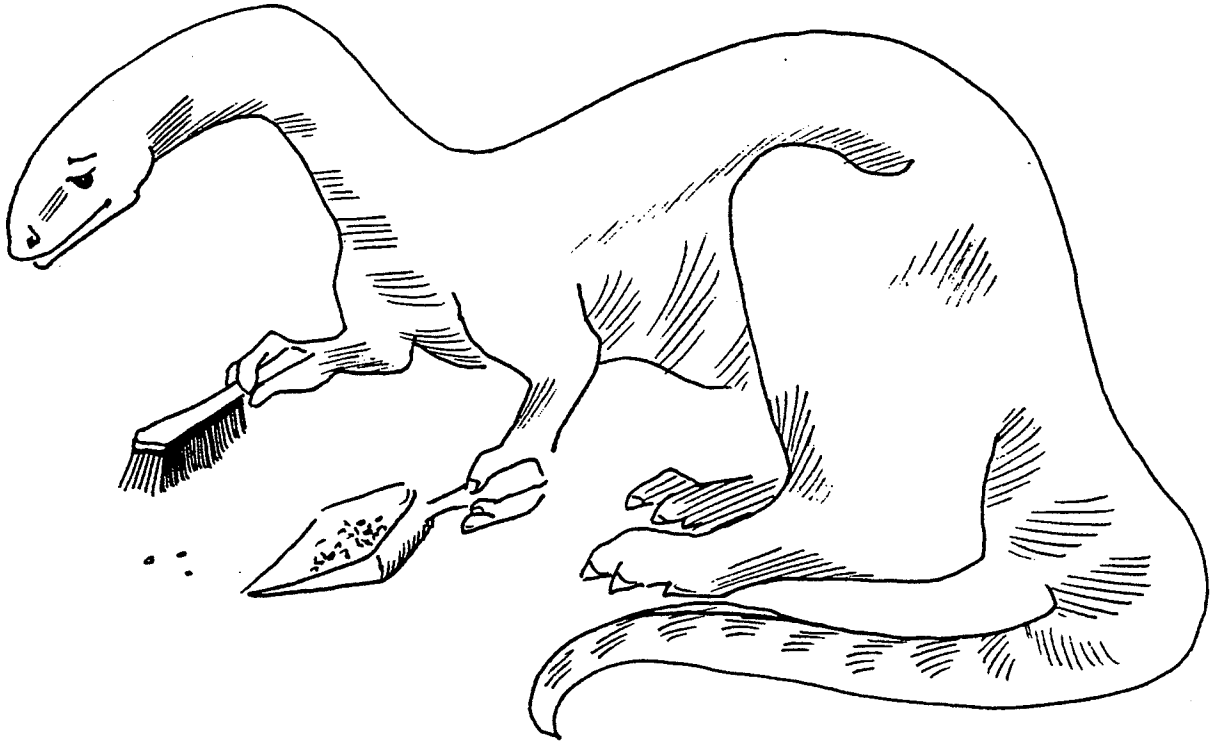
PHYTODEBRIS



Notes for a Workshop on the Study of Fragmentary Plant Remains

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DISPERSED ANGIOSPERM CUTICLES

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ABSTRACT

Dispersed leaf cuticles are the second most abundant component of the angiosperm fossil record, but the study of dispersed angiosperm cuticles is still in its infancy. This paper reviews previous studies of dispersed angiosperm cuticles and discusses important features of cuticular anatomy and the occurrence of dispersed cuticles in the rock record. The stratigraphic density of dispersed cuticles is second only to that of pollen and spores, and dispersed cuticles more closely reflect local flora than palynomorphs. This means that dispersed cuticles are best suited to studies that require both greater facies resolution than what is obtainable for palynomorphs and greater sampling density than what is obtainable for megafossils. Reconstruction of paleocommunities/paleovegetation has been the most common use of dispersed cuticles, but other potentially productive uses include the reconstruction of paleofloras and analyses of angiosperm diversification and extinction.

INTRODUCTION

Studies of ancient organisms rely on two distinct but complementary sets of characters. Intrinsic characters comprise those features of morphology, anatomy, physiology, and chemistry preserved in the geologic record, either within whole organisms or within parts of organisms. Extrinsic characters comprise the vertical and horizontal distributions of fossil remains in the rock record, which are used to infer the distribution of ancient organisms in space and time. Intrinsic characters are the major source of data on evolutionary relationships, functional morphology, physiology, and paleobiogeochemistry. Extrinsic characters are combined with intrinsic characters to reconstruct paleofloras, paleovegetation, paleoclimate, and patterns of biotic change. In order to put detailed studies of fossil organisms within a temporal and geographic context, most paleontological studies strike a compromise between maximizing information from intrinsic characters and maximizing information from extrinsic characters.

Paleobotanists typically have achieved such compromises by studying one of two organs: (1) leaves (i.e., leaf megafossils), or (2) pollen and spores. In well-preserved leaf megafossils, venation and other features of foliar architecture can yield 50 or more distinct characters, many of which are taxonomically useful (e.g., Hickey, 1973; Wolfe, 1973; Hickey and Wolfe, 1975), and others of which reflect environment (e.g., Wolfe, 1978; Wolfe and Upchurch, 1987b). This suits leaf megafossils well for studies of systematics, vegetational physiognomy, and patterns of community diversity, but sporadic preservation limits the value of leaf megafossils for studies that require numerous large samples or fine stratigraphic resolution. Dispersed pollen and spores, in contrast, are the most abundant character-rich remains in the plant fossil record, and they occur in diverse rocks of continental and marine origin. However, the ease with which palynomorphs are transported by wind and water complicates the recognition of local diversity patterns, and the structural features of pollen and spores are perhaps best suited for the recognition of clades at generic and higher taxonomic levels, especially when studied under light microscopy (e.g., Leopold and MacGinitie, 1972; Wolfe, 1973; J. Walker, oral comm.).

Dispersed fossil leaf cuticles offer a compromise between leaf megafossils and pollen and spores that has at least five advantages, especially when the study of dispersed cuticles is combined with the study of megafossils and palynomorphs.

1. Dispersed leaf cuticles can be recovered from many rock types that otherwise preserve only palynomorphs and highly fragmented plant material. This means that dispersed cuticles can sample a wider range of sedimentary environments than megafossils.

2. The transport potential of leaf fragments is greater than the transport potential of whole leaves. All other factors being equal, small plant fragments have a lower settling velocity than whole leaves (R. A. Spicer, oral comm., 1987). This means that dispersed cuticle assemblages probably sample a larger area of source vegetation than leaf megafossils.

3. Identifiable plant cuticles and leaf fragments show less transport potential than pollen and spores because of their larger size. This suits them well for the reconstruction of paleocommunities in autochthonous coals, where water transport of plant remains is minimal and leaf megafossils typically are not preserved.

4. Many taxa well represented in the record of dispersed cuticles are poorly understood in the record of other plant organs. This can result from poor preservation potential (e.g., the virtual absence of Lauraceae and closely related families in the pollen record), organs that are difficult to identify at lower taxonomic levels because of generalized external morphology (e.g., leaf impressions of palms), or a combination of the above.

5. The systematic significance of characters preserved in dispersed cuticles can be checked readily through comparisons with the cuticular anatomy of megafossils. Identifiable leaf megafossils that preserve cuticle are more abundant and diverse than identifiable floral remains that preserve pollen, especially in highly compacted and thermally mature rocks.

The following pages provide an overview of dispersed angiosperm cuticles that describes their collection, preparation, systematic analysis, and application to paleobiologic and geologic problems. Because the study of dispersed angiosperm cuticles is still in its infancy, this paper should be considered a state-of-the-art report, rather than the sole formula for a research program in dispersed angiosperm cuticles.

HISTORY

Prior to the 1960's, most research on leaf cuticles was restricted to megafossils. The first wave of research on the cuticular anatomy of angiosperm leaf megafossils began in Europe during the 1920's and continued through the 1930's with work on Tertiary remains (e.g., Johnson and Gilmore, 1921; Bandulska, 1923, 1926, 1931; Krä usel, 1938). Following interruption by the second World War, this wave of research on fossil angiosperm cuticles continued, particularly for remains of Tertiary age associated with coal deposits (e.g., Kräusel and Weyland, 1950, 1954, 1959; Kirchheimer, 1957). Today, the analysis of cuticular anatomy forms an integral part of leaf megafossil investigations in Europe (e.g., Kvaček and Walther, 1981, 1984; Kvaček, 1984; Kovar, 1982; numerous other references could be cited). In North America and Asia, paleobotanists were slower to use cuticular anatomy for the identification of leaf megafossils. Here, comprehensive studies of cuticular anatomy in fossil angiosperms did not begin until the late 1960's and early 1970's (e.g., Dilcher, 1963; Dilcher and Mehrota, 1969; Dilcher and Dolph, 1970; Krassilov, 1973; 1979; Chourey, 1974; Tanai, 1979), and many North American and Asian studies of angiosperm leaf megafossils still incorporate little or no data from cuticular anatomy.

