT IMMERSE FINGERS OR SPLASH) for a brief period of time (30 seconds to 3 minutes). Start with 1 minute and adjust time based on results. The time will vary with the specimens. This part of the process dissolves the matrix leaving the plant remains—cell walls—standing in relief.
5. Do not touch the etched surface or you will destroy the plant remains. Carefully rinse the etched surface under a gentle stream of water to stop the etching process and remove the excess acid.
6. Rinse the etched surface with a gentle stream of acetone to promote rapid drying.
7. Place the coal-ball, etched surface up, level in a pan of coarse gravel to dry. While it dries, cut out a piece of clear cellulose acetate slightly larger than the surface of your coal-ball (about ½–1 inch larger in all directions).
8. Flood the surface of your coal-ball with acetone and lay your sheet of cellulose acetate on the wet surface. Starting at one end helps to eliminate air bubbles. The acetone serves to dissolve the plastic which then melts down in and around the exposed plant material.

9. Let dry approximately ½ to 1 hour. If the surface feels cold to the touch it is probably still not dry.
10. When dry, carefully peel off the plastic sheet starting at the edge (or edges). The plant parts exposed by the acid are now embedded in the plastic, and you have a thin section to examine under the microscope.
11. Examine your peel under both the dissecting and compound microscopes and consider the questions below.
12. To make another peel of the same coal-ball, simply repeat steps 2 through 10.

Questions

1. How many different plant parts can you recognize? Do they all have the same orientation?
2. Can you distinguish different tissues? Different cell types?
3. What types of cells and tissues seem to have been preserved better?
4. Are there any differences in the cells and tissues observed in the peel from the cells and tissues observed in modern plants? If so, describe.
5. What might be some of the disadvantages of this method?
6. Approximately how long ago were the various organisms in your peel alive?
7. What other organisms may have populated the world at that time? (Include both plants and animals).
8. What was the world climate like then? What about the probable local climate?
9. In what ways are these fossil plants similar to present day plants? How are they different? What other pieces of information would you like to have at your disposal?

NOTE: If the petrifaction matrix is not soluble in HC1, the acid to use would be HF; however, this acid is extremely hazardous and requires special facilities, containers and handling. It is not suitable for situations other than research.

Microexcavation method for compressions not confined to a narrow bedding plane

1. Select a likely looking specimen (one with a branch or leaf that disappears into the rock). Does the axis or stem branch again? Are there sporangia at its tip? How does the leaf terminate? Check to make sure that the desired branch or leaf isn't exposed on the back surface of the specimen.
2. Stabilize the specimen on a sandbag on the stage of a dissecting microscope.
3. Using a sewing needle (or very small chisel) and a light hammer, carefully chip away the rock that is covering the part of the specimen in question.

Tapping with the needle vertical seems to do the least accidental damage to the plant material, but with practice you will develop your own techniques to avoid scratching, puncturing or popping out the plant material. The technique is tedious but often the only way to get the information.

Questions

1. Were you able to answer the questions you asked yourself in step one?
2. Approximately how long ago were the various organisms in existence?
3. What other organisms may have populated the world at this time? (Include both plants and animals.)
4. What was the world climate like then? The local climate?
5. In what ways are the fossil plants similar to present day plants? How are they different? What other pieces of information would you like to have at your disposal?
6. Is there information here that this method has not liberated? If so, what information?

Other Methods of Fossil Preparation

Bulk maceration

This is a method where entire chunks of rock containing compression material are placed in acid. The rock matrix is dissolved to a sludge, liberating the plant parts which can then be isolated for further treatment (such as clearing) and investigation. Often intact sporangia with spores *in situ* can be obtained in this way. All surfaces of the stem can be exposed in this manner.

Transfer

In this technique plant parts exposed on the surface of a rock are coated with a resin or plastic and then the rock is dissolved away. This helps to avoid breakage and loss of parts during the maceration process. The results obtained are similar to those of bulk maceration.

Ground thin section

This technique is used for petrifactions in rock matrices which do not respond well to the peel method. Sections of rock are cut as thin as possible with a diamond saw, and then one side is polished as smooth as possible. This polished surface is then glued to a glass slide and the other surface is cut as thin as possible, then polished and ground until the whole section is thin enough to see through. Occasionally the rock matrix is so crumbly that entire portions have to be embedded in plastic or resin before grinding can proceed.

Silicon-rubber pulls

This is a good technique for surface features of impressions and compressions. A film of silicon-rubber is applied to the desired surface, allowed to dry, and peeled off to be observed with a microscope.

Instructor's Materials

To be effective this exercise does require that appropriate background information be presented to the students before the actual laboratory period. The background material should at the very least include coverage of basic plant organs, tissues and cell types, as well as concepts concerning fossils and fossilization. The remainder of the material and the questions on the lab sheet can be tailored to the level of the students and the desired learning objectives. It is useful to have reference materials pertinent to the questions available in the laboratory so that the students can peruse them while waiting for peels to dry. The first part of the laboratory can be used separately to stimulate problem recognition and problem solving.

The techniques are fairly straight-forward as presented in the section on student materials, but a few words of caution do seem indicated.