Studies of dispersed angiosperm cuticles lagged behind studies of in situ cuticle. Much of the initial motivation for studies of dispersed angiosperm cuticles was the analysis of coal, a lithology that preserves few leaf megafossils. Early studies of dispersed cuticle, such as those of Miner (1935), were largely descriptive and included analyses of megaspores and seeds. These early descriptive studies led the way to more synthetic studies of dispersed cuticles that began in the 1960's (summarized in Roselt and Schneider, 1969; and Kovach and Dilcher, 1984). These early synthetic studies were linked to the study of coal geology and emphasized facies analysis and paleoecology (e.g., Peters, 1963; Litke, 1966; Schneider, 1969), but some investigators also used the taxonomic composition of dispersed cuticle assemblages for stratigraphic correlation (e.g., Peters, 1963) and the physiognomy of dispersed cuticle assemblages to infer paleoclimate (Litke, 1967). During this period a structure-based classification system was proposed for dispersed cuticles, in order to facilitate their use in geological studies (Roselt and Schneider, 1969). Despite a promising beginning, few synthetic studies of dispersed angiosperm cuticles were published in Europe after the mid-1970's.

In North America, research on dispersed angiosperm cuticles was largely not published until the 1980's. Unlike much of the European research, North American investigations of dispersed cuticle were linked to the study of megafossils and had a strong biological emphasis. These studies are still in their infancy, but they already have addressed such topics as structural evolution in early flowering plants (Upchurch, 1984a, b), mass extinction (Upchurch et al., 1985; Wolfe and Upchurch, 1987a), and potential of dispersed cuticle for determing patterns of species diversity (Kovach and Dilcher, 1984).

OCCURRENCE, COLLECTION, AND PREPARATION OF DISPERSED CUTICLE

OCCURRENCE AND COLLECTION

Dispersed plant cuticles occur in both continental and marine rocks. They are most abundant in continental rocks because of proximity to source vegetation. Coal, carbonaceous shale, and carbonaceous mudstone are the most productive lithologies, but organic-rich siltstones and argillaceous sandstones also can yield well-preserved cuticle (e.g. Upchurch et al., 1985; Wolfe and Upchurch, 1987a). In marine rocks, dispersed plant cuticles have been reported from a variety of lithologies in depositional settings that were probably not far removed from land. These lithologies include include argillaceous, organic-rich sandstones deposited in marginal marine to nearshore marine environments (Upchurch and Askin, submitted manuscript), carbonate concretions that formed in shales from outer continental shelf to upper continental slope environments (the carbonate nodules reported by Blome and Nairn, 1985; Upchurch, unpublished), and deepwater organic muds (Clark et al., 1986). Dispersed plant cuticles have been recovered from well cores drilled on land (e.g., Benda, 1962) and in the deep ocean (Batten, 1979; Clark et al., 1986).

The collecting techniques for dispersed cuticles are virtually identical to those used for palynomorphs. For outcrop material, the stratigraphic section should be trenched to fresh bedrock. In areas of humid temperate climate, such as the eastern United States, the depth of trenching is usually no more than a few inches unless the exposure is covered with slumped material. In wet tropical climates, the depth of trenching probably would be much greater. In areas of semi-arid climate, such as the Western Interior of North America, the depth of trenching should be greater than for areas of humid temperate climate. This is because deep alkaline weathering readily destroys cuticles and palynomorphs. In dry regions where the sediments are relatively unconsolidated, the rule of thumb is to dig a hole large enough to sit in before collecting the sample (R. H. Tschudy, oral comm., 1985). Heavy plastic freezer bags are best for collecting and storing samples because they minimize the odds of contamination. If the sediment contains a large quantity of moisture, as is the case for rocks from the Atlantic and Gulf Coastal Plains, the sample may need to be dried in order to retard mold growth.

The total amount of sample collected depends on many factors, including lithology, sampling interval, and whether the rock was deposited under continental or marine conditions. For continental rocks, I recommend collecting at least 100 grams of coal and at least 200 grams of clastic rock. For marine rocks, I recommend collecting at least 400 grams of material. This will provide enough sample to yield a reasonable number of cuticle fragments, yet still leave material available for archival and analysis by other scientists.

The amount of sample initially prepared for cuticle will depend on many factors, including the abundance of terrestrial organic matter in the rock, the quality of organic preservation, and whether or not cuticles will be picked for scanning electron microscopy. As a rule of thumb for continental rocks, I recommend processing 40 to 60 grams of coal and 50 to 100 grams of carbonaceous shale and

mudstone; this should yield a minimum of 4 to 6 slides of cuticle. When cuticle is sparse, poorly preserved, or extra material is needed for scanning electron microscopy, more sample should be processed. As a rule of thumb for marine rocks, I recommend processing at least 200 to 300 grams of sample; this should yield a minimum of 2 to 4 slides of cuticle. Usually much less material is available from well cores than from outcrops; therefore, take as much sample as possible. The major advantages of core material relative to outcrop material are a minimum of weathering and the absence of covered intervals in stratigraphic sections.

The following criteria will maximize the probability of collecting productive samples.

Color.

Organic content has to be high enough to impart a gray, black, or dark brown color to the rock; in sandstones, the color of sediment between sand grains is the important criterion. Yellow, orange, red, green, and tan rocks rocks are typically barren. Medium brown rocks are often marginal and necessitate processing large quantities of sample.

Quality of organic preservation

This can be assessed by exposing a fresh surface of rock and examining the plant fragments at 5 to 10X magnification. When preservation is good to excellent, leaf fragments are smooth and shiny, and vitrainized wood is dark, shiny, and lacks a crumbly texture. When preservation is poor, leaf fragments lack sheen and have a powdery texture, and vitrainized wood is typically light colored, lacks sheen, and has a crumbly texture. Samples with poor organic preservation may contain much fusain but little else in the way of organic material. Replacement of organic material by zeolites and other minerals can be detected through subtle differences in color and sheen.

Thermal maturity

Increasing temperature decreases the chemical distinctness of cuticle relative to other types of organic matter. In coals, cuticle is chemically distinct from other macerals up through medium-volatile bituminous rank. Semianthracites and anthracites are too chemically homogeneous to yield cuticle (or palynomorphs) upon maceration; recovery of cuticle and palynomorphs from low-volatile bituminous coals is variable. In carbonaceous shales and mudstones, cuticle can be recovered from rocks showing thermal maturation up through anthracite rank. Cuticle from rocks of semianthracite to anthracite rank is usually quite dark, making observation of systematically important features difficult.

Abundance of plant fragments

Abundant comminuted plant debris can often indicate that a sample will be productive. However, cuticle can be hard to see in unprepared samples when it is naturally macerated or is preserved in the pore space of sandstones.

PREPARATION

Once a sample is collected and selected for preparation, plant fragments must be released from the matrix, then macerated, stained, and mounted. The following pages provide an overview of procedures that I currently use. Typically, 8 samples are processed simultaneously to increase time efficiency; I usually can process them at the rate of 1 sample every 1 to 2 hours. Sieves are used at various stages of processing and are the most likely source of sample contamination. To minimize chances of

sample contamination, use only one sieve per sample. For proper cleaning, sieves should be scrubbed with detergent, rinsed well, then placed in an ultrasonic cleaning bath for 5 to 10 minutes.

Release of fragments from matrix

(1). Break the rock into fragments 1 to 2 cm in diameter, splitting along bedding planes whenever possible. Breaking the rock into smaller pieces will badly fragment the cuticle.

(2). Place the pieces of rock in a polyethylene beaker. The volume of the beaker in ml should be 4 to 5 times sample weight in grams.

(3). Place a few drops of concentrated HCl on the sample. Observe the resulting reaction.

A. No reaction--Go to step 4.

B. Vigorous reaction--Fill beaker with a saturated solution of EDTA, pH no greater than 7. When bubbling stops, pour off the EDTA, rinse 2 times, then pour off the liquid. Add HCl and observe. Repeat EDTA step if the reaction with HCl is still vigorous.

(4). Cover sample with concentrated HCl. If more than a few large bubbles form, dilute with distilled water. Leave 2 to 4 hours overnight. Vigorous bubbling will badly fragment cuticle.

(5). Fill beaker with water and let settle. Pour off liquid. Repeat both steps.

(6). Add a saturated solution of sodium pyrophosphate in water (pH = 9) until the beaker is full or pH = 7, whichever comes first. Stir sample with stirring rod. Leave for 1 to 2 hours.

(7). Test sample for disaggregation by poking with stirring rod.

A. Sample still consolidated--Rinse 3 to 4 times with distilled water. The liquid can be poured through a 150-mesh sieve (pore size = 105 microns) to catch cuticle and small pieces of rock.

B. Sample turned into mud--Sieve sample through a 150-mesh seive. Wash sample until all the clay is removed. Return sample to beaker. Then add 50 to 100 ml of distilled water. (Note: Failure to wash out the clay or add the distilled water can result in explosive boiling when HF is added!)

(8). Add HF gradually to beaker until the volume of HF in ml is 3 to 4 times sample weight in grams. It is usually best to add HF in increments of 15 to 20 ml, with a period of time in between, to avoid boiling the sample. HF can be added in larger increments to coals and well-consolidated samples. If the sample starts to boil, immediately pour on distilled water!

(9). Cover beakers and swirl by hand once each hour. Avoid mechanical agitation; this fragments cuticles badly.

(10). After several days, pour off HF. Repeat steps 8 through 10 if sample is not fully disaggregated or sediment grains will not pass through a sieve. For coals, overnight in HF is usually sufficient unless ash content is high.

(11). Fill beakers with distilled water. Let settle 1 to 2 hours. Pour off liquid. Repeat 3 times.

(12). Fill beaker with water and pour sample through seive. Wash sample well. (Note: I advise wearing gloves in case the sample contains residual HF.)

(13). Pour sample into a clean glass beaker for maceration. Use a 200 to 400 ml beaker for plant fragments from clastic rocks. Use a 1,000-ml tall beaker for coals.

Maceration of cuticle

Maceration procedure varies according to type of sample (coal vs. other lithologies) and degree of thermal maturation. Two different procedures are given. Procedure 1 is for plant fragments from clastic rock where thermal maturation is no higher than lignite grade. Procedure 2 is for all coals and for plant fragments from clastic rock where thermal maturation is sub-bituminous or higher.

Procedure 1 (adapted from Kovach and Dilcher, 1984):

(1). Add 50 to 100 ml of fresh household bleach (sodium hypochlorite). Leave on plant fragments until bleached.

(2). Fill beaker with water and pour the sample through a sieve. Rinse fragments well. Then return cuticle to beaker with as little water as possible.

(3). Pour cuticle sample into a 50-ml beaker with as little water aspossible. Then pour sample into a labeled 2-dram vial with poly-seal cap.

Procedure 2 (adapted from Doher, 1980, p. 7):

(1). Barely cover coals with a saturated solution of potassium chlorate. Add a larger volume of potassium chlorate to plant fragments from clastic rock.

(2). Add nitric acid. The exact procedure depends on whether coals or plant fragments from clastic rock are being macerated. Use 70% nitric acid for lignites and sub-bituminous grade organic matter, 90% (fuming) nitric acid for higher-rank organic matter.

A. Coals--Put 50 ml of nitric acid into an empty beaker. Pour 5 to 10 ml of nitric acid from this beaker onto the sample. Stir sample with a glass stirring rod. If sample does not produce a frothy, vigorous reaction within 1 to 2 minutes, add a little more nitric acid. Test the firmness of the lumps of coal by pressing them with a glass stirring rod. Maintain a vigorous reaction by adding nitric acid until no hard lumps remain. Fill beaker with distilled water and allow to settle 1 to 2 hours. (Note: If the beaker starts to boil over, slow the reaction by adding some water to the foam with a squeeze bottle.)

B. Plant fragments from clastic rocks--Add a volume of nitric acid equal to that of the potassium chlorate. Macerate plant fragments until they turn yellow to light golden brown. If needed, add additional nitric acid to speed up reaction. Fill beaker with water and allow to settle.

(3). Pour off water. Fill beakers with water and allow to settle. Pour off water and repeat. If desired, samples can be rinsed through sieves to save time. Wear rubber gloves and eye protection.

(4). Add 5% KOH. Leave KOH on samples for 30 minutes or until all oxidized humic material has been dissolved, whichever comes first. Rinse several times as in step 3.

(5). Check a sample of macerated cuticle under the microscope. If the cuticle is fully macerated, cell outlines will be readily visible, and the cuticles will be mostly free of adhering brown debris. If the cuticle is still undermacerated, go to step 6. If the cuticle is fully macerated, go to step 7.

(6). Cover sample with a 20% solution of chromium trioxide. Leave sample in chromium trioxide until the adhering debris is oxidized (1 day to 1 week). Rinse as in step 3.

(7). Pour cuticle into a 50 ml beaker with as little water as possible. Pour cuticle sample into a labeled 2-dram vial with poly-seal cap.

Staining and Mounting

Each person has a preferred technique for staining and mounting cuticle. I use the following technique, which relies on a discontinued brand of alcohol-soluble, polystyrene plastic (AYAF) to mount the cuticle on the cover slip. "Cellosize"® probably can be substituted for AYAF; if so, use a water-based, rather than methanol-based, stain. Mounting the cuticles on a coverslip places all specimens near the objective lens, which permits the use of high-resolution, low-working-distance objectives.

(1). Fill each 2-dram vial with 100% methanol. Screw on cap and shake vial to mix solvents. Allow the cuticle gravity to settle (centrifugation often does not help!), then use a pipette to withdraw the liquid. Repeat 2 times.

(2). Cover cuticle with a 1% mixture of Safranin O in 100% methanol. Leave for 1-several hours or overnight

(3). Fill each vial with 100% methanol, screw on cap, and agitate. Let the cuticle gravity settle. Repeat until the stain stops bleeding into the methanol.

(4). Withdraw as much methanol as possible with a pipette. Then add a solution of AYAF to each vial. Screw on cap. For best results, use 3 to 4 times as much AYAF as cuticle.

(5). Mix AYAF and cuticle by rapidly spinning the vial back and forth between the palms of your hands. Allow the samples to sit for 1 hour.

(6). When you are ready to mount the sample, agitate the vial as in step 5. Then pour a few drops of the AYAF/cuticle mixture onto a cover slip. Spread the mixture with a clean toothpick, using as few strokes as possible. Place the cover slip, AYAF side up, on a labeled slide. Dry the AYAF on a warming tray for at least 1 hour. Wipe the mouth of the vial with a Kimwipe before screwing on the cap.

(7). When the AYAF is dry, mount the cover slip, AYAF side down, on the slide with Canada Balsam. Put the slide on a hot plate for a few seconds to improve the flow of balsam. (Note: Because AYAF is slightly soluble in toluene, balsam produces less bubbling during the curing process than toluene-based synthetic resins. Bubbling probably will not be a problem when Cellosize is used.)

(8). Cure the slides at 30°C until the balsam is hard.

CHARACTERISTICS OF ANGIOSPERM CUTICLES

RECOGNITION OF DISPERSED ANGIOSPERM CUTICLES

Within Cretaceous and Tertiary rocks, virtually all megafossils that preserve cuticle belong to angiosperms and gymnosperms; the cuticle of ferns and other pteridophytes is usually very thin and readily destroyed by diagenesis and maceration in nitric acid. Thus, one of the major problems in identifying Cretaceous and Tertiary dispersed plant cuticles is the problem of distinguishing angiosperms from gymnosperms. Most angiosperm cuticles can be distinguished readily from gymnosperm cuticles, despite the fact that no single epidermal character uniquely defines angiosperms as a group. This is because certain combinations of characters are restricted to angiosperms and because certain individual characters are restricted to one or more subgroups of angiosperms.

The following characters are considered the most reliable for establishing probable angiospermous affinities.

Height of the stomatal poles

Each pair of guard cells encloses a **stomatal pore**, and the two regions where the end walls of the guard cells meet are known as the **stomatal poles.** The stomata of both monocots and dicots are characterized by stomatal poles that are the same height as the stomatal pore (or "level" guard cells; Harris, 1932; unpublished observations). In contrast, the stomata of nearly all gymnosperms have stomatal poles that are raised relative to the stomatal pore.

Within gymnosperms, the only taxa that have level guard cells are Caytoniaceae and possibly Furcula, a taxon for which level guard cells were reported (but not illustrated) by Harris (1932). The guard cells of Gnetum and a few cycads have stomatal poles that are only slightly higher than the stomatal pore (Pant and Nautiyal, 1963; personal observations), a feature that can be difficult to discern with only light microscopy.

Cuticular thickenings on the guard cells

The stomata of most angiosperms show well developed cuticular thickenings on the outer walls of the guard cells. These thickenings are either ridge shaped (**outer stomatal ledges**) or flat (**lamellar thickenings**). Most gymnosperms lack well developed cuticular thickenings on the outer walls of the guard cells but have lignified cell walls (e.g., Harris, 1932). The most notable exceptions to this rule are Cordaites, which has well developed outer stomatal ledges (Florin, 1932), and Bennettitales, which have strong lameller thickenings on the cuticle of the guard cells (e.g., Harris, 1932). Gnetum, which has venation similar to that of dicots and stomata with only slightly raised poles, does not possess these cuticular thickenings.