1. Personal protection and care are always necessary when handling acids, but one should also consider the plumbing and thoroughly flush the drains to prevent undue corrosion.
2. If the instructor stresses proper polishing technique the life of the plate glass will be prolonged and the quality of preparations will remain uniform.
3. If any microexcavation is done without using the dissecting microscope, proper eye protection should be provided.
4. Problems which may be encountered in preparing coal-ball peels—such as difficulty in removing the peel from the specimen—are usually due to either etching too deeply or not letting the preparation dry long enough (if the cellulose acetate stretches, it is not dry). Specimens do vary, so trial and error is necessary.
5. Coal-ball drying can be hastened by *judicious* use of a small fan or hair dryer. Remember however that sudden changes in temperature can cause cracking and breaking of the coal-ball. Such changes of temperature could occur when moving an acetone-evaporation-cooled rock into a hot stream of air, or by flooding an overheated rock with acetone. The fire hazard should also be considered.
6. Coal-balls should be stored with a cellulose acetate sheet in place—*i.e.* stop at step 9 of the peel procedure.

Supplies and Materials

Sufficient dissecting and compound microscopes for a class of 20
Fossils (possible sources are listed in Appendix A):
- Coal-balls—4 or more
- Compressions suitable for microexcavation—10 or more
- Prepared coal-ball peels—as desired
- Examples of various types of fossilization—as desired

Tack hammers or other light hammers—10 or more
Steel sewing needles, surgical needles, and/or very small chisels—2 dozen
Corrosion resistant pans (approximately 12 × 18 × 2″)—minimum of 2
Scissors—1 or 2 pair
Large pipette bulbs and/or soft brushes (for dust removal)—2 or more
Disposable protective gloves—2 dozen
Plate glass (¼″ thick, at least 18 × 18″)—at least 1
Small sandbags (approximately 4 × 6″)—10 or more
Wash bottles for acetone—2 or more
#400 or #600 carborundum powder
Acetone—1 liter
Cellulose acetate sheets or roll (approximately 0.003″ thick)—24′
Hydrochloric acid (2%–5%)—enough to fill a pan to a depth of approximately ¼ inch.

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APPENDIX A
Sources of Materials

Fossils

Prepared and mounted coal-ball peels of a variety of plant groups can be purchased from Carolina Biological Supply Company (Cat. Nos. B-5000 to B-5260). Fossil collections (mixed animal and plant) and collections of fossil types, as well as projection slides of plant fossils are also available through Carolina Biological Supply Company (Cat. Nos. GEO-5265 to GEO-7180 and 48-3041) and through Ward's Biology and Earth Science Catalog. Ward's also offers coal-balls suitable for peeling.

Unprepared fossil specimens of various kinds can be obtained either by personal collection (see Appendix B) or by requesting them (in reasonable quantities) from scientists in the field of paleobotany. A list of such possible sources can be compiled relatively easily by going through the abstracts of a recent national meeting such as the American Institute of Biological Sciences or the Botanical Society of America (often held jointly with AIBS). A letter with your request, an explanation of your purposes, and an offer to pay postage or shipping costs (remember, rocks are heavy) may not get you the specimens immediately, but you should at least be referred to a more likely source. It is my understanding that Dr. David Dilcher at Indiana University (Bloomington) has a grant specifically for the purpose of specimen dissemination, but as you know grants have finite limits.

Supplies

Carborundum powder (#400 or #600) can be obtained in ½-lb. lots from Carolina Biological Supply Company (Cat. No. 60-2055). Hobby shops may also carry such polishing powders.

Cellulose acetate can be obtained in 100′ rolls from Southern School Service, P. O. Box 867, Canton, North Carolina 28716. Some types of sheets or rolls commonly used for overhead projection may serve as substitutes, but are generally thicker than is optimal. Always make sure such substitutes are soluble in acetone before using them.

APPENDIX B
Collecting Your Own Fossils

Locating a Site

1. Go on one of the annual paleobotanical field trips which are usually held in conjunction with the AIBS or BSA meetings. This is also a good way to meet potential fossil donors and to start your own fossil collection.
2. Locate the nearest professional paleobotanist, explain your desires, and ask to go along on trips that seem feasible.
3. Ask the above professional for locations or directions to sites which would be suitable for your purposes. ACTIVE RESEARCH SITES SHOULD NOT BE DISTURBED. If you do find something particularly nice or potentially exciting take it to your professional (be prepared to relinquish it). Your cooperation and responsibility will be appreciated and usually not unrewarded.

4. If none of the above are feasible, check with a local geologist. Geologists frequently know of fossil locations or can at least direct you to areas of high possibility. In such areas check places like roadside cuts where sedimentary rock layers are exposed, or similar streambank cuts. Quarries, newly-cut roads, and newly-excavated dam sites or building sites are also areas of potential. Red stains or black seams often indicate plant material.
5. Local rockhounds or other natural history buffs are often good sources of site information.

What to Do When You Get There (for Compression and Impression Material)

1. Equipment: You will need a rock or masonry hammer (one with a flat head on one end and a chisel tip on the other) or a separate hammer and chisel, a pocket knife for splitting small samples, a small shovel or at least a trowel for clearing away overburden and overly-weathered rock. Newspapers and marking pens are necessary for wrapping and labelling specimens. Boxes may be needed for transport and storage. Sturdy shoes are suggested (falling or sliding rock slabs hurt).
2. Clear away the overburden and weathered material from your site until a relatively unweathered portion of sediment is exposed. Look for dark lines or patches indicative of organic matter.
3. Remove a slab or chunk of dark-veined rock and orient it with the bedding planes perpendicular to the supporting surface. Strike the rock one or more times with the chisel tip of the hammer along the bedding plane. The rock should split along the bedding plane revealing the plant parts (if any) lying parallel to that plane. This process may be continued until the rock is too thin or the specimens are satisfactory.
4. Once at least one productive site is located students generally enjoy fossil hunting field trips. Use them to explore new areas as well, but always include a "sure-fire" spot. Do remember to report any good spots to professionals.