Patterns of subsidiary cell arrangement

Certain patterns of subsidiary cell arrangement appear to be restricted to angiosperms. These include **anisocytic, diacytic,** and **helicocytic.**

Types of hairs

Both angiosperm and gymnosperm epidermises can possess hairs, but multiseriate hairs and peltate scales have not been reported for gymnosperms.

CHARACTERS OF SYSTEMATIC IMPORTANCE WITHIN ANGIOSPERMS

Many features of the leaf cuticle in angiosperms have systematic significance at specific, generic and higher taxonomic levels. This conclusion is based on more than 100 years of systematic anatomical studies, which have concentrated on individual genera and families but are beginning to concentrate on orders and higher taxa of flowering plants. The systematic anatomical literature provides a firm basis for the weighting of characters in fossil angiosperm cuticles but is too incomplete to allow the paleobotanist to assign dispersed cuticles to extant taxa with confidence. As a result, the paleobotanist must initially classify dispersed cuticles using a system of weighted characters, revise initial classifications after incorporating data from megafossils, then relate dispersed cuticle types to extant taxa using pertinent data from organically preserved leaf megafossils and reference collections of extant angiosperm leaf cuticles. Epidermal characters that show systematic significance within extant angiosperms have been reviewed by Stace (1965a, 1965b), Roselt and Schneider (1969), Dilcher (1974), Theobald et al. (1979), and Wilkinson (1979); some of these characters are briefly described below.

Stomata and **trichomes** are major sources of systematic information. In studies of extant fragmented leaf material, such as those conducted in the Composition Analysis Laboratory at Colorado State University, both stomata and trichomes are typically needed to make positive identifications of species and genera (T. Foppe, oral comm., 1986). Systematically important stomatal characters occur in both the **guard cells** and **neighboring** (adjacent) **cells**, which together comprise the **stomatal complex** (Fig. 1). Features of the guard cells that tend to distinguish species and genera include size, shape, outline of the guard cell walls, shape of the stomatal poles, and pattern of surface sculpture. At higher taxonomic levels, the most important feature of the guard cells is pattern of cuticular thicking. In monocotyledons and most subclasses of dicotyledons, the guard cells typically possess ridge-shaped thickenings known as **stomatal ledges**, which are borne on the outer and inner sides of the guard cells and enclose regions known as the **outer** (front) and **inner** (back) **chambers** (Fig. 1). The shape, number, and pattern of cuticular thickenings on the outer stomatal ledges have systematic importance within genera and families. In the primitive dicot subclass Magnoliidae and the enigmatic family Myrothamnaceae, outer stomatal ledges are typically absent and the guard cells possess flat cuticular thickenings known as **lamellae** or **lamellar cuticular thickenings** (Baranova, 1972; unpublished observations). The shape of these lamellae, their position within the stomatal complex, and the number of lamellae per guard cell together are useful for distinguishing genera and families within Magnoliidae (unpublished observations).

The number of neighboring cells and their pattern on specialization form the basis for stomatal classification in angiosperms. Unlike the situation for gymnosperms, stomata on an individual angiosperm leaf are typically assigned to one, or at most two, distinct types, which are based on the number and arrangement of specialized neighboring cells (or **subsidiary cells**). Within the dicotyledons as a whole, the most commonly encountered stomatal types are **anomocytic** (Fig. 2a), or with no specialized neighboring cells; **paracytic** (Fig. 2b), or with one lateral specialized neighboring cell per guard cell; **anisocytic** (Fig. 2c), or with three specialized neighoring cells, one of which is distinctly smaller than the others; **laterocytic** (Figs. 2d, 2e), or with at least three specialized neighboring cells lateral to each pair of guard cells; **cyclocytic** (Fig. 2f), or with a ring of specialized neighboring cells encircling each pair of guard cells; and **diacytic** (not pictured), or

with a pair of specialized neighboring cells oriented at right angles to the guard cell pair. When the stomatal complex possess more than one order of specialized (or subsidiary) cells, the prefix **amphi** or **complex** is added to the stomatal type (Fig. 2e). The epidermis of some angiosperm leaves shows high variation in the construction of the stomatal complex (Fig. 3), a condition that characterizes Early Cretaceous dicotyledons (Upchurch, 1984a,b) and many Late Cretaceous-early Tertiary Magnoliidae. For these groups, a semi-quantitative analysis of stomatal variation shows greater promise than typological categorizations of the stomatal complex (Upchurch, 1984b). Within monocotyledons, plant anatomists have tended not to give names to the various stomatal types. Instead, each of the four major types has been given a number designation (e.g., Stebbins and Kush, 1961).

Trichomes and **trichome bases** possess numerous systematically useful characters. Trichomes have been intensively studied by systematists and systematic anatomists, but trichomes often are abscissed during the life of the leaf, decay readily because of poor cutinization, or are destroyed during preparation of the cuticle. Features used in the classification of trichomes include the presence or absence of branching, pattern of trichome branching, the number of cell rows at the base of the hair (uniseriate vs. multiseriate), shape of the hair, and the presence or absence of glandularity. Glandularity in fossilized hairs cannot be observed directly but often can be inferred by the presence of dark contents within the hairs or the presence of a thin, punctate cuticle over the terminal cell(s) of the hair. The structure and classification of trichomes is complex and beyond the scope of this paper; for details see Uphof et al. (1962), Dilcher (1974), Radford et al. (1974), Theobald et al. (1979), and others.

A **trichome base** comprises the region of trichome attachment to the epidermis and the immediately adjacent epidermal cells, termed **base cells** (Stace, 1965a). The area of trichome attachment consists of either a **pore** or a surficial **trichome abscission scar**. The size and shape of the pore and trichome abscission scars can be important systematic characters, along with the positioning of the trichome abscission scar relative to the underlying cells. Often, the region adjacent to the point of trichome attachment has thicker cuticle than the other regions. The base cells generally show a tendency to be radially aligned, and they often differ from the surrounding cells in being highly elongate and in having a different pattern of cuticular sculpture. The terminology of trichome bases is extensively reviewed by Stace (1965a) and Dilcher (1974), with some additional features of systematic importance illustrated by Upchurch (1984b).

Secretory structures are a third group of information-rich characters. Some secretory structures originate from the epidermis, while others originate from the mesophyll tissue. Epidermal secretory structures can comprise individual secretory cells, glandular trichomes, or multicellular glands; they are recognized in cuticular preparations by the presence of dark cellular contents, the occurrence of extremely large or otherwise abnormal stomata ("**water stomata**"), the presence of structurally distinct cells that have thinner and more punctate cuticle than the adjacent cells, or structural similarities with the secretory structures of extant relatives. Mesophyll secretory structures typically preserve in cuticular preparations as either resin bodies or individual spherical cells with dark contents, and sometimes regions of the epidermis show modified structure adjacent to mesophyll secretory structures (e.g., the lid cells of many Myrtaceae). The classification of secretory structures and criteria for the recognition of secretory structures in macerated cuticle are reviewed and illustrated by Roselt and Schneider (1969). Macerated mesophyll secretory cells are well illustrated by Jähnichen (1976).

Many other features of the cuticle can have systematic significance at specific and higher taxonomic levels but have been well reviewed elsewhere. These features

include the topography of the exterior and interior surfaces of the cuticle (**surface sculpture** and **internal sculpture**, respectively), cell size, patterns of variation in the length to width ratio of cells, the pattern of cutinization of the anticlinal walls (the **cuticular flanges**), and contour of the anticlinal (or lateral) walls (Fig. 4). The interested reader should consult Stace (1965a), Dilcher (1974), and Wilkinson (1979) for discussions of terminology and systematic significance.

RECOGNITION OF MAJOR ANGIOSPERMOUS GROUPS

Epidermal/cuticular anatomy in extant flowering plants is not yet known in sufficient detail to make definitive statements about the systematic distribution of individual features within angiosperms as a whole or combinations of features that diagnose major clades of angiosperms. Nevertheless, certain combinations of features do appear to characterize major groups and can be used for tentative systematic placement of dispersed cuticle types.

Monocotyledons and dicotyledons generally can be distinguished using cuticle with stomata. Monocotyledons are characterized by stomata and unspecialized epidermal cells that are longitudinally oriented. Usually, recognizable ontogenetic lineages of epidermal cells form well defined rows many cells in length, and each stomatal complex possesses a pair of lateral neighboring cells. Dicotyledons, in contrast, are characterized by stomata and unspecialized epidermal cells that show no one preferred orientation in regions between large veins ("random" orientation). Recognizable ontogenetic lineages of cells show no one preferred orientation and often are only a few cells in length, and each stomatal complex can possess more than one pair of lateral neighboring cells. Notable exceptions to the above generalizations include: (1) monocotyledons with "reticulate" rather than "parallel" venation (e.g., Dioscoreales), and (2) dicotyledons with reduced and often linear leaves (e.g., some Caryophyllaceae).

Within the dicotyledons as a whole, a distinction can be made between woody Magnoliidae and other groups of dicots. Woody Magnoliidae are characterized by lamellar cuticular thickenings on the guard cells and a pattern of subsidiary cell arrangement on an individual leaf that is either highly variable, uniformly paracytic, or anomocytic. Many leaves of Magnoliidae also possess epidermal secretory cells that are not strongly underthrust by the adjacent cells, and spherical mesophyll secretory cells often occur in cuticular preparations. Other groups of dicots, especially the subclasses of "higher" dicots, are characterized by outer stomatal ledges on the guard cells and often posess different stomatal types from woody Magnoliidae, including anisocytic, laterocytic, and diacytic. Mesophyll secretory cells typically do not occur in cuticular macerations; resinous exudates from multicellular mesophyll secretory bodies are another matter.

Differences in epidermal structure between the various subclasses of "higher" dicots are not yet well known, but certain families appear to be distinctive. See the modern resources section for initial orientation to this large body of dispersed literature.

CLASSIFICATION OF DISPERSED ANGIOSPERM CUTICLE

The classification of dispersed angiosperm cuticles is problematic relative to the classification of dispersed gymnosperm cuticles and leaf megafossils for two reasons.

(1). Cellular patterns typically vary between stem and leaf, between the upper and lower leaf surfaces, and between areas associated with major veins and areas not associated with veins. These regions dissociate from one another through (a) fragmentation of stems and leaves during abscission, transport, and fossilization, and (b) separation of adaxial and abaxial cuticles during maceration. This

dissociation of epidermal regions produces a diversity of dispersed cuticle types within an individual assemblage, all of which could represent different parts of the same shoot.

(2). The systematic distribution of cuticular features in extant angiosperms is poorly known relative to the systematic distribution of cuticular features in extant and fossil gymnosperms. Thus, the character combinations that diagnose many angiosperm clades are unknown.

Two research strategies have been designed to cope with these problems, both of which use some artificial system of classification.

The strategy proposed by Roselt and Schneider (1969) was an artificial system of classification for dispersed plant cuticles (mostly angiospermous), which was modeled after Potonié's system for dispersed pollen and spores. In the Roselt and Schneider system, the primary groups of cuticles were based on the presence or absence of cell outlines, the presence or absence of stomata, and the pattern of cell organization. Cuticles with stomata were further subdivided based on pattern of neighboring cell specialization and the presence or absence of trichomes and trichome bases. All distinctive cuticle types were given a binomial name, even if two or more associated cuticle types could have been derived from the same plant. This system of classification has been followed in many subsequent studies of dispersed angiosperm cuticles. The major features of the system are outlined in Kovach and Dilcher (1984, Table 1).

I have devised a somewhat different strategy for my own research on Cretaceous to early Tertiary dispersed cuticles. In this system, dispersed cuticles are assigned to one of five artificial types, based on cellular patterns and abundance of stomata. These five types are then organized into three groups based on probable position on the parent plant, then analyzed taxonomically and/or paleoecologically.

Type 1 cuticle is most characteristic of herbaceous or young woody stems, petioles, leaf rachises, and regions of the leaf associated with major veins. The cells are all organized into distinct rows or longitudinal files, alternating bands of cells are absent, and stomata are rare to absent.

Type 2 cuticle characterizes the adaxial (upper) epidermis of "parallel-veined" monocot leaves. Like Type 1 cuticle, the cells are oriented into distinct rows or longitudinal files, and stomata are rare to absent. In contrast to Type 1 cuticle, alternating bands of cells are present.

Type 3 cuticle characterizes the abaxial (lower) surface of "parallel-veined" monocot leaves. Type 3 cuticle is characterized by common stomata and longitudinally-oriented cells that are often organized into distinct bands.

Type 4 cuticle characterizes the adaxial (upper) epidermis of dicot leaves and "reticulate-veined" monocot leaves. Type 4 cuticle is characterized by large regions of unoriented cells and rare to absent stomata.

Type 5 cuticle characterizes the abaxial (lower) epidermis of dicot leaves and "reticulate-veined" monocot leaves. Type 5 cuticle is characterized by large regions of unoriented cells and abundant stomata.

For purposes of data analysis, the above five cuticle types are organized into three groups: (A) Type 1, (B) Types 2 and 4, and (C) Types 3 and 5.

Each group is analyzed somewhat differently, because each potentially contains different information about the individual parent plants and the assemblage as a whole.

For Type 1 cuticle, only abundance relative to other cuticle types is calculated. The relative abundance of Type 1 cuticle is used to estimate the extent to which a dispersed cuticle assemblage represents cuticles from petioles, rachises, and herbaceous stems. A high relative abundance of Type 1 cuticle can result from a high abundance of petioles, rachises, and herbaceous stems in the assemblage at the time of deposition (due to derivation from herbaceous vegetation), preferential decay of other cuticle types (which typically are thinner and therefore less resistant to oxidation), or a combination of the two. Many assemblages dominated by Type 1 cuticle show other lines of evidence for local or regional dominance of herbaceous plants. This includes the interval of fern spore abundance immediately above the Cretaceous-Tertiary boundary clay (the "fern spike interval"), taken as evidence for widespread ecological disruption following a bolide impact (Upchurch et al., 1985; Wolfe and Upchurch, 1987a).

Type 3 and Type 5 cuticles are given full taxonomic recognition. Prior to publication of Linnean binomials, Type 3 and Type 5 cuticles are grouped using an artificial binomial system in which the name contains abbreviations for many of the diagnostic features. For example, a cuticle characterized by paracytic stomata with outer stomatal ledges, striate cuticle, and glands would have the characterization "Oslpara-striate/glands" followed by a number. These groupings of cuticles are based largely on cuticular features summarized by Wilkinson (1979) and, hence, on a larger number of characters than Roselt and Schneider's genera. Emphasis is placed on features that circumscribe genera and higher taxa of extant angiosperms (e.g., the presence of lamellar thickenings or outer stomatal ledges on the guard cells). The exact groupings are based on patterns of congruence between individual cuticular features, rather than a set formula, and are modified when taxonomic information becomes available from megafossils.

For Type 2 and Type 4 cuticles, all distinct types with hairs or hair bases are noted for each assemblage, but no formal taxonomic status is given. The diversity of Type 2/Type 4 cuticles with trichomes or trichome bases is then calculated relative to the diversity of Type 3/Type 5 cuticle for each assemblage. This calculation is used as a tentative estimate of the openness of vegetation, based on the fact that plants of dry regions and shade-intolerant plants of wet climates tend to show the highest percentage of species with hairs on the upper epidermis (e.g., Fahn, 1967; Coley, 1983). Distinctive cuticle types with hairs are photographed.

In order to facilitate identification of unknown dispersed cuticles, I glue 2 to 4 photographs of each known cuticle type onto a 5" by 8" index card. The cards are filed alphabetically according to my parataxonomic system.

SCIENTIFIC USES OF DISPERSED ANGIOSPERM CUTICLES

Ultimately, the degree to which paleobotanists study dispersed angiosperm cuticles will be determined by how readily dispersed cuticles can contribute useful data for the solution of important scientific problems. The limited number of studies conducted to date indicate that the greatest potential of dispersed angiosperm cuticles may lie in the areas of paleocommunity reconstruction, plant diversity and extinction, and possibly paleoclimate.

Most commonly, dispersed angiosperm cuticles have been used to reconstruct paleocommunities and variation in vegetational physiognomy, especially in coal swamps (e.g. Peters, 1963; Schneider, 1969; Upchurch et al., 1985). All authors have noted marked variation in the taxonomic composition of dispersed cuticle assemblages in vertical section, taken as evidence for changes in climate and/or changes in local ecological conditions such as depth of water table. Several authors (Schneider, 1969; Juchniewicz, 1975b; Upchurch et al., 1985) have noted that some assemblages are

characterized by an abundance of either masses of elongate cells (e.g., Marcoduria assemblages) or non-stomatal cuticles where the cells tend to be organized into rows (Type 1 cuticle), while other assemblages are characterized by an abundance of cuticles with unoriented cells and abundant stomata (Type 5 cuticle). These two types of assemblages have been inferred to represent the remains of herbaceous and woody vegetation, respectively. In one study of a laterally extensive coal seam (Upchurch et al. 1985), sample by sample comparisons were made between the relative abundance of cuticles and palynomorphs. Here, changes in the taxonomic composition of cuticle assemblages paralleled changes in the relative abundance of palynomorphs, which indicates that dispersed cuticles were more sensitive indicators of paleocommunities. However, the combined analysis of cuticles and palynomorphs provided a more complete picture of ecological and environmental change than either type of remain could provide in isolation.

The stratigraphic distribution of dispersed angiosperm cuticles has been used in conjunction with data from leaf megafossils to study plant extinction patterns during the terminal Cretaceous mass extinctions (Wolfe and Upchurch, 1987a; Upchurch and Wolfe, 1987; Kauffman et al., in press). Dispersed angiosperm cuticles corroborate data from palynology for rapid extinction of many plant taxa at the end of the Cretaceous, because many dispersed cuticle types characteristic of the latest Cretaceous have their highest stratigraphic occurrence immediately beneath the Cretaceous-Tertiary boundary clay. Some taxa that show rapid extinction, such as Laurales, have yet to be reported from the pollen record for the same stratigraphic sections. Dispersed angiosperm cuticles also indicate that palynology may underestimate true biological extinction, because many surviving clades of cuticles show reduced species diversity during the earliest Tertiary, and groups such as monocots show major changes in specific and generic composition that have yet to be noted in the pollen record. Both leaf megafossils and dispersed cuticles indicate reduced species diversity and stereotyped leaf structure during the earliest Tertiary relative to the latest Cretaceous (Fig. 5).

Few studies of paleoclimate have used dispersed cuticles, but the results from these studies are consistent with evidence from other paleoclimatic indicators. Litke (1967) inferred climatic warming during the Miocene (floral zones XI and XII of Mai) based on the physiognomy of dispersed cuticle assemblages. The assemblage from Zone XI was characterized by a majority of species with thin cuticles that came from leaves with toothed margins, while the assemblage from Zone XII was characterized by a majority of species with thick cuticles, which characterize entire-margined leaves from the tropics and subtropics. Coeval fruit and seed assemblages (cf. Mai, 1967) showed a higher proportion of tropical and subtropical elements during the inferred warm interval. For the latest Cretaceous and earliest Tertiary of the southern Rocky Mountains, Wolfe and Upchurch (1987a, 1987b) inferred a major increase in precipitation across the Cretaceous-Tertiary boundary. Wolfe and Upchurch (1987a) noted that the percentage of Type 2/Type 4 cuticle with trichomes and trichome bases showed an inverse correlation with average leaf size and the percentage of species with drip tips, which implied that "hairy" assemblages probably represented open-canopy vegetation of subhumid climates and "hairless" assemblages probably represented closed-canopy vegetation of wetter climates. Stratigraphic plots of dispersed cuticle assemblages indicate a rapid decrease in the hairiness of dispersed cuticle assemblages at the Cretaceous-Tertiary boundary (Fig. 6), which implies that the inferred increase in precipitation occurred rapidly.

RESOURCES FOR THE IDENTIFICATION OF DISPERSED CUTICLE

Major resources for the identification of dispersed angiosperm cuticle include the literature and comparative collections. One major means for identifying dispersed

angiosperm cuticle is by comparison with the cuticular anatomy of coeval leaf megafossils. For Cretaceous angiosperms, systematic coverage exists for only a small fraction of the known species. Papers that illustrate in situ or dispersed angiosperm cuticle include those of Miner (1935), Krassilov (1973; 1979), Němejc and Kvaček (1975), Tanai (1979), Kvaček (1984), Upchurch (1984a,b), Upchurch and Dilcher (in press), and Kauffman et al. (in press).

For Tertiary angiosperms, systematic coverage is far more complete, especially for remains from Europe. Citation of all relevant papers would be an impossible task and is not attempted here. Papers on Tertiary dispersed cuticles include those of Peters (1963), Litke (1966, 1967), Roselt and Schneider (1969), Schneider (1969), Doubinger and Pons (1973), Juchniewicz (1975a,b), Anzotegui (1980), and Kovach and Dilcher (1984). Major papers on in situ angiosperm cuticle include those listed in the history section; references to much of the literature on Tertiary angiosperm leaf cuticles can be found in Roselt and Schneider (1969), Dilcher (1974), and Kovach and Dilcher (1984).

The literature on the systematic anatomy of extant angiosperm epidermises is incomplete, but enough data currently exist to guide the budding cuticle specialist to possibly related modern taxa. Monocotyledons currently have the best systematic coverage at the level of subclass and order. This largely has been accomplished through the Anatomy of the Monocotyledons series, published by Clarendon Press at Oxford University. In these works, epidermal anatomy is illustrated in both surface view and transverse section by line drawings. Systematic coverage includes Graminae (Metcalfe, 1960), Cyperaceae (Metcalfe, 1971), Palmae (Tomlinson, 1961), Commelinales and Zingiberales (Tomlinson, 1969), Juncales (Cutler, 1969), and Dioscoreales (Ayensu, 1972). Comprehensive coverage for dicotyledons is currently limited to the first edition of Metcalfe and Chalk's Anatomy of the Dicotyledons (1965) and tables in the second edition of Anatomy of the Dicotyledons, Volume 1 (1979). Use of the first edition of Anatomy of the Dicotyledons is complicated by few illustrations of epidermal anatomy in surface view, no analysis of many cuticular features, and pidgeonholing of dicot stomata into only four types. These problems will be alleviated with full publication of the second edition of Anatomy of the Dicotyledons. Both general volumes in the Second Edition have been published as well as Volume III, Magnoliales, Laurales, and Illiciales (Metcalfe, 1987). Numerous papers on individual genera and families also exist; references can be found in the above volumes and through computer searches of bibliographic data bases such as Biosis.

North American comparative collections of extant angiosperms include macerated cuticles and foliage that has been cleared and stained for venation. The largest collection of macerated extant angiosperm leaf cuticle is at Indiana University, Bloomington (soon to be housed at the Florida State Natural History Museum in Gainesville). The IU collection comprises approximately 4,000 species of monocot and dicot leaves, of which about one-half have been prepared for cuticular anatomy. Slides typically have been prepared in triplicate to facilitate loans. Interested researchers should contact either Dr. David L. Dilcher or Dr. Steven R. Manchester.

Foliage that has been cleared and stained for venation is much more difficult to use in studies of epidermal anatomy than either cuticular macerations or epidermal peels. This is because the clearing process often removes the cuticle, and the staining procedure often stains epidermis and mesophyll equally, which makes observation of epidermal features difficult. Nevertheless, cleared exant angiosperm leaves can be a valuable source of information on epidermal anatomy in extant flowering plants, if care is exercised in making anatomical interpretations and only good clearings are used (approximately 1 in 4 clearings is good for epidermal anatomy). The two major comparative collections of cleared extant angiosperm leaves are the U.S. National Cleared Leaf Collection (over 8,000 species), housed at Yale University, and the U.S.

Geological Survey Cleared Leaf Collection (over 17,000 species), housed at the U.S. Geological Survey, Denver. Researchers interested in using the U.S. National Cleared Leaf Collection should contact Dr. Leo J. Hickey at Yale University. Researchers interested in using the U.S. Geological Survey Cleared Leaf Collection should contact Dr. Jack A. Wolfe at the U.S. Geological Survey in Denver. The two collections have complementary strengths and only partial taxonomic overlap; I recommend use of both.

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Figure 1. Surface (a) and transverse (b) views of the stomatal complex in angiosperms. In b, the cuticle is shown in heavy black. Abbreviations: EW = end wall of guard cell; OSL = outer stomatal ledge; PW = poral wall of guard cell; TP = T-piece; EpW = epidermal wall of guard cell; RW = radial wall of neighboring cell; TW = tangential wall of neighboring cell; OW = outer wall of epidermal cell; IW = inner wall of epidermal cell; FC = front (or outer) cavity; BC = back (or innner) cavity; SsC = substomatal chamber.

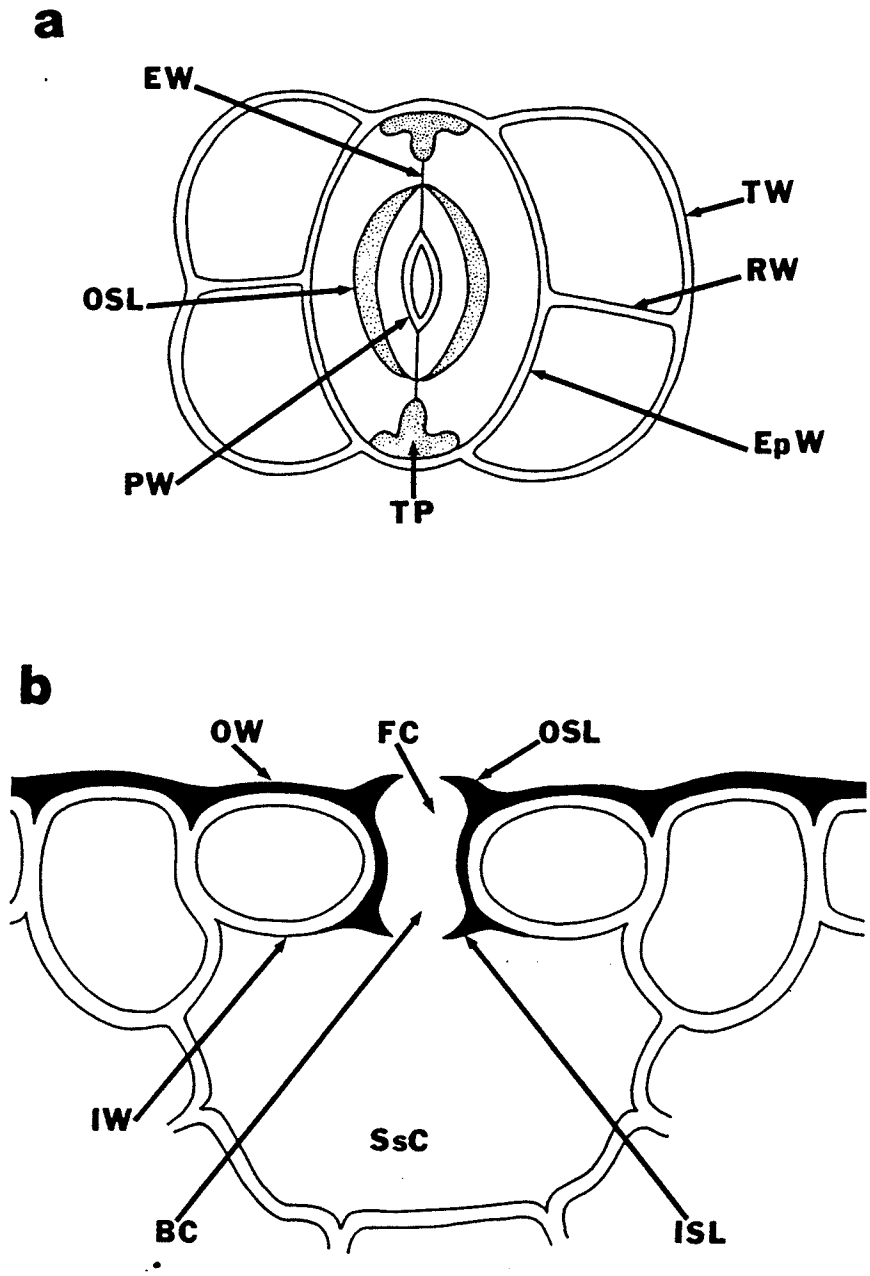

Figure 2. Six types of stomatal complexes commonly found in dicotyledons. a. Anomocytic b. Paracytic c. Anisocytic d. Laterocytic e. Complex (or amphi) Laterocytic f. Cyclocytic.

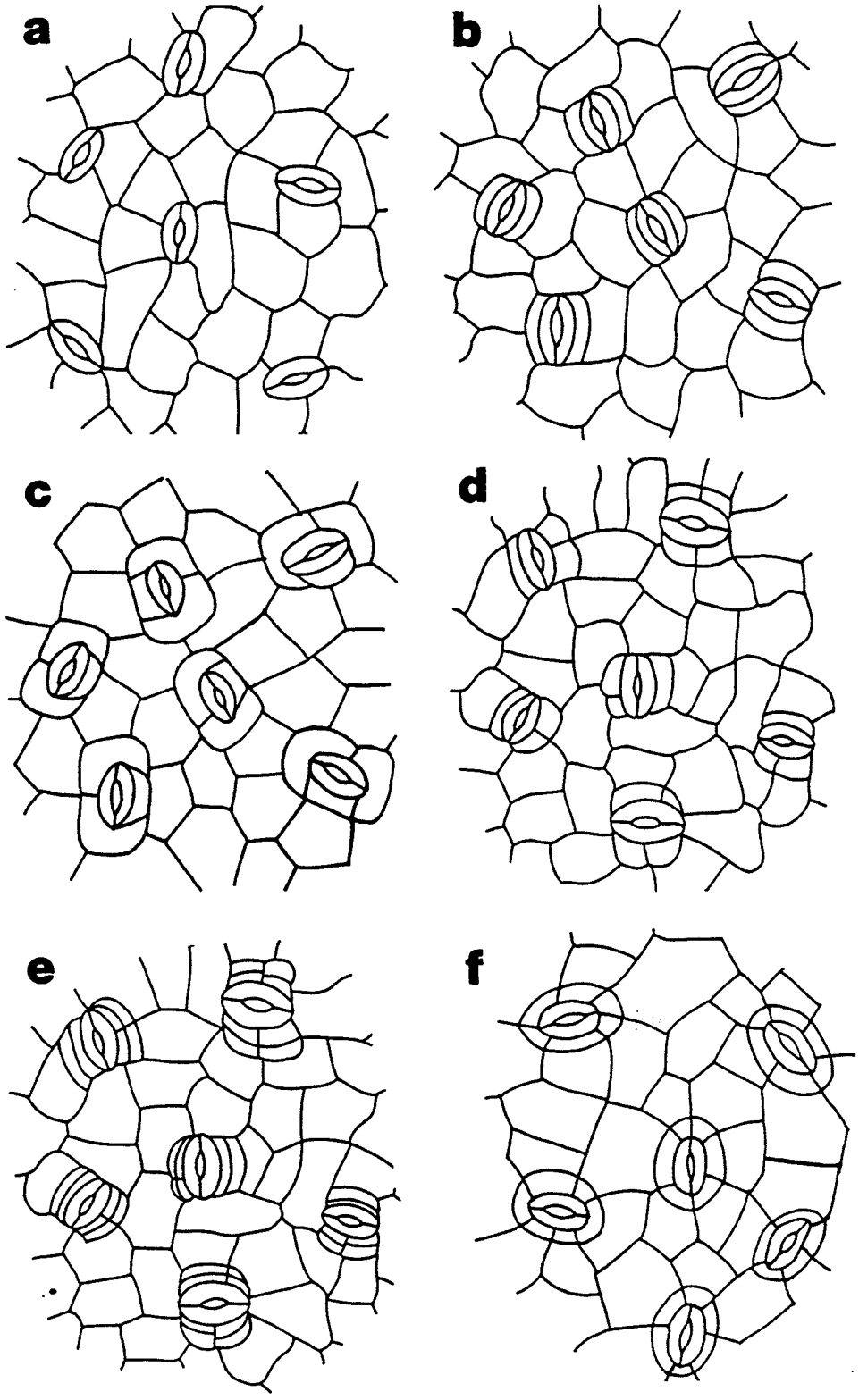

Figure 3. Six stomata from the lower epidermis of a single leaf of Early Cretaceous angiosperms (Drewrys Bluff Leaf Type 1 of Upchurch, 1984a, b). Note paracytic (lower middle), anomocytic (upper right), and additional unnamed stomatal types.

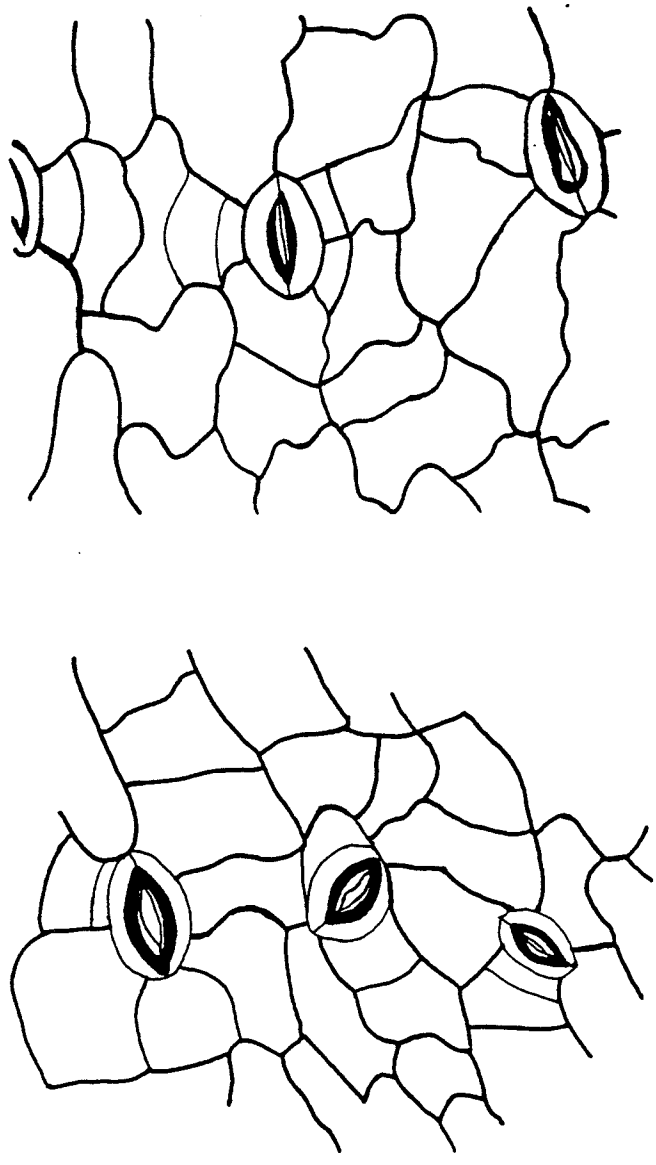

Figure 4. Some major components of anticlinal wall contour. a. Straight walls b. Curved walls c. Sinuous walls d. Two components of sinuosity.

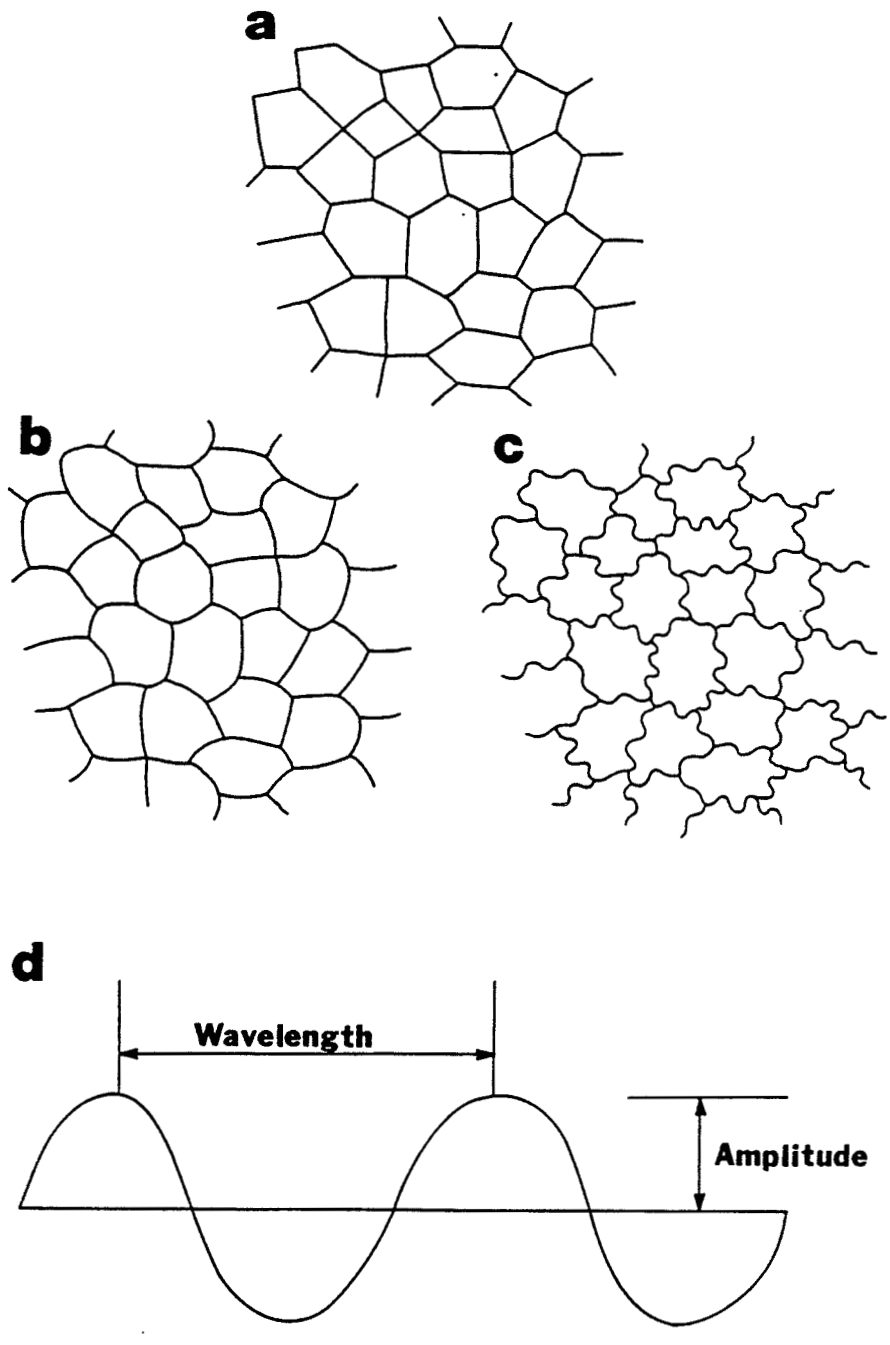

Figure 5. Sequence of important angiosperm leaf megafossils and dispersed cuticles across the Cretaceous- Tertiary boundary, Raton Basin (from Wolfe and Upchurch, 1987a, Fig. 2.). Note the large size of leaf megafossils and the absence of leaf cuticles with hairs in the Tertiary.

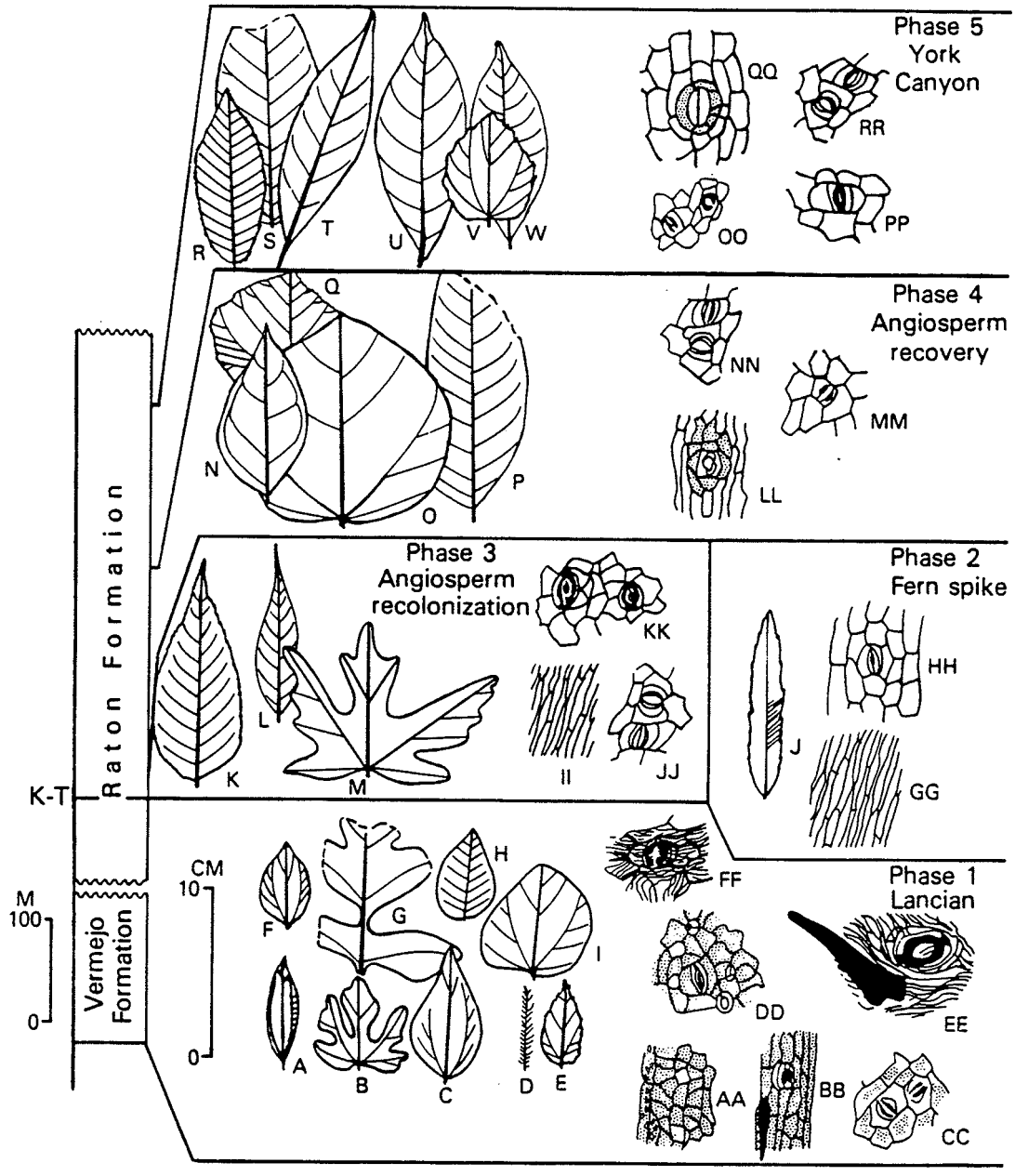

Figure 6. Composite section of the Cretaceous part of the Raton Formation showing the percentage of Type 2/Type 4 cuticles with trichomes or trichome bases relative to Type 3/Type 5 cuticles. Note the sharp decrease at the Cretaceous-Tertiary boundary. Early Tertiary assemblages are generally (but not always) hairless, unlike latest Cretaceous assemblages. All percentages are based on assemblages with more than 20 species of Type 3/Type 5 cuticle; stratigraphically contiguous assemblages were pooled if the total number of Type 3/Type 5 species was less than 20